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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

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Re: Expediente No. 09.149.219 ✓

Solicitud de patente a nombre de **WORLD-TRADE IMPORT-EXPORT, WTIE, AG**

Yo, DILIA MARIA RODRIGUEZ D'ALEMAN identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, piso 6, obrando como apoderada de la sociedad **WORLD-TRADE IMPORT-EXPORT, WTIE, AG** domiciliada en Zug, Suiza, solicitante de la patente de la referencia, en respuesta al oficio No. 15165 notificado por fijación en lista el 28 de agosto de 2012, me permito manifestar:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio que consta de 2 cláusulas dirigidas a la composición farmacéutica que comprende la combinación específica de paroxetina hemidrato del clorhidrato de paroxetina en y alprazolam. (ANEXO 1)

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de superar las objeciones de claridad planteadas por el examinador en su concepto técnico.

2. **De la claridad de la solicitud**

En relación con la objeción planteada por el señor examinador, atentamente nos permitimos manifestar que el nuevo pliego reivindicatorio describe una composición específica que contiene cantidades exactas de los dos principios activos presentes en la misma.

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3. De la novedad de la invención

En este sentido, respetuosamente nos permitimos decir que el artículo 16 de la Decisión 486 establece que: “Una invención se considera nueva cuando no está comprendida en el estado de la técnica”.

El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida....” (El resaltado es nuestro) y de acuerdo con el espíritu de la norma legal indicada, la invención no se considera como nueva si está “comprendida” en el estado de la técnica, dicha norma se está refiriendo, naturalmente, a la totalidad del invento y no a alguno o a algunos de los componentes que hacen parte de la nueva solicitud.

En efecto, para que la invención pierda su calidad de novedosa, es claro que ella, TODA ELLA, ha de estar contenida en el “*conjunto de conocimientos existentes que son aplicables al proceso industrial*” como define el estado de la técnica el Tribunal de justicia del Acuerdo de Cartagena (Proceso No. 6-IP-89, sentencia de 31 de octubre de 1989, publicada en Jurisprudencia del Tribunal de Justicia del Acuerdo de Cartagena, Tomo 11, BID-INTAL, Buenos Aires, 1994, página 60).

Esto se conoce como **NOVEDAD ABSOLUTA**, de conformidad con lo cual para desvirtuar el requisito de novedad de una invención será necesario que lo reivindicado por la invención bajo estudio corresponda de forma total con lo revelado en un documento o anterioridad señalada, conclusión a la que se llega después de un análisis exhaustivo mediante una comparación entre el capítulo reivindicatorio de la invención en estudio y la divulgación citada por ese despacho como anterioridad.

Ahora bien, si un determinado elemento, condición o circunstancia de la nueva creación ya hace parte del estado de la técnica no quiere ello decir, desde luego, que la invención deba ser rechazada por carecer del requisito de novedad. No sin razón ha dicho el mencionado Tribunal: “*hay que advertir que toda creación es en cierta forma, producto del estado de la técnica existente, en el sentido de que el inventor ha de tener en cuenta un conjunto de conocimientos técnicos que le permitan crear nuevos procedimientos aplicables a la industria*” (Ibidem, páginas 53 y 54) (El subrayado es nuestro).

Por lo tanto y teniendo en cuenta lo anteriormente expuesto, como mínimo la novedad debe contemplar los siguientes aspectos:

-Que la invención en estudio esté contenida de forma **TOTAL** y **ABSOLUTA** en el documento que se menciona como anterioridad; y

-Que la invención en cuestión puede partir de conocimientos conocidos, caso en el cual habrá que hacer la comparación no de lo conocido sino de lo inédito y novedoso, teniendo que analizarse el valor agregado que aporta la invención

con sus nuevas características al campo técnico al cual pertenece la solicitud en estudio.

Aplicando lo anterior al caso concreto, para que las razones que aduce ese despacho para desvirtuar la novedad fuesen ciertas, sería forzoso que se demostrara que la invención de mi mandante está comprendida en el estado de la técnica de forma **TOTAL**, lo cual no sucede toda vez que las características mostradas en el capítulo reivindicatorio modificado son totalmente diferentes de aquellas enseñadas en el documento D1.

En ese orden de ideas, el señor examinador considera que las características técnicas esenciales de la composición de la reivindicación 1 habían sido divulgadas previamente en el siguiente documento D1:

D1.- "Lack of Pharmaceutical Interaction Between Paroxetine and Alprazolam at Steady in Healthy Volunteers", Gonzalo Calvo, MD, PhD*, Consuelo García-Gea, BSc., Antonio Luque, MD, Adelaida Morte, MD, Rafael Dal-Ré, MD, PhD, and Manel Barbanoj, MD, PhD. Journal of Clinical Psychopharmacology, Mol. 3, págs. 268-76. Junio 2004."

Dicho documento da a conocer un estudio en donde se evaluó la administración combinada de los principios activos Paroxetina y Alprazolam en dosis de 20 mg/día y 1 mg/día respectivamente, en las dosis descritas una vez al día, durante 15 días.

Dicho estudio se realizó en 25 voluntarios, adultos jóvenes sanos a los cuales se les administraron 4 secuencias de tratamiento las cuales corresponden a Paroxetina-Alprazolam placebo, Alprazolam-Paroxetina placebo, Paroxetina-Alprazolam, y Paroxetina Placebo-Alprazolam placebo. Esto con el fin de evaluar la interacción farmacocinética de la terapia combinada, y se observó que no se producía interacción entre los principios activos.

En razón de lo anterior, el Examinador concluye lo siguiente:

"En consecuencia, la reivindicación 1 carece de novedad, según lo divulgado en el documento D1."

Sin embargo, aunque en dicho documento se menciona la administración de los mismos principios activos contemplados en la presente invención y se aborda el mismo problema, **no es así con la solución propuesta.**

La terapia descrita en el documento D1 consiste en la **CO-ADMINISTRACION** de Alprazolam y Paroxetina, no así el desarrollo de un medicamento que combine Paroxetina con Alprazolam **en una sola unidad de dosificación** (ver página 275, primer párrafo segundo renglón "*As expected, coadministration of Paroxetine and Alprazolam...*" énfasis añadido). Se adjunta al presente como **Anexo 2** el texto completo de dicho documento D1 para mayor referencia.

Aunado a lo anterior, el nuevo capítulo reivindicatorio acota la dosis de la presente invención, con lo cual la dosis utilizada en la referencia citada por el examinador no

corresponde a la descrita en la presente invención, por lo que resulta ser novedosa y con nivel inventivo.

Asimismo, debemos tener en cuenta que para obtener una composición farmacéutica eficaz y segura que comprenda dos o más fármacos contenidos en una misma unidad de dosificación es necesario contar con conocimiento desarrollado acerca de las propiedades fisicoquímicas (reología, estabilidad química, etc.) que caracterizan a cada uno de los compuestos activos que componen la formulación, así como de las incompatibilidades químicas e interacciones farmacológicas (farmacocinéticas y farmacodinámicas) que pueden generarse entre dichos compuestos, ya que estos son factores que pueden imposibilitar la mezcla de dos o más compuestos activos en una misma forma farmacéutica para conseguir su administración conjunta y/o provocar variaciones en su efecto terapéutico.

Con los resultados de ambos estudios (preclínico y clínico) relacionados con la presente invención, los cuales han sido presentados en el presente procedimiento (oposición y solicitud, respectivamente), se demuestra que cuando se administra la combinación de los fármacos Paroxetina y Alprazolam en una misma unidad de dosificación y con menores concentraciones con respecto a las conocidas para cada uno de los fármacos administrados de forma individual, se lograron estabilizar (*in vitro*) las propiedades fisicoquímicas que los caracterizan, tal como pH, densidad, tamaño de partícula, punto de fusión, solubilidad, etc., además de mejorar (*in vivo*) los tiempos de realización de los procesos de absorción, distribución, metabolismo y excreción de la formulación que contiene la combinación de fármacos, dando como resultado un efecto terapéutico eficaz en menor tiempo y con menores efectos adversos.

En cambio, cuando son administrados en forma concomitante, los fármacos "compiten" entre sí para lograr individualmente el efecto deseado o alcanzar una concentración plasmática máxima en menor tiempo, por lo que NO ES EL MISMO RESULTADO, es decir, NO ES EL MISMO EFECTO el que se presenta farmacocinética y farmacodinámicamente con la administración concomitante y la administración de ambos fármacos en una sola unidad de dosificación.

La razón de lo anterior se debe a que al combinar Paroxetina y Alprazolam en una misma unidad de dosificación se consigue un equilibrio entre ambos fármacos en sus propiedades fisicoquímicas (PREVIO A SU ADMINISTRACIÓN), farmacocinéticas y farmacodinámicas (POSTERIOR A SU ADMINISTRACIÓN), el cual se demuestra con un efecto terapéutico eficaz y con la incidencia de menores efectos adversos, de tal manera que la administración concomitante de dichos fármacos nunca tendrá el mismo efecto que la administración en una única unidad de dosificación, ya que, por separado ambos activos presentan diferencias significativas en sus características fisicoquímicas, farmacocinéticas y farmacodinámicas, que pudieran dar como resultado efectos individualizados mínimos o poco eficaces al emplear menores concentraciones de dichos fármacos.

Por otra parte, el Examinador menciona que es conocido también en el estado de la técnica un estudio en el cual se evaluaron los efectos adversos provocados con la administración simultánea de inhibidores de la recaptación de serotonina y

antipsicóticos. En donde se administraron diariamente de forma conjunta Paroxetina 20 mg y Alprazolam 1.2 mg en el tratamiento de la depresión y de la ansiedad en donde se evidenció la producción del síndrome maligno neuroléptico como una complicación en el tratamiento antipsicótico.

Precisamente, una de las ventajas que reporta la presente invención es la **disminución de concentración**, lo cual trae como resultado la reducción de los efectos adversos, por lo que dicho documento mencionado en el Examen de Patentabilidad (Tani H, Ichihashi K, Inoue K, Fujita K, Okazaki Y. "Possible neuroleptic syndrome related to concomitant treatment with Paroxetina and Alprazolam". Vol. 30, págs. 1176-1178) refuerza el requisito de nivel inventivo de nuestra solicitud.

Información Complementaria.

En la descripción de la presente solicitud se presentó el "**Estudio de Interacción entre Paroxetina Hemidrato y Alprazolam en voluntarios sanos**", con el cual, a efecto de acreditar la disminución de dosis en la combinación materia de la presente invención, queremos hacer notar que, tal y como se menciona en la metodología descrita, el medicamento solo necesita ser administrado **1 UNA VEZ AL DÍA**, siendo que en el estado de la técnica aplicable a la presente invención **era necesaria la administración de dosis más altas dosificadas** en varias tomas diarias a fin de conseguir el efecto terapéutico deseado. En dicho estudio se presentaron los siguientes resultados que se adjuntan al presente bajo el número de **Anexo 3**.

Asimismo, se adjuntan al presente diversos estudios (**Anexos 4 a 9**) en donde podemos apreciar la dosis conocida del activo Alprazolam, con lo cual se evidencia que, cuando éste es prescrito en forma individual habrá que administrar dosis más elevadas comparadas con la utilizada en la presente invención.

ANEXO 4.- "Alprazolam plasma concentrations and treatment response in panic disorder and agoraphobia." (2 a 6 mg diarios) Lesser IM, Lydiard RB, Antal E, Rubin RT, Ballenger JC, DuPont R. Department of Psychiatry, Harbor-UCLA Medical Center, Torrance 90509.

Se evaluó la relación entre la concentración plasmática de alprazolam y los efectos adversos, y la respuesta al tratamiento en pacientes con desorden de pánico y agorafobia. 96 noventa y seis pacientes con desorden de pánico y agorafobia fueron tratados en un estudio de 6 semanas, con dosis mixta, doble ciego, controlado con placebo, dosis-respuesta de 2 mg/día o 6 mg/día de alprazolam.

ANEXO 5.- "The relationship of alprazolam dose to steady-state plasma concentrations." Ciraulo DA, Antal EJ, Smith RB, Olson DR, Goldberg DA, Rand EH, Raskin RB, Phillips JP, Shader RL, Greenblatt DJ. J Clin Psychopharmacol. 1990 Feb; 10(1):27-32. Department of Psychiatry, Tufts University School of Medicine, Boston, Massachusetts. AmJ Psychiatry. 1992 Nov; 149(11):1556-62..

Este estudio examina la relación entre el amplio rango de dosis terapéuticas de Alprazolam, que van desde 1 mg hasta 10 mg diarios.

ANEXO 6.- "Drug treatment of panic disorder. Comparativa efficacy of alprazolam, imipramine, and placebo." Cross-National Collaborative Panic Study, Second Phase Investigators. J Clin Psychiatry. 1993 Oct; 54 Suppl: 25-45; discussion 46-8.

Se comparó alprazolam, imipramina y placebo en una muestra de 1,168 sujetos asignados randomizadamente. El estudio se condujo en 12 centros, valorando los cambios clínicos durante 8 semanas de tratamiento doble ciego. Se administró desde 1 mg de alprazolam hasta 10 mg, según el estado del paciente.

ANEXO 7.- Cambridge University Press 0521011698 - Essential Psychopharmacology: **The Prescriber's Guide** Stephen M. Stahl Excerpt. En este documento se publica una dosis de Alprazolam que va desde 1 mg hasta 6 mg diarios.

ANEXO 8.- "Clinical Pharmacology, Clinical Efficacy, and Behavioral Toxicity of Alprazolam: a review of the literature." Joris C. Verster and Edmund R. Volkerts, Utrecht Institute for Pharmaceutical Sciences, Department of Psychopharmacology, University of Utrecht, Utrecht, The Netherlands. CNS Drug Reviews Vol. 10, No. 1, pp. 45-76, 2004 Neva Press, Branford, Connecticut.

Se hizo una revisión de la literatura en donde se evaluó la actuación de Alprazolam en diversos trastornos, así como las dosis conocidas de este activo que, por ejemplo, en el caso del Desorden de Pánico la dosis media fue de 4.2 mg diarios después de 3 semanas y 5.7 después de 8 semanas de tratamiento.

ANEXO 9.- "Sertraline and alprazolam in the treatment of panic disorder."
Fisekovic S, Loga-Zec S. Bosn J Basic Med Sci. 2005 May; 5(2): 78-81. Pschiatric Clinic. University of Sarajevo, Clinics Center, Bolnicka 25, 71 000, Sarajevo, Bosnia and Herzegovina. Describe un estudio de 12 semanas controlado con placebo, con dosis mixta, con pacientes diagnosticados con desorden de pánico con y sin agorafobia, para evaluar la eficacia y seguridad de sertralina y alprazolam. El estudio incluyó 40 pacientes divididos en 2 grupos. Sertralina fue administrado en dosis de 20 mg/día y Alprazolam en dosis de 1-1.5 mg/día.

Como podemos apreciar el compuesto activo Alprazolam es administrado, hasta antes de la presente invención, en dosis que van desde 1 mg hasta 6 mg diarios. Aunado a lo anterior, podemos apreciar que, aunque es administrado en concentraciones de 0.25 mg, es dosificado 4 veces al día, es decir, 1 mg diario.

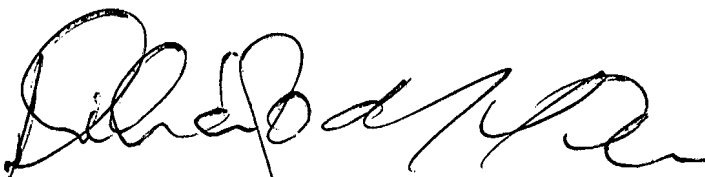
Lo anterior evidencia que la presente invención aporta una significativa disminución de dosis y también una mejora en los resultados conseguidos con la combinación en comparación a cuando los activos son administrados separadamente, así como una considerable disminución de efectos adversos.

Finalmente, por medio del presente y en relación con el estudio preclínico presentado como parte de la contestación a la oposición interpuesta por LAFRANCOL, S.A., solicitamos atenta y respetuosamente que el mismo sea tomado en consideración como evidencia del efecto sinérgico que presenta la combinación materia de la presente invención.

Habiendo atendido cabalmente las objeciones presentadas por ese despacho, atentamente solicitamos se conceda el privilegio de patente de invención a la solicitud en estudio o si el señor examinador considera que subsisten razones para objetar la presente invención, rogamos expedir otro examen de fondo con los debidos motivos y razones que sustenten su objeción con el fin de que nuestro mandante tenga la posibilidad de exponer sus argumentos con base en la interpretación de los artículos 45 de la Decisión 486 -el cual otorga la facultad al examinador para emitir los exámenes de fondo-, 35 y 59 del Código Contencioso Administrativo que señala que: *“habiéndose dado oportunidad a los interesados para expresar sus opiniones, y con base en las pruebas e informes disponibles, se tomará la decisión que será motivada al menos en forma sumaria si afecta a particulares”* y *“(.) deberá proferirse la decisión definitiva. Esta se motivará en sus aspectos de hecho y de derecho, y en los de conveniencia si es el caso”*, respectivamente (El subrayado es nuestro).

En consecuencia, solicitamos a ese despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

Señor Superintendente,



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ANEXO 1

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NOVEDAD DE LA INVENCION

Habiendo descrito la presente invención, se considera como novedad y, por lo tanto, se reclama como propiedad lo contenido en las siguientes

REIVINDICACIONES

1.- Una composición farmacéutica caracterizada porque consiste en la combinación de Paroxetina hemidrato del Clorhidrato de Paroxetina en una cantidad de 20.0 mg. y Alprazolam en una cantidad de 0.25 mg, además de excipientes farmacéuticamente aceptables, los cuales se encuentran formulados en una sola unidad de dosificación para ser administrada por vía oral.

2.- La composición farmacéutica de conformidad con la reivindicación 1, caracterizada además porque se encuentra en la forma farmacéutica de tabletas o cápsulas.

ANEXO 2

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Lack of Pharmacologic Interaction Between Paroxetine and Alprazolam at Steady State in Healthy Volunteers

Gonzalo Calvo, MD, PhD,* Consuelo García-Gea, BSc,† Antonio Luque, MD,‡ Adelaida Morte, MD,‡ Rafael Dal-Ré, MD, PhD,‡ and Manel Barbanj, MD, PhD,‡

Abstract: This investigation aimed to provide evidence on the lack of pharmacokinetic interaction of paroxetine (20 mg/d) and alprazolam (1 mg/d) in combined therapy. In addition, the central effects of both drugs when administered alone and in combination were assessed to rule out any relevant synergistic depressant central effect. Twenty-five healthy young adult volunteers participated in a double-blind, double-dummy, placebo-controlled, repeated dose (15 days), 4-period crossover study. Each subject received each of 4 treatment sequences (ie, paroxetine-alprazolam placebo, alprazolam-paroxetine placebo, paroxetine-alprazolam, and paroxetine placebo-alprazolam placebo) in randomized order. The ratios for area under the curve within a dosing interval and maximum plasma concentration of the paroxetine plus alprazolam sequence to single agent paroxetine were 1.07 (90% confidence interval = 0.99 to 1.16) and 1.05 (90% confidence interval = 0.97 to 1.13), respectively, with no statistically significant differences between the 2 treatments. Similarly, for alprazolam, ratios for the combined to the single treatment sequence were 0.99 (90% confidence interval = 0.93 to 1.05) and 1.00 (90% confidence interval = 0.94 to 1.07) for area under the curve within a dosing interval and maximum plasma concentration, respectively, showing no evidence for interaction. Comparative pharmacodynamics on the combination was assessed using 6 Psychomotor Performance Tests and 5 Visual Analogue Scales focused on mood variables. Alprazolam and paroxetine plus alprazolam induced similar and significant performance impairment and sedation after both single and repeated dose administration, being less evident on day 15. After dosing, paroxetine plus alprazolam showed a lower recovery pattern than alprazolam alone, especially

on day 15. No treatment sequence showed cumulative effects after repeated dose administration. Psychomotor Performance Tests and Visual Analogue Scales data suggested lack of pharmacodynamic interactions. Accordingly, study results showed no evidence for pharmacologic interactions between paroxetine and alprazolam at steady state. The most commonly reported adverse event was drowsiness, with a higher incidence under both single and combined alprazolam treatments.

(*J Clin Psychopharmacol* 2004;24:268-276)

The use of benzodiazepines for the control of anxiety symptoms is clearly stated nowadays, offering a prompt onset of action and relief to the patient after a brief period of treatment.¹ However, their prolonged use leads to both physical and psychologic dependencies among other problems.² Moreover, rebound or withdrawal syndromes may also occur.³ Antidepressants, in general, and selective serotonin reuptake inhibitors (SSRIs), in particular, have shown to be more effective than benzodiazepines,⁴ and their long-term use seems to be associated with a lower tolerance to the treatment effect.⁵ Paroxetine, an antidepressant belonging to the SSRI group, is the only drug of its class indicated in adults for obsessive-compulsive disorder, panic disorder, social anxiety disorder (social phobia), generalized anxiety disorder, and posttraumatic stress disorder in addition to major depressive disorder.⁶ Although there is evidence that paroxetine achieves therapeutic efficacy after the second week of treatment, the onset of effects of benzodiazepines is shorter (within the first week). Hence, their use as adjunctive treatment during the initial phase of paroxetine therapy has been suggested,⁷ with alprazolam the benzodiazepine of widest use. Among SSRIs, paroxetine seems to be concurrently used with benzodiazepines (alprazolam, clonazepam, diazepam, midazolam, and triazolam) in 10% of cases and specifically with alprazolam in 8% of overall paroxetine prescriptions.⁸

It is well known that enzymes of the human liver cytochrome P450 (CYP) are involved in the metabolism of several medications from a variety of therapeutic classes, including most antidepressant agents.^{9,10} Some SSRIs may

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Presented in abstract form at the 14th European College of Neuropsychopharmacology Congress, Istanbul, Turkey, October 13-17, 2001 [*Eur Neuropsychopharmacol*, 2001;11(suppl 3):S345].

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also inhibit different CYP subfamilies, thus potentially decreasing the hepatic clearance of comedications also metabolized by CYP. CYP3A4 accounts for 30% of the total body CYP enzymes and is responsible for the metabolism of ~50% of drugs that undergo oxidative biotransformation,¹¹ including some antidepressants and benzodiazepines.¹⁰ As CYP3A4 is densely located in the small intestine and in the liver, it plays an important role in first-pass metabolism and, consequently, in the oral bioavailability of these drugs. Thus, any change in CYP3A4 activity may have clinical implications, especially for those drugs with a narrow therapeutic window.

Although data from *in vitro* studies indicate that different CYP isoforms actively participate in the metabolism of paroxetine and alprazolam (ie, CYP2D6 and CYP3A, respectively) and, consequently, no pure metabolic drug-drug interaction should be anticipated, there are no currently available *in vivo* data supporting such *in vitro* findings. Moreover, considering the central pharmacodynamic action of paroxetine and alprazolam and to close out any relevant synergistic depressant central effect, any new study focusing on drug interactions should also evaluate the effect of both drugs in psychomotor performance and mood status. Therefore, this study was conducted to determine the potential for *in vivo* pharmacologic interactions between paroxetine and alprazolam after single and repeated administration following a dosing schedule that mimics the concomitant use of both drugs in clinical practice.

METHODS

Study Population

Healthy young adult volunteers of either sex were to be selected for participation in the study. Within a 21-day period before study initiation, volunteers underwent a clinical interview, physical and electrocardiogram examinations, and clinical chemistry, hematology, and urinalysis tests to rule out any relevant physical and/or psychologic abnormality. The prestudy examinations also included a screening on drugs of abuse and viral serologic testing. Female volunteers were asked to follow an effective contraceptive method and were to have a negative plasma pregnancy test at study entry. Subjects with a history of alcohol and/or caffeine-containing beverages abuse and heavy smokers were excluded from the study. The Research Ethics Committee of the study center authorized the study, and all subjects had to provide written informed consent before entering into the trial. The study was conducted in accordance with the Declaration of Helsinki.

Study Design

This was a phase I randomized, double-blind, double-dummy, placebo-controlled, repeated dose, 4-period crossover study. Data on paroxetine indicate that the within-subject coefficients of variation for area under the curve within a

dosing interval [$AUC_{(0-24)}$ or AUC_{∞}] and maximum plasma concentration (C_{max}) of paroxetine at steady state (the drug with the higher pharmacokinetic variability) would be no greater than 30%. On this basis, it was estimated that a sample size of 18 eligible subjects would provide at least 90% power to detect a true difference of $\geq 30\%$ between sequences (true ratio = 0.70 to 1.43). These calculations were based on a 2-tailed procedure on log-transformed data with a type I error rate of 5%. Each volunteer received each of the following treatment sequences: P [active paroxetine (20 mg) plus placebo alprazolam], A [placebo paroxetine plus active alprazolam (1 mg)], P + A [active paroxetine (20 mg) plus active alprazolam (1 mg)], and PLA [placebo paroxetine plus placebo alprazolam]. Study subjects were randomly assigned to 1 of 4 possible treatment sequences according to a Williams design.¹² In each treatment sequence, study medication was administered early morning, once daily, in the fasting state for 15 consecutive days together with 120 mL water. Thereafter, subjects remained fasting until 2 hours after dosing. There was a 1-week treatment-free window between treatment sequences. On days 13 to 15 of each treatment sequence, predose blood samples were taken to ensure that paroxetine and alprazolam plasma concentrations had reached steady state. On day 15, serial blood samples were taken at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours postdose for subsequent assay of paroxetine and alprazolam plasma concentrations. A single daily sample was also taken on the morning of days 16 to 19 inclusive. On each sampling, 8 mL of blood samples were collected into 2 precoated EDTA tubes and were immediately centrifuged (3000 rpm, 10 minutes, 4°C). The resulting plasma was transferred to individual plain polypropylene tubes. All tubes were frozen at -20°C .

On days 1 and 15 of each treatment sequence, the influence of the different treatment sequences on pharmacodynamic effects and mood status was assessed at predose and at 1, 2, 4, and 8 hours postdosing using a set of Psychomotor Performance Tests (PPT) and Visual Analogue Scales (VAS); the latter focused on mood variables.

Daily recording of adverse events and vital signs and measurement of safety laboratory parameters at the prestudy and last study visit assessments evaluated clinical safety.

Pharmacokinetic Analysis

According to standard guidelines,¹³ plasma samples were analyzed for alprazolam using a validated high-performance liquid chromatography method with tandem mass spectrometric detection. An aliquot of 100 μL plasma was mixed with 250 μL of 25 mM ammonium acetate buffer (pH 5), containing 5 ng/mL triazolam (Sigma, St. Louis, MO), the internal standard, and the mixture was extracted with 2 mL *tert*-butyl methyl ether for 10 minutes. After centrifugation at $\sim 3200 \times g$ for 10 minutes, the organic phase was separated

from the aqueous phase and evaporated under a stream of nitrogen at 50°C. The residue was reconstituted into 100 μ L of mobile phase, a mixture of 10 mM ammonium acetate buffer (pH 2.5) and acetonitrile (55:45 v/v). An aliquot of 25 μ L was injected into a 50 $\times$ 4 mm id YMC (Kyoto, Japan) AQ C₁₈ column (3 μ m particle size) kept at 40°C and at a mobile phase flow rate of 1.0 mL/min. Detection was performed using a PE Sciex (Concord, Canada) API III plus tandem mass spectrometer in positive ion mode and using a turbo ion-spray interface. For alprazolam (Sigma), the mass transition of the m/z 309 ion to the m/z 281 ion was monitored as well as the mass transition of the m/z 343 to the m/z 308 ion for triazolam. The calibration range was from 0.10 to 50.00 ng/mL. After dilution with analyte-free plasma, quantitation was valid up to 200.00 ng/mL. Similarly, plasma concentrations of paroxetine were determined using a validated high-performance liquid chromatography method with tandem mass spectrometric detection method. Briefly, 300 μ L of plasma were mixed with 150 μ L of 0.1% acetic acid containing the internal standard, 20 ng/mL ²H₆-paroxetine (GlaxoSmithKline, Harlow, UK). Further sample cleanup was performed by online solid phase extraction using the Prospekt system (Spark Holland, Emmen, The Netherlands). Separation was performed using a 50 $\times$ 4 mm id YMC AQ C₁₈ column (3 μ m particle size) kept at 40°C. The mobile phase was delivered at a flow rate of 1.0 mL/min and consisted 10 mM ammonium acetate (pH 2.5) and methanol (45:45 v/v) followed by a step gradient to a mixture of water and methanol (10:90 v/v). Detection was performed using a PE Sciex API 365 tandem mass spectrometer, in positive ion mode and using a turbo ion-spray interface, by monitoring the m/z 330 to the m/z 192 mass transition for paroxetine versus the m/z 336 to the m/z 198 mass transition for the internal standard. The calibration range was from 0.10 to 50.00 ng/mL; after dilution with analyte-free plasma, quantitation was valid up to 200.00 ng/mL. For both assays, with each analytical run of ~60 samples, quality control samples were analyzed in triplicate at 3 concentration levels to assess the day-to-day performance of the assay. Steady-state pharmacokinetic parameters of both paroxetine and alprazolam were determined using standard noncompartmental techniques with the pharmacokinetic analysis program PCModfit.¹⁴ Nominal sampling times were used in all calculations because no relevant deviations from the scheduled times occurred. The peak plasma concentration (C_{max}) and the time to peak (T_{max}) were defined by the highest measured concentration, and areas under the plasma concentration versus time curve were calculated up to 24 hours postdose (ie, within 1 dosing interval) using the combined linear-logarithmic trapezoidal rule. The apparent terminal elimination rate constant (λ_z) was determined using linear regression analysis (after log transformation) on those concentration-time pairs visually assessed to be in the terminal phase. The degree of fluctuation of the plasma concentration within a dosing interval was calculated as $(C_{max} - C_{min}) / C_{av}$, where C_{av} is the average plasma concentration over the 24-hour dosing interval. $AUC_{(0-24)}$ and C_{max} were log transformed (base e) and analyzed using analysis of variance (ANOVA) fitting terms for sequence, subject within sequence, period, and treatment (ie, P and A vs. P + A). The residual variance from the model was used for calculating 90% confidence intervals (CIs) for the differences between single and combined regimens on the log_e scale and then back transformed to obtain point estimates and 90% CIs for the ratio of the combined regimen relative to the single regimens. T_{max} was analyzed nonparametrically using the method outlined by Hauschke et al.¹⁵

Pharmacodynamic Assessments

Measurement of pharmacodynamic activity comprised 6 PPT and 5 VAS applied consecutively in the same order.

Several of these evaluations were conducted using a microcomputer (Multipsy 821, BIO-DATA, Version 2-1988, Steinback, Frankfurt, Germany): (a) critical flicker-fusion frequency test (CFF), an indicator of cortical arousal, measures the speed at which information is processed at the central nervous system; primary parameter was mean threshold of flicker-fusion frequency after 4 trials; (b) finger-tapping rate measures quick movements of the wrist and fingers; primary parameter was mean number of strike(s) during 30 seconds; (c) simple reaction time test (SRT) measures reaction time to a sensory cue (visual) by pressing a counting knob; primary parameter was mean reaction time to 40 stimuli randomly triggered in the range 500 to 3000 milliseconds; (d) Pauli test measures sustained concentration in cognitive tasks; primary parameter was the number of arithmetic problems correctly solved during 3 minutes. The remaining evaluations involved paper-and-pen-based tasks: (e) fine motoric test (FMT) measures ability for quick and accurate movements whereby a good eye-hand coordination is essential; primary parameter was the number of squares correctly dotted in 15 seconds by each hand; and (f) "d2" cancellation test measures attention and recognition of sensory information; primary parameter was the number of correct responses during 280 seconds. All subjects underwent training sessions before initiating the study to avoid a learning effect during the experimental phase.

Symptoms of depression, anger, anxiety, activity, and drowsiness were self-recorded using a 100 mm longitudinal VAS in the range "euphoric-depressed," "happy-annoyed," "relaxed-anxious," "passive-active," and "awake-sleepy," respectively.

The statistical analysis was conducted using PC SAS Version 6.0 on a DEC VAX 3100 computer. Univariate analysis was based in the comparisons of baseline performance (predose on day 1), single dose effects (day 1), and

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TABLE 1. Plasma Concentrations (ng/mL) of Paroxetine and Alprazolam at Predose on Days 13 to 15 (n = 22)

Treatment Regimen	Time	Mean ± SD	Coefficient of Variation (%)
P	Day 13	28.3 ± 16.3	74
	Day 14	29.6 ± 16.1	71
	Day 15	26.3 ± 14.6	73
P in P + A	Day 13	31.0 ± 25.6	71
	Day 14	31.8 ± 28.8	70
	Day 15	28.8 ± 26.3	68
A	Day 13	4.9 ± 2.0	41
	Day 14	5.1 ± 2.2	42
	Day 15	5.1 ± 2.4	46
A in P + A	Day 13	5.3 ± 1.8	34
	Day 14	5.2 ± 1.9	37
	Day 15	5.1 ± 2.0	42

P indicates active paroxetine (20 mg) + placebo alprazolam; P + A, active paroxetine (20 mg) + active alprazolam (1 mg); A, placebo paroxetine + active alprazolam (1 mg).

repeated dose effects (day 15). Baseline performance was compared using ANOVA, modeled to include treatment sequence and subject as main effects. Changes from predose on day 15 to baseline on day 1 were analyzed to assess residual morning effects after repeated dosing. On day 1, single dose effects were evaluated for both original values (effects of time and of interaction sequence by time) and changes to baseline (effects of sequence) using ANOVA that included treatment sequence, time point, and subject as main effects. The repeated dose effects were analyzed in the same way. When suitable, pairwise comparisons were performed using the Tukey-Kramer procedure to adjust for multiple testing. To provide an overall perspective of results, a multivariate, nonparametric analysis was also performed. Effect of treatment sequences (ie, changes from predose values to 1, 2, 4, and 8 hours postdosing in PPT and VAS) was assessed using Friedman rank ANOVA and Wilcoxon tests. Transformations were performed in a way that low Friedman rank results indicate high psychomotor impairment (ie, a decrease in CFF, finger-tapping rate, FMT, "d2" cancellation test, Pauli test, and an increase in SRT scorings) and high impact on mood status (ie, a decrease in the anxiety activity and an increase in the depression-anger-drowsiness self-assessments). Statistical significance was defined for *P* < 0.05.

RESULTS

Study Population

Twenty-five healthy subjects (9 male and 16 female; aged 26 ± 4 years; height 168 ± 7 cm; weight 64 ± 9 kg) were

enrolled. Two subjects dropped out of the study owing to personal reasons, and another one was lost to follow-up after ending the first treatment period.

Pharmacokinetics

Paroxetine

Predose plasma concentrations of paroxetine observed on days 13 to 15 of each treatment sequence suggested that steady-state conditions were reached in all subjects and all treatment sequences (Table 1). Some fluctuation was seen for some subjects, but no case of increasing plateau concentrations was observed. On day 15, the 2 paroxetine sequences (ie, P and P + A) resulted in similar mean plasma concentration profiles (Fig. 1). Additionally, the derived steady-state pharmacokinetic parameters for paroxetine were similar between both treatment sequences (Table 2). Although, numerically, the AUC and C_{max} values were slightly increased after the combined regimen administration, both 90% CIs were fully contained within the prespecified interval of nonrelevant pharmacokinetic interaction (ie, 0.70 to 1.43). Moreover, both pharmacokinetic parameters lied within the 0.80 to 1.25 interval, signifying pharmacokinetic bioequivalence of paroxetine when administered either alone or in combination. Paroxetine T_{max} was slightly delayed after the combined treatment. Arithmetic means of 25.4 hours (12.2 to 51.7) and 27.4 hours (9.31 to 63.7) were obtained for the paroxetine t_{1/2} after dosing with P and P + A, respectively. A wide variation of paroxetine t_{1/2} was apparent, but the ranges and means were very similar under both treatment conditions. The mean degree of fluctuation was 0.78 (0.33 to 1.55) after P administration and 0.72 (0.29 to 1.48) after P + A.

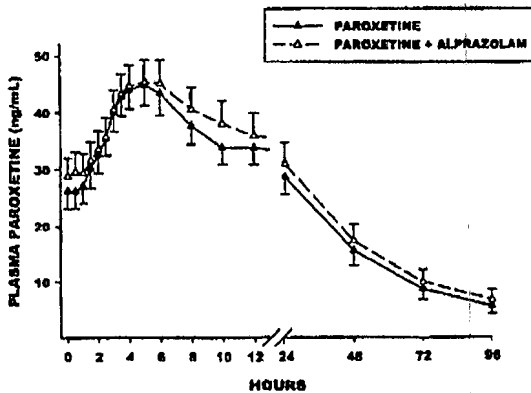


FIGURE 1. Mean paroxetine plasma concentrations (ng/mL) on day 15 (steady state) after administration of 20 mg paroxetine single agent (P) and in combination with 1 mg alprazolam (P + A; n = 22).

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TABLE 2. Pharmacokinetic Parameters of Paroxetine and Alprazolam on Day 15 (steady state): Descriptive Statistics and Summary of ANOVA

Parameter	Treatment Regimen*	Mean ± SD	CV (%)	Comparison	Ratio [†]	90% CI	Difference [‡]	95% CI
AUC ₍₀₋₂₄₎ (ng h/mL)	P	813 ± 362	51	(P + A): P	1.07	(0.99 to 1.16)	—	—
	P in P + A	875 ± 424	55	(P + A): A	0.99	(0.93 to 1.05)	—	—
	A	238 ± 66	25	—	—	—	—	—
	A in P + A	233 ± 52	22	—	—	—	—	—
C _{max} (ng/mL)	P	47.6 ± 17.7	38	(P + A): P	1.05	(0.97 to 1.13)	—	—
	P in P + A	49.7 ± 19.2	42	(P + A): A	1.00	(0.94 to 1.07)	—	—
	A	20.9 ± 3.6	18	—	—	—	—	—
	A in P + A	20.9 ± 3.4	16	—	—	—	—	—
T _{max} (hours)	—	—	—	(P + A) - P	—	—	0.25	(-0.25 to 1.00)
	—	—	—	(P + A) - A	—	—	0.25	(-0.25 to 0.75)

AUC₍₀₋₂₄₎ indicates area under the plasma concentration-time curve from 0 to 24 hours after the last administered dose; C_{max}, peak plasma concentration; T_{max}, time to peak drug concentration in plasma; CV, coefficient of variation.

*P indicates active paroxetine (20 mg) + placebo alprazolam; P + A, active paroxetine (20 mg) + active alprazolam (1 mg); A, placebo paroxetine + active alprazolam (1 mg).

[†]Data represent the ratio of the adjusted geometric means from ANOVA.

[‡]Data represent the median difference.

Alprazolam

Plasma concentrations of alprazolam on days 13 to 15 indicated that steady-state conditions had also been attained (Table 1). On day 15, the 2 alprazolam sequences (ie, A and P + A) resulted in almost superpose plasma concentration profiles (Fig. 2). In addition, similar results for the derived steady-state pharmacokinetic parameters were also obtained (Table 2). Again, numerically, differences in AUC and C_{max} values after A and P + A were not relevant, as both 90% CIs were fully contained within the 0.80 to 1.25 interval, showing bioequivalence. Alprazolam T_{max} was slightly delayed after the combined administration. Similar values were also observed for alprazolam t_{1/2} steady state: arithmetic means of

12.6 hours (7.3 to 20.1) and 11.8 hours (8.1 to 18.9) were found for A and P + A, respectively. The mean degree of fluctuation was 1.70 (0.90 to 2.71) after A and 1.76 (0.96 to 2.41) after P + A.

Psychomotor Performance and Mood Changes-Induced Pharmacodynamics

Baseline scores, either for the PPT or for the VAS did not differ across treatment sequences. Changes from predose values on day 15 to baseline values on day 1 did not achieve significant differences between treatment sequences, suggesting no carryover effects were induced in the repeated dosing of study medication.

Effects on Single Dose Administration

The 2-way ANOVA evidenced significant effects for the factors treatment sequence and time and the interaction of both factors (Table 3). In all PPT, maximum impairment was attained at 1 hour, improving thereafter, except for CFF that reached a peak at 2 hours. Comparing to PLA, both A and P + A gave similar results: CFF and "d2" cancellation test showed the longest impairment followed by SRT, FMT, and Pauli test. A or P + A did not show significant differences in any test at any time point. Comparisons of P versus A or P + A showed alike significant effects for CFF, finger-tapping rate, and SRT. The 2-way ANOVA for the VAS achieved peak changes between 1 and 4 hours (Table 3). Comparing to PLA, A or P + A described same significant effects. Changes in "drowsiness" were significant from 1 to 4 hours, while changes in "anxiety" were significant also at 8 hours. Once again, statistical significance was not reached

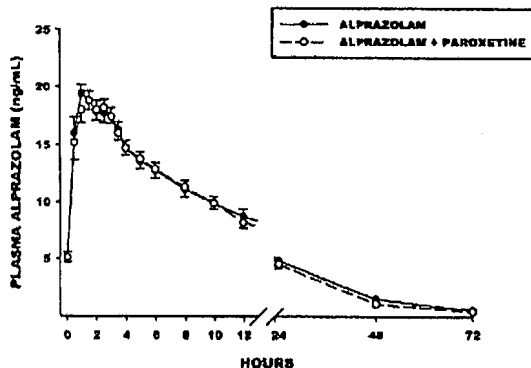


FIGURE 2. Mean alprazolam plasma concentrations (ng/mL) on day 15 (steady state) after administration of 1 mg alprazolam single agent (A) and in combination with 20 mg paroxetine (P + A; n = 22).

Summary

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TABLE 3. Acute Dosing Effects (Day 1) on PPT and VAS

Test	ANOVA (a)				Tukey-Kramer Procedure (b)					
	Drug	Time	Drug × Time	Pla vs. P	Pla vs. A (Hours)	Pla vs. P + A (Hours)	P vs. A (Hours)	P vs. P + A (Hours)	A vs. P + A (Hours)	
CFF	15.01*	21.65*	3.90*	—	1, 2, 8	1, 2, 4	1, 2, 4	1, 2, 4	—	
TR	6.18*	15.13*	4.30*	—	1	1	1, 2	1, 2	—	
SRT	10.23*	18.82*	3.56*	—	1, 2, 4	1, 2, 4	1, 2	1, 2	—	
FMT	13.20*	17.77*	5.38*	—	1, 2	1, 2, 4	1, 2	1, 2, 4	—	
D2T	23.81*	23.13*	5.79*	—	1, 2, 4, 8	1, 2, 4, 8	1, 2, 4	1, 2, 4, 8	—	
PT	9.06*	10.45*	2.78*	—	1, 4	1, 2, 4	1, 4	1, 2, 4, 8	—	
Depression	0.49	2.01	1.21	—	—	—	—	—	—	
Anger	2.16	3.33 [†]	1.40	—	—	—	—	2	—	
Anxiety	5.42*	4.99*	2.03 [†]	—	1, 2, 4, 8	1, 2, 4, 8	—	—	—	
Activity	2.49	4.22 [†]	2.92*	—	—	—	1, 2	2	—	
Drowsiness	14.5*	13.33*	4.62*	—	1, 2, 4	1, 2, 4	1, 2, 4	1, 2, 4, 8	—	

(a) Δ values (ie, changes from predose scoring) for the drug effects and row values for time and for the interaction between drug and time effects; (b) pairwise comparisons between treatments (data on table show time points where statistical significance was reached).

Pla indicates placebo; P, paroxetine (20 mg); A, alprazolam (1 mg); P + A, paroxetine (20 mg) + alprazolam (1 mg); TR, finger-tapping rate; D2T, "d2" cancellation test; PT, Pauli test.

*P < 0.05.

[†]P < 0.10.

out between A and P + A in any VAS at any time point. Finally, when comparing P versus A or P + A, significantly more "activity" and less "drowsiness" was observed.

Effects on Repeated Dose Administration

In any PPT, the 2-way ANOVAs showed significant effects for the factors treatment sequence and time

or the interaction between one and the other (Table 4). Induced psychomotor impairment reached a peak at 1 hour, diminishing thereafter except for CFF (maximum impairment at 2 hours). On day 15 and for any PPT, the magnitude of the psychomotor detriment was lower than the observed on day 1. In comparison with PLA, A and P + A get to exchangeable results: "d2" cancellation and Pauli tests were significantly

TABLE 4. Superimposed Dosing Effects (Day 15; After the Last Administered Dose) on PPT and VAS

Test	ANOVA (a)				Tukey-Kramer Procedure (b)					
	Drug	Time	Drug × Time	Pla vs. P	Pla vs. A (Hours)	Pla vs. P + A (Hours)	P vs. A (Hours)	P vs. P + A (Hours)	A vs. P + A (Hours)	
CFF	11.41*	20.36*	4.07*	—	1, 2	1, 2	1, 2, 4	1, 2, 4, 8	—	
TR	2.12	6.53*	2.85*	—	1	1	1	1, 2	—	
SRT	9.38*	13.39*	3.34*	—	—	1, 2, 4	2	1, 2, 4	1	
FMT	1.81	7.88*	2.43 [†]	—	1, 2	1, 2	1, 2	1, 2	—	
D2T	10.48*	15.27*	3.51*	—	1, 2	1, 2, 4	1, 2	1, 2, 4	—	
PT	6.52*	11.52*	2.55 [†]	—	1, 2, 4	1, 2, 4	2	1, 2, 4	—	
Depression	0.89	2.50 [†]	1.09	—	—	—	—	—	—	
Angers	1.24	1.76 [†]	1.52	—	—	—	—	—	—	
Anxiety	2.63	2.77 [†]	0.93	—	—	—	—	—	—	
Activity	1.65	3.33 [†]	1.77	—	—	—	—	—	—	
Drowsiness	3.53 [†]	9.48*	2.74*	—	1, 2	1, 2	2	2, 4	—	

(a) Δ values (ie, changes from predose scoring) for the drug effects and row values for time and for the interaction between drug and time effects; (b) pairwise comparisons between treatments (data on table show time points where statistical significance was reached).

Pla indicates placebo; P, paroxetine (20 mg); A, alprazolam (1 mg); P + A, paroxetine (20 mg) + alprazolam (1 mg); TR, finger-tapping rate; D2T, "d2" cancellation test; PT, Pauli test.

*P < 0.05.

[†]P < 0.10.

impaired up to 4 hours, CFF and FMT between 1 and 2 hours, and finger-tapping rate only at 1 hour, with SRT being the only exception to this rule (PLA vs. A: nonsignificant; PLA vs. P + A: significant from 1 to 4 hours; A vs. P + A: significant only at 1 hour). Also comparing with PLA, P got through nonsignificant results in any test at any time point. Finally, although P described significant effects for the FMT in comparison with A or P + A (with no differences between the latter sequences), for the remaining test, the impairment induced by A was significantly shorter than that induced by P + A. Regarding the VAS assessment, only "drowsiness" achieved significant results (Table 4), with a peak to maximum changes between 1 and 4 hours. Lastly, in comparison with PLA, A or P + A showed equivalent significant effects between 1 and 2 hours, although no differences were detected between these active treatment sequences.

Multivariate Nonparametric Approach

Based on Friedman rank sums, the analysis showed significant differences in psychomotor performance impairment between sequences on day 1 ($\chi^2 = 21.93$; $P = 0.0001$) and day 15 ($\chi^2 = 15.53$; $P = 0.0014$; Fig. 3). Comparing with PLA or P, A or P + A reached statistical significance on both days. Also on day 15, results on the combination P + A significantly differed from those observed with A. In line with this results, rank sums achieved significant differences between treatment sequences also in VAS scorings on day 1 ($\chi^2 = 9.72$; $P = 0.021$) and day 15 ($\chi^2 = 9.00$; $P = 0.029$; Fig. 3), again being more evident for A or P + A but also arriving at differences between these 2 regimens on both days.

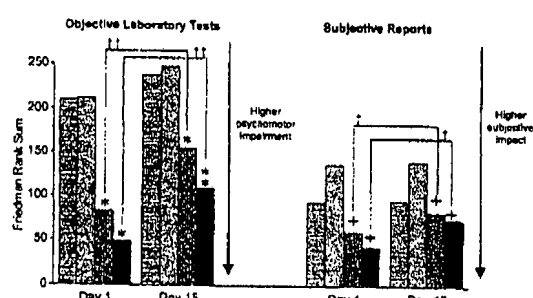


FIGURE 3. Friedman rank sums applied to changes from premedication (at +1, +2, +4, and +8 hours) to 6 objective psychomotor and 5 subjective self-reported combined variables. Lower values means higher psychomotor impairment and higher subjective impact. Placebo (□), paroxetine (▨), alprazolam (▩), and combination of paroxetine + alprazolam (■). Wilcoxon test: ** $P < 0.01$, differences from placebo, paroxetine, and alprazolam; * $P < 0.05$, differences from placebo and paroxetine; † $P < 0.01$, differences between days 1 and 15; ‡ $P < 0.05$ differences between days 1 and 15.

Clinical Safety

All subjects reported at least 1 adverse event, mainly drowsiness—although anecdotic cases of ataxia, insomnia, and diplopia were also observed—with a higher incidence following treatment with A and P + A. Some adverse events were reported as severe—drowsiness, dizziness, diplopia, and increased appetite; however, all had resolved by the end of the study without withdrawals owing to them.

DISCUSSION

Comparisons of the $AUC_{(0-24)}$ and C_{max} indicated the steady-state pharmacokinetics of paroxetine and alprazolam remain unchanged when administered alone or in combination. In fact, the CIs for the mean ratios of these parameters were fully contained within the interval 0.80 to 1.25 (predefined nonrelevant interaction range = 0.7 to 1.43), thus allowing to conclude there is bioequivalence of the combination treatment with the single agent administration. Although a very slight increase in T_{max} was observed for the combined treatment, these small changes were considered clinically unmeaning. The elimination half-lives and degree of fluctuation at steady state were reported descriptively, and again, there was no evidence for either compound of any difference between the single and the combined sequence.

Members of the CYP3A subfamily are the principal enzymes involved in the metabolism of alprazolam¹⁶ and, more specifically, the 3A4 isoform.^{17,18} Many SSRIs have been reported to inhibit CYP3A4-mediated alprazolam hydroxylation in vitro with varying degrees of potency,¹⁹⁻²¹ although for paroxetine, inhibition was reportedly very weak ($K_i \sim 40 \mu M$). As was suggested previously,^{19,22-24} the results of this study confirm that paroxetine does not affect either the CYP3A4-mediated alprazolam hydroxylation pathway or its overall pharmacokinetic profile. In the broad sense, there is evidence suggesting that nefazodone,²⁵ fluoxetine,^{26,27} and fluvoxamine²⁸ are significant in vivo inhibitors of alprazolam metabolism, thus potentially increasing the risk of inducing alprazolam central nervous system side effects. Conversely, venlafaxine,²⁹ sertraline,^{30,32} and, according to the present study, paroxetine have demonstrated no in vivo inhibition of CYP3A4-mediated alprazolam metabolism.

In relation to psychomotor-induced effects, 1 mg alprazolam induced a significant psychomotor impairment in most tests, with a peak between 1 and 4 hours postdosing. The absence of significant differences between treatment sequences at predose on day 15 when comparing with baseline values on day 1 suggests lack of cumulative effects after repeated dosing, although superimposed effects with alprazolam last administered dose showed a significant psychomotor performance impairment. This late profile was similar to that observed after single dosing, although of lesser magnitude, suggesting a low degree of tolerance was developed. In

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addition, reporting on impact in mood status assessments showed equal effects. As expected, coadministration of paroxetine and alprazolam mimics the effects observed with alprazolam alone. This coincides with reports on in vivo central nervous system effects of paroxetine in combination with benzodiazepines,^{33,34} which contrasts with published results of other SSRIs with higher in vitro inhibitory CYP3A activity.²⁵⁻²⁸

With regard to safety and tolerability outcomes, the observed side effect profiles were similar to those described previously for paroxetine and alprazolam,^{6,35} and the incidence of adverse events was essentially similar during the combined sequence to the incidence reported during the single active treatments.

In summary, there was no evidence found for any pharmacologic interaction between oral daily doses of 20 mg paroxetine and 1 mg alprazolam under pharmacokinetic steady-state conditions. These findings may be useful to clinicians who face the need of prescribing an anxiolytic together with an antidepressant. The conduction of further controlled trials would elucidate the real risk/benefit ratio of using this combined therapy in patient populations.

ACKNOWLEDGMENTS

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ANEXO 3

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ESTUDIO DE INTERACCION ENTRE PAROXETINA HEMIDRATO Y ALPRAZOLAM EN VOLUNTARIOS SANOS.

RESULTADOS.

Voluntarios.

-Género.

Femenino: 9 sujetos.

Masculino: 16 sujetos.

*1 sujeto desistió del estudio por motivo no especificado.

-Edad. Entre 18 y 30 años.

-Peso. Aproximado desde 47 kg. hasta 84 kg.

-Talla. Aproximada desde 146 cm. hasta 181 cm.

La administración de Paroxetina, Alprazolam y la combinación de Paroxetina + Alprazolam en dosis fija fue de 20 mg., 0.25 mg., y 20/0.25 mg., respectivamente; en forma de tableta una vez al día por la mañana.

Las tablas 1 a 5 muestran los resultados obtenidos de las visitas de valoración inicial y final en las cuales fueron evaluados lo sujetos de estudio empleando la Escala EVEA, la cual valora 4 estados de animo: ansiedad, hostilidad, depresión y alegría.

Asimismo, la tabla 6 muestra la relación de efectos adversos manifestados por los sujetos de estudio. Los eventos adversos reportados fueron No Serios, ninguno de los sujetos discontinuó el tratamiento.

TABLA 1.

Puntaje Inicial y Final (Promedio ± Desviación Estándar) en la Escala EVEA para valorar: Ansiedad, Hostilidad, Depresión y Alegría.

Se anota la probabilidad con la Prueba de Wilcoxon (p1) y la probabilidad con la prueba t de Student para muestras dependientes (p2) que evalúa si los cambios ocurridos entre los puntajes iniciales y finales son estadísticamente significativos.

Grupo 1.

1.- Paroxetina hemidrato / Placebo.

Preguntas del Test EVEA	Valoración Inicial		Valoración Final		p1	p2
1) Me siento nervioso	8.67	± 0.52	5.83	± 1.17	0.031*	p<0.001†
2) Me siento irritado	9.00	± 0.00	5.00	± 1.26	0.031*	p<0.001†
3) Me siento alegre	0.00	± 0.00	3.33	± 0.82	0.031*	p<0.001†
4) Me siento melancólico	9.50	± 0.55	5.33	± 0.52	0.031*	p<0.001†
5) Me siento tenso	9.50	± 0.55	6.50	± 0.55	0.031*	p<0.001†
6) Me siento optimista	0.00	± 0.00	2.67	± 0.52	0.031*	p<0.001†
7) Me siento desanimado	9.00	± 0.00	6.67	± 0.52	0.031*	p<0.001†
8) Me siento enojado	10.00	± 0.00	7.67	± 0.52	0.031*	p<0.001†
9) Me siento ansioso	10.00	± 0.00	6.50	± 0.55	0.031*	p<0.001†
10) Me siento apagado	9.00	± 0.00	7.33	± 0.52	0.031*	p<0.001†
11) Me siento molesto	9.17	± 0.41	7.50	± 0.55	0.031*	p<0.001†
12) Me siento jovial	0.00	± 0.00	2.00	± 0.63	0.031*	p<0.001†
13) Me siento intranquilo	9.00	± 0.00	6.17	± 0.41	0.031*	p<0.001†
14) Me siento enfadado	9.00	± 0.00	3.00	± 0.00	0.031*	-----
15) Me siento contento	0.00	± 0.00	2.67	± 0.52	0.031*	p<0.001†
16) Me siento triste	9.00	± 0.00	5.67	± 0.52	0.031*	p<0.001†

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Dimensión: Ansiedad	37.17 ± 0.75	25.00 ± 1.26	0.031*	p<0.001†
Dimensión: Hostilidad	37.17 ± 0.41	23.17 ± 1.17	0.031*	p<0.001†
Dimensión: Depresión	36.50 ± 0.55	25.00 ± 0.89	0.031*	p<0.001†
Dimensión: Alegría	0.00 ± 0.00	10.67 ± 1.21	0.031*	p<0.001†

* p<0.05 ** p<0.01 † p<0.001

TABLA 2.

Puntaje Inicial y Final (Promedio ± Desviación Estándar) en la Escala EVEA para valorar: Ansiedad, Hostilidad, Depresión y Alegría.

Se anota la probabilidad con la Prueba de Wilcoxon (p1) y la probabilidad con la prueba t de Student para muestras dependientes (p2) que evalúa si los cambios ocurridos entre los puntajes iniciales y finales son estadísticamente significativos.

Grupo 2.

2.- Alprazolam / Placebo.

Preguntas del Test EVEA	Valoración Inicial	Valoración Final	p1	p2
1) Me siento nervioso	9.17 ± 0.75	3.50 ± 0.55	0.031*	p<0.001†
2) Me siento irritado	8.67 ± 0.52	5.50 ± 0.55	0.031*	p<0.001†
3) Me siento alegre	0.00 ± 0.00	0.67 ± 0.82	0.250	0.102
4) Me siento melancólico	8.83 ± 0.75	6.60 ± 1.52	0.125	0.063
5) Me siento tenso	9.83 ± 0.41	5.00 ± 0.89	0.031*	p<0.001†
6) Me siento optimista	0.00 ± 0.00	2.67 ± 0.52	0.031*	p<0.001†
7) Me siento desanimado	8.83 ± 0.41	8.83 ± 0.75	1.000	1.000
8) Me siento enojado	10.00 ± 0.00	9.50 ± 0.55	0.250	0.076
9) Me siento ansioso	9.67 ± 0.52	3.33 ± 0.52	0.031*	p<0.001†
10) Me siento apagado	9.00 ± 0.00	9.00 ± 0.00	1.000	----
11) Me siento molesto	9.33 ± 0.52	9.00 ± 0.00	0.500	0.175
12) Me siento jovial	0.00 ± 0.00	0.17 ± 0.41	1.000	0.363
13) Me siento intranquilo	9.33 ± 0.52	4.50 ± 1.64	0.031*	p<0.001†
14) Me siento enfadado	9.50 ± 0.55	9.33 ± 0.52	1.000	0.363
15) Me siento contento	0.00 ± 0.00	0.00 ± 0.00	1.000	----
16) Me siento triste	9.33 ± 0.52	9.17 ± 0.41	1.000	0.363
Dimensión: Ansiedad	38.00 ± 0.89	16.33 ± 1.37	0.031*	p<0.001†
Dimensión: Hostilidad	37.50 ± 1.05	33.33 ± 0.82	0.031*	p<0.001†
Dimensión: Depresión	36.00 ± 1.10	32.50 ± 2.95	0.063	0.044*
Dimensión: Alegría	0.00 ± 0.00	3.50 ± 1.05	0.031*	p<0.001†

* p<0.05 ** p<0.01 † p<0.001

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TABLA 3.
Puntaje Inicial y Final (Promedio $\pm$ Desviación Estándar) en la Escala EVEA para valorar: Ansiedad, Hostilidad, Depresión y Alegría.
Se anota la probabilidad con la Prueba de Wilcoxon (p1) y la probabilidad con la prueba t de Student para muestras dependientes (p2) que evalúa si los cambios ocurridos entre los puntajes iniciales y finales son estadísticamente significativos.

Grupo 3.
3.- Paroxetina hemidrato / Alprazolam.

Preguntas del Test EVEA	Valoración Inicial	Valoración Final	p1	p2
1) Me siento nervioso	9.33 $\pm$ 0.52	1.33 $\pm$ 0.52	0.031*	p<0.001†
2) Me siento irritado	9.50 $\pm$ 0.55	1.67 $\pm$ 0.82	0.031*	p<0.001†
3) Me siento alegre	0.00 $\pm$ 0.00	8.50 $\pm$ 0.55	0.031*	p<0.001†
4) Me siento melancólico	9.50 $\pm$ 0.55	2.33 $\pm$ 0.52	0.031*	p<0.001†
5) Me siento tenso	9.83 $\pm$ 0.41	1.83 $\pm$ 0.75	0.031*	p<0.001†
6) Me siento optimista	0.00 $\pm$ 0.00	8.50 $\pm$ 0.55	0.031*	p<0.001†
7) Me siento desanimado	9.00 $\pm$ 0.00	2.33 $\pm$ 1.03	0.031*	p<0.001†
8) Me siento enojado	10.00 $\pm$ 0.00	1.67 $\pm$ 0.52	0.031*	p<0.001†
9) Me siento ansioso	9.83 $\pm$ 0.41	0.50 $\pm$ 0.55	0.031*	p<0.001†
10) Me siento apagado	9.33 $\pm$ 0.52	1.00 $\pm$ 0.63	0.031*	p<0.001†
11) Me siento molesto	9.33 $\pm$ 0.52	0.83 $\pm$ 0.75	0.031*	p<0.001†
12) Me siento jovial	0.00 $\pm$ 0.00	8.33 $\pm$ 0.82	0.031*	p<0.001†
13) Me siento intranquillo	9.17 $\pm$ 0.41	1.00 $\pm$ 0.00	0.031*	p<0.001†
14) Me siento enfadado	9.17 $\pm$ 0.41	1.00 $\pm$ 0.00	0.031*	p<0.001†
15) Me siento contento	0.00 $\pm$ 0.00	8.83 $\pm$ 0.41	0.031*	p<0.001†
16) Me siento triste	9.50 $\pm$ 0.55	0.83 $\pm$ 0.75	0.031*	p<0.001†
Dimensión: Ansiedad	38.17 $\pm$ 0.75	4.67 $\pm$ 1.03	0.031*	p<0.001†
Dimensión: Hostilidad	38.00 $\pm$ 0.63	5.17 $\pm$ 1.17	0.031*	p<0.001†
Dimensión: Depresión	37.33 $\pm$ 1.21	6.50 $\pm$ 2.59	0.031*	p<0.001†
Dimensión: Alegría	0.00 $\pm$ 0.00	34.17 $\pm$ 1.60	0.031*	p<0.001†

* p<0.05 ** p<0.01 † p<0.001

TABLA 4.
Puntaje Inicial y Final (Promedio $\pm$ Desviación Estándar) en la Escala EVEA para valorar: Ansiedad, Hostilidad, Depresión y Alegría.
Se anota la probabilidad con la Prueba de Wilcoxon (p1) y la probabilidad con la prueba t de Student para muestras dependientes (p2) que evalúa si los cambios ocurridos entre los puntajes iniciales y finales son estadísticamente significativos.

Grupo 4.
4.- Placebo / Placebo.

Preguntas del Test EVEA	Valoración Inicial	Valoración Final	p1	p2
1) Me siento nervioso	9.33 $\pm$ 0.52	8.67 $\pm$ 0.52	0.250	0.102
2) Me siento irritado	9.50 $\pm$ 0.55	8.83 $\pm$ 0.75	0.125	0.025*

3) Me siento alegre	0.00 ± 0.00	0.33 ± 0.52	0.500	0.175
4) Me siento melancólico	9.50 ± 0.55	9.50 ± 0.55	1.000	-----
5) Me siento tenso	9.67 ± 0.52	5.33 ± 3.72	0.031*	0.038*
6) Me siento optimista	0.00 ± 0.00	0.17 ± 0.41	1.000	0.363
7) Me siento desanimado	9.33 ± 0.52	9.17 ± 0.75	1.000	0.363
8) Me siento enojado	9.83 ± 0.41	9.00 ± 0.00	0.063	0.004**
9) Me siento ansioso	9.83 ± 0.41	8.67 ± 0.52	0.063	0.013*
10) Me siento apagado	9.33 ± 0.52	9.17 ± 0.41	1.000	0.363
11) Me siento molesto	9.67 ± 0.52	9.00 ± 0.00	0.125	0.025*
12) Me siento jovial	0.00 ± 0.00	0.00 ± 0.00	1.000	-----
13) Me siento intranquilo	9.33 ± 0.52	1.00 ± 0.00	0.031*	p<0.001†
14) Me siento enfadado	9.33 ± 0.52	1.00 ± 0.00	0.031*	p<0.001†
15) Me siento contento	0.00 ± 0.00	0.00 ± 0.00	1.000	-----
16) Me siento triste	9.17 ± 0.41	9.17 ± 0.41	1.000	-----
Dimensión: Ansiedad	38.17 ± 0.75	23.67 ± 3.14	0.031*	p<0.001†
Dimensión: Hostilidad	38.33 ± 1.21	27.83 ± 0.75	0.031*	p<0.001†
Dimensión: Depresión	37.33 ± 1.21	37.00 ± 1.55	1.000	0.363
Dimensión: Alegría	0.00 ± 0.00	0.50 ± 0.84	0.500	0.203

* p<0.05 ** p<0.01 † p<0.001

TABLA 5.

Aumento o Disminución en el puntaje de las preguntas y de las dimensiones que evalúa la Escala EVEA en pacientes con cuatro tratamientos durante 15 días. Se anota el promedio del aumento (+) o de la disminución (-) para cada pregunta y para la puntuación de cada una de las dimensiones de la escala. Se anota también la probabilidad de la prueba de Kruskal Wallis que evalúa si los cambios observados entre los tratamientos presentaron diferencias estadísticamente significativas.

Cambio en Puntuación

Preguntas de la Escala EVEA	Paroxetina hemidrato / Placebo	Alprazolam / Placebo	Paroxetina hemidrato / Alprazolam	Placebo / Placebo	p
1) Me siento nervioso	-2.83 ± 0.75	-5.67 ± 0.82	-8.00 ± 0.63	-0.67 ± 0.82	p<0.001†
2) Me siento irritado	-4.00 ± 1.26	-3.17 ± 0.98	-7.83 ± 0.98	-0.67 ± 0.52	p<0.001†
3) Me siento alegre	3.33 ± 0.82	0.67 ± 0.82	8.50 ± 0.55	0.33 ± 0.52	p<0.001†
4) Me siento melancólico	-4.17 ± 0.41	-2.20 ± 1.92	-7.17 ± 0.75	0.00 ± 0.00	p<0.001†
5) Me siento tenso	-3.00 ± 0.89	-4.83 ± 1.17	-8.00 ± 0.63	-4.33 ± 3.78	0.006**
6) Me siento optimista	2.67 ± 0.52	2.67 ± 0.52	8.50 ± 0.55	0.17 ± 0.41	p<0.001†
7) Me siento desanimado	-2.33 ± 0.52	0.00 ± 0.63	-6.67 ± 1.03	-0.17 ± 0.41	p<0.001†
8) Me siento enojado	-2.33 ± 0.52	-0.50 ± 0.55	-8.33 ± 0.52	-0.83 ± 0.41	p<0.001†
9) Me siento ansioso	-3.50 ± 0.55	-6.33 ± 0.52	-9.33 ± 0.52	-1.17 ± 0.75	p<0.001†
10) Me siento apagado	-1.67 ± 0.52	0.00 ± 0.00	-8.33 ± 1.03	-0.17 ± 0.41	p<0.001†
11) Me siento molesto	-1.67 ± 0.52	-0.33 ± 0.52	-8.50 ± 0.55	-0.67 ± 0.52	p<0.001†
12) Me siento jovial	2.00 ± 0.63	0.17 ± 0.41	8.33 ± 0.82	0.00 ± 0.00	p<0.001†
13) Me siento intranquilo	-2.83 ± 0.41	-4.83 ± 1.60	-8.17 ± 0.41	-8.33 ± 0.52	p<0.001†
14) Me siento enfadado	-6.00 ± 0.00	-0.17 ± 0.41	-8.17 ± 0.41	-8.33 ± 0.52	p<0.001†
15) Me siento contento	2.67 ± 0.52	0.00 ± 0.00	8.83 ± 0.41	0.00 ± 0.00	p<0.001†
16) Me siento triste	-3.33 ± 0.52	-0.17 ± 0.41	-8.67 ± 1.21	0.00 ± 0.00	p<0.001†
Dimensión: Ansiedad	-12.17 ± 1.17	-21.67 ± 1.51	-33.50 ± 1.05	-14.50 ± 3.73	p<0.001†
Dimensión: Hostilidad	-14.00 ± 1.26	-4.17 ± 1.47	-32.83 ± 1.17	-10.50 ± 0.55	p<0.001†
Dimensión: Depresión	-11.50 ± 1.05	-3.50 ± 3.21	-30.83 ± 3.37	-0.33 ± 0.82	p<0.001†
Dimensión: Alegría	10.67 ± 1.21	3.50 ± 1.05	34.17 ± 1.60	0.50 ± 0.84	p<0.001†

** p<0.01 † p<0.001

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TABLA 6.

Número y porcentaje de Pacientes que presentaron el Evento Adverso indicado al menos un Evento Adverso, por grupo de tratamiento. En cada tratamiento se incluyeron 6 pacientes. Se anota la probabilidad (p) con la Prueba de Exacta de Fisher para la comparación de las frecuencias de presentación de los Eventos Adversos.

Eventos Adversos	1.- Paroxetina hemidrato / Placebo.		2.- Alprazolam / Placebo.		3.- Paroxetina hemidrato / Alprazolam.		4.- Placebo / Placebo.		p
Somnolencia	2	33.3%	3	50.0%	3	50.0%	0	0.0%	0.241
Agitación	5	83.3%	0	0.0%	0	0.0%	6	100.0%	p<0.001*
Sudoración	2	33.3%	3	50.0%	0	0.0%	4	66.7%	0.154
Pacientes con Presencia de Evento Adverso	6	100.0%	5	83.3%	3	50.0%	6	100.0%	0.066

* p<0.001

Promedio y Desviación Estándar del Número Total de Eventos Adversos por Paciente y por Tratamiento. Se anota la probabilidad al comparar los grupos con la Prueba de Kruskal Walls.

Tratamiento	1.- Paroxetina hemidrato / Placebo.	2.- Alprazolam / Placebo.	3.- Paroxetina hemidrato / Alprazolam.	4.- Placebo / Placebo.	p
Prom.± Desv.Est.	1.50 ± 0.84	1.00 ± 0.63	0.50 ± 0.55	1.67 ± 0.52	0.293

CONCLUSIONES.

Al comparar los resultados de todos los grupos se observó que el tratamiento correspondiente al Grupo 3 que comprende Paroxetina + Alprazolam redujo de manera significativa los estados de ansiedad, depresión y hostilidad en los sujetos de estudio, manifestando una mejor actitud y comportamiento que se evidenció al mostrarse durante la entrevista más joviales, optimistas, contentos, alegres; a diferencia de los demás grupos en los que los sujetos manifestaron sentirse, en su mayoría, nerviosos, irritados, ansiosos, melancólicos, tristes, enfadados.

En relación a los efectos adversos reportados éstos fueron NO Serios. Se observó que para el Grupo 3 (Paroxetina + Alprazolam), los efectos adversos manifestados fueron menores en comparación con los demás grupos de tratamiento en los cuales se les administró a los sujetos de estudio el tratamiento farmacológico de Paroxetina y Alprazolam por separado.

Con el presente estudio se demostró que con la combinación de Paroxetina + Alprazolam administrada en una sola unidad de dosificación con menores concentraciones de dichos principios activos comparadas con las prescritas en la práctica médica, produce un efecto terapéutico eficaz que el originado por la Paroxetina y el Alprazolam administrados separadamente y durante periodos prolongados de tiempo.

ANEXO 4

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Alprazolam Plasma Concentrations and Treatment Response in Panic Disorder and Agoraphobia

Ira M. Lesser, M.D., R. Bruce Lydiard, Ph.D., M.D., Edward Antal, Ph.D., Robert T. Rubin, M.D., Ph.D., James C. Ballenger, M.D., and Robert DuPont, M.D.

Objective: The authors' goal was to evaluate the relationship between plasma concentrations of alprazolam and both treatment response and side effects in patients with panic disorder and agoraphobia. **Method:** Ninety-six patients with panic disorder and agoraphobia were treated at three sites in a 6-week, fixed-dose, double-blind, placebo-controlled, dose-response study of 2 mg/day or 6 mg/day of alprazolam. Assessments were made of panic attacks, avoidance behavior, generalized anxiety, and global response. Blood samples were collected throughout the study and analyzed for alprazolam and other benzodiazepines. **Results:** Patient compliance with the protocol was judged to be good on the basis of plasma concentrations. According to logistic regression analysis, the relationships between plasma alprazolam concentration and response, as reflected by number of panic attacks reported, phobia ratings, physicians' and patients' ratings of global improvement, and the emergence of side effects, were significant. However, there was no significant relationship between plasma alprazolam concentration and the degree of generalized anxiety symptoms. **Conclusions:** The authors conclude that plasma concentration of alprazolam is related to treatment response, particularly in panic attacks. The alprazolam concentration associated with treatment response or with emergence of a given side effect varied widely among individuals, highlighting the necessity for individualized dose adjustment to obtain optimal treatment response while minimizing side effects.

(Am J Psychiatry 1992; 149:1556-1562)

Studies of the triazolobenzodiazepine alprazolam in the treatment of panic-related disorders have repeatedly demonstrated the efficacy of this compound, but they have indicated that a wide range of doses may be necessary for control of panic attacks (1-4). This wide range could be due to a number of factors that are not mutually exclusive, such as 1) pharmacokinetic variability, resulting in variable alprazolam plasma concentrations from a given dose, 2) differences in severity of the panic-related disorder, 3) intersubject vari-

ability in response to alprazolam, and 4) self-medication with other benzodiazepines.

Intersubject differences in alprazolam pharmacokinetics may be a major factor in the variability of effective dose, since administration of the same dose to homogeneous patient samples can result in a three- to four-fold variability in steady-state plasma alprazolam concentration (3, 5). Although the results of previous studies have demonstrated a relationship between alprazolam dose and plasma concentration (6, 7), the relationship between alprazolam plasma concentration and clinical outcome has not been clearly elucidated.

The present study was a three-site, placebo-controlled trial. It was undertaken to evaluate the relationships between alprazolam dose, plasma concentration, and treatment response in patients with panic disorder. A fixed-dose study design was used to evaluate a relatively low and a relatively high dose of the drug. This design may be more appropriate for evaluating relationships between concentration and treatment response (8) than the adjustable-dose design used in previous alprazolam efficacy studies. This report presents the analysis of the relationship between treatment response measures and plasma alprazolam levels in pa-

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tients with panic disorder who were receiving 2 mg/day of alprazolam, 6 mg/day of alprazolam, or placebo.

We also address the use of plasma benzodiazepine concentrations to assess protocol compliance. Efficacy and side effect results from the study have been reported elsewhere (9). A preliminary report on the logistic regression methodology also has been presented elsewhere (10), and the concentration data obtained from that study have been used to evaluate the feasibility of subjecting clinical efficacy trial data to population pharmacokinetic analyses (11).

METHOD

Patients and Study Design

The complete study design, including details of patient recruitment and screening and descriptions of the clinical evaluation scales and diagnostic criteria, has been described in a previous report (9). In brief, this was a double-blind, placebo-controlled, fixed-dose study evaluating placebo, 2 mg/day of alprazolam, and 6 mg/day of alprazolam. The subjects were 96 patients who met *DSM-III* criteria for panic disorder or agoraphobia with panic attacks after evaluation with the Structured Clinical Interview for *DSM-III*, Upjohn Version (12). Seventy percent of the subjects were women and 30% were men; their mean age was 37.1 years. From 77% to 90% in each treatment group met criteria for agoraphobia (the difference among the groups in the proportion of patients with agoraphobia was not significant). The protocol was approved by the institutional review board of each study site, and all subjects gave written informed consent before participating in the trial.

After diagnostic, medical, and laboratory examinations were completed, subjects were randomly assigned to three treatment groups: 30 were given 2 mg/day of alprazolam, 31 were given 6 mg/day of alprazolam, and 35 were given placebo. The groups were comparable in age; there were more male subjects in the group given 2 mg/day of alprazolam. A psychotropic drug washout period of at least 1 week preceded the baseline evaluation. Patients then received either placebo or 1 mg/day of alprazolam, with a dose escalation schedule ensuring that the target dose was achieved by day 3 for the patients given 2 mg/day and by day 18 for the patients given 6 mg/day. The trial lasted 6 weeks and was followed by a 3-week drug taper. Evaluations were performed at baseline and after 1, 2, 3, 4, and 6 weeks of treatment; 15 (45.5%) of the patients given placebo, 23 (76.7%) of the patients given 2 mg of alprazolam, and 16 (48.4%) of the patients given 6 mg of alprazolam completed the 6-week trial. The methods of dealing with this differential dropout rate are discussed later in this paper and elsewhere (9).

Blood samples were obtained from the subjects at each evaluation time. In addition to the times blood was drawn, the actual times the medication or placebo was administered on the day of sampling and the previous

TABLE 1. Criteria for Classification of Response to Alprazolam Treatment for Panic Disorder

Measure of Treatment Response	Response Classification		
	Major	Moderate	None
Reduction from baseline in total number of panic attacks (%)	>75	25-75	<25
Reduction from baseline in Hamilton anxiety score (%)	>50	25-50	<25
Overall phobia score	0-3 ^a	4-6 ^b	7-10 ^c
Change from baseline in main phobia fear and avoidance score (%)	>50	25-50	<25
Change from baseline in agoraphobia fear and avoidance score (%)	>50	25-50	<25
Physician-rated global improvement scale score	9-10 ^d	7-8 ^e	0-6 ^f
Patient-rated global improvement scale score	9-10 ^d	7-8 ^e	0-6 ^f

^aNot at all to mildly anxious.

^bModerately anxious.

^cMarkedly to extremely anxious.

^dMarkedly improved or back to normal.

^eModerately improved.

^fWorse to a little better.

day were recorded. To generate a random selection of collection times after the previous alprazolam dose, no time of blood sample collection was specified within the protocol. Ten-ml blood samples were collected in heparinized tubes and centrifuged, and the plasma was separated and frozen until analysis.

Benzodiazepine Assays

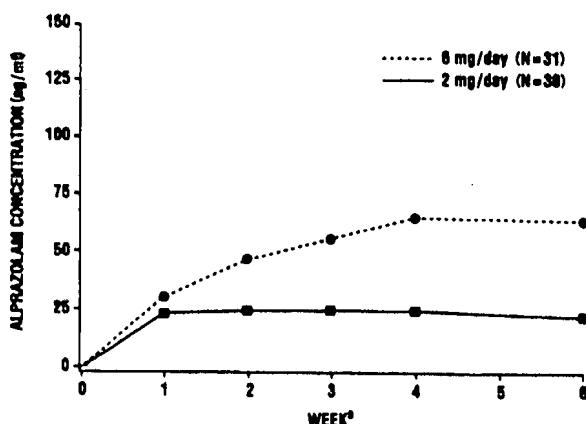
Plasma samples from all patients were analyzed for not only alprazolam but also diazepam and desmethyldiazepam to determine if patients were medicating themselves with other benzodiazepines (excluding lorazepam). Alprazolam, diazepam, and desmethyldiazepam concentrations were determined by gas-liquid chromatography with electron-capture detection by previously reported methods (13, 14).

Data Analysis

Treatment response measures. Treatment response was classified for each of the following efficacy measures: total number of major panic attacks per week, total score on the Hamilton Rating Scale for Anxiety (15) (used to measure overall anxiety, apart from specific panic attacks), overall phobia rating, fear and avoidance factors of the main phobia and agoraphobia ratings derived from the work of Marks and Mathews (16), physician-rated global improvement scale score, and patient-rated global improvement scale score. We defined categories of major, moderate, or no response for each measure, as shown in table 1.

Logistic regression analysis. The relationship between treatment response and plasma alprazolam concentrations was examined with logistic regression analysis (17, 18) of data from the three treatment groups. Patient response classification and plasma alprazolam

FIGURE 1. Mean Plasma Alprazolam Concentrations of Patients With Panic Disorder Given 2 mg/day or 6 mg/day



*Significant difference between groups at week 2 ($t=3.86$, $df=52$, $p=0.0003$), week 3 ($t=6.02$, $df=49$, $p=0.0001$), week 4 ($t=7.04$, $df=47$, $p=0.0001$), and week 6 ($t=6.63$, $df=37$, $p=0.0001$).

concentrations were fitted to a logistic response function with the CATMOD procedure of the Statistical Analysis System (19). Regression coefficient estimates generated from the fitting procedure then were used to calculate the probability of a patient's having a particular response (e.g., major, moderate, none) at a given alprazolam plasma concentration. The details of the analysis have been reported elsewhere (10).

RESULTS

Data for some of the 96 patients could not be included in some of the analyses due to the incompleteness of concentration and/or response data. The number of individuals for whom data were analyzed by each method is reported in each analytical section.

Compliance With Treatment

Only one patient in the placebo group had a positive alprazolam level at any evaluation time; this patient had a positive level at week 2. At baseline, six (20%) of the 30 placebo patients for whom this information was available had positive diazepam levels, and nine (30%) had positive desmethyldiazepam levels. The percent of placebo patients with positive levels for diazepam and/or desmethyldiazepam remained relatively constant throughout the study.

No patients given either 2 mg or 6 mg of alprazolam treatment for whom this information was available ($N=55$) had a positive alprazolam level at baseline. After therapy was initiated, positive alprazolam levels were detected in virtually all alprazolam-treated patients (96%–100% at different evaluation times). At baseline, nine (33%) of the available 27 patients given 2 mg/day of alprazolam and five (18%) of the available

28 patients given 6 mg/day of alprazolam had detectable diazepam and/or desmethyldiazepam concentrations. These percentages decreased during the study; at week 6 only two (8.7%) of 23 patients given 2 mg/day of alprazolam and one (6.2%) of 16 patients given 6 mg/day had detectable levels.

Mean Plasma Alprazolam Concentrations

Alprazolam plasma concentration data were available for 61 patients treated with alprazolam (30 of the patients given 2 mg/day and 31 of the patients given 6 mg/day). On average, each subject had four plasma level determinations. The majority of the samples were collected between 0 and 6 hours after medication administration. Mean plasma alprazolam concentrations for the two alprazolam treatment groups at each week of evaluation are shown in figure 1. Alprazolam concentrations differed significantly between the patients given 2 mg/day of alprazolam and those given 6 mg/day at weeks 2, 3, 4, and 6 (figure 1). At week 4, when all patients had been receiving their maximum alprazolam dose for at least 9 days, the mean alprazolam concentrations were 24.5 ng/ml ($SD=15.6$) for the patients given 2 mg/day and 65.2 ng/ml ($SD=24.4$) for the patients given 6 mg/day. Only 10% of the patients given 2 mg/day had plasma levels above 40 ng/ml, and none had a plasma level above 80 ng/ml. Conversely, approximately 80% of those given 6 mg/day had plasma levels above 40 ng/ml.

Response Relationship to Alprazolam Plasma Concentration

Data from the last evaluation period in the three treatment groups were analyzed by logistic regression. This is roughly equivalent to a week-6 endpoint-imputed analysis (i.e., the last observation was carried forward). The mean times of the last evaluations were 4.8 weeks ($SD=1.6$) for the placebo group, 5.1 weeks ($SD=1.2$) for the patients given 2 mg/day of alprazolam, and 4.8 weeks ($SD=1.4$) for the patients given 6 mg/day of alprazolam (there were more dropouts among the patients given placebo and those given 6 mg/day than among the patients given 2 mg/day). At these evaluation times, all patients were receiving their scheduled alprazolam dose except for three patients in the 6 mg/day group who had reached a dose of only 4 mg/day. These patients were included in the analysis because plasma concentration, not dose, was being evaluated as a predictor of response.

Number of Major Panic Attacks

At baseline, the mean number of panic attacks per week was 7.5 ($SD=9.9$) for the placebo group, 5.3 ($SD=4.9$) for the patients given 2 mg/day of alprazolam, and 6.6 ($SD=7.7$) for the patients given 6 mg/day of alprazolam. Among patients who completed the 6-week trial, the mean number of panic attacks per week

was 3.7 (SD=4.9) for the placebo group, 1.0 (SD=2.7) for the patients given 2 mg of alprazolam, and 1.0 (SD=2.8) for the patients given 6 mg of alprazolam. According to endpoint analysis, the mean number of panic attacks per week was 5.4 (SD=6.2) for the placebo group, 2.0 (SD=4.6) for the patients given 2 mg/day of alprazolam, and 1.9 (SD=3.9) for the patients given 6 mg/day of alprazolam. Both treatment groups improved more than the patients given placebo (9).

Plasma alprazolam concentration was a significant predictor of response assessed by the reduction of major panic attacks ($\chi^2=70.58$, $df=90$, $p=0.003$, likelihood ratio=0.935). A steady-state plasma alprazolam concentration of 15 ng/ml was necessary to achieve a 50% probability of being classified as a major responder, and a concentration of 48 ng/ml was necessary to achieve a 75% probability of being a major responder.

The calculated probability curves, based on the best-fit parameter estimates from the logistic regression model, and the actual response frequencies are presented in figure 2. The three individual graphs represent the predicted probabilities for responder classification over a range of drug concentrations for the three categories of response: major (greater than 75% reduction in panic attacks), moderate (25%–75% reduction), and none (less than 25% reduction). From these graphs, one can determine the probability of attaining the level of response associated with a particular alprazolam steady-state concentration by selecting the concentration and reading the numerical value associated with the curve at that concentration on the respective response graph of interest. At any given alprazolam concentration, the sum of the three probability values for the response classifications equals one.

Using the more stringent criterion for treatment response of having no panic attacks at the last visit, we found that 18 (67%) of 27 subjects with alprazolam concentrations greater than 40 ng/ml, eight (57%) of the 14 subjects with concentrations between 20–40 ng/ml, and eight (42%) of the 19 subjects with concentrations less than 20 ng/ml met this criterion.

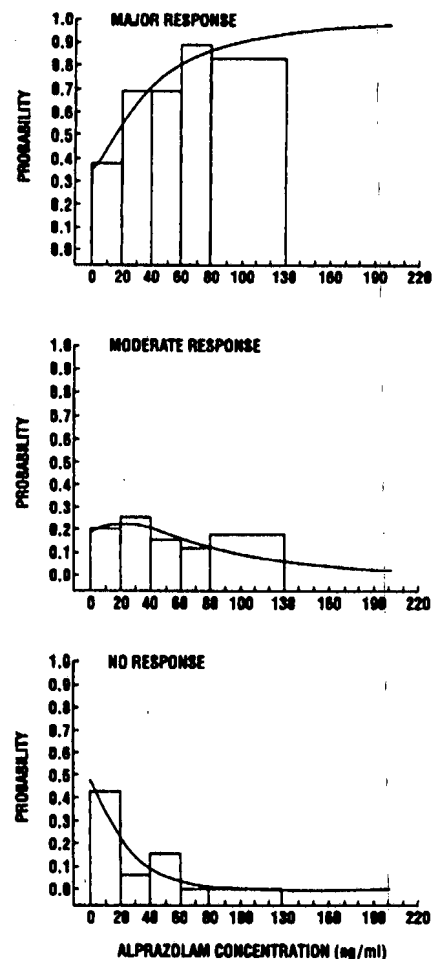
Hamilton Rating Scale for Anxiety

Logistic regression analysis showed no significant relationship between steady-state alprazolam plasma concentration and response classified on the basis of change in Hamilton anxiety score. According to this scale, approximately 70% of the patients were classified as either major or moderate responders at the lowest alprazolam concentrations.

Phobia Ratings

The five phobia ratings were 1) the overall phobia rating, 2) the main phobia fear score, 3) the main phobia avoidance score, 4) the agoraphobia fear score, and 5) the agoraphobia avoidance score. Alprazolam plasma concentration was a significant predictor for response based on the main phobia fear score ($\chi^2=76.24$,

FIGURE 2. Calculated Probability Curves and Observed Frequencies of Patients With Major, Moderate, or No Response to Alprazolam for Panic Disorder



$df=76$, $p=0.04$, likelihood ratio=0.471) and the agoraphobia fear score ($\chi^2=78.4$, $df=76$, $p=0.03$, likelihood ratio=0.403). However, the actual response frequencies on these scales varied considerably and inconsistently with increasing alprazolam concentrations. Therefore, the probability values should be interpreted cautiously. An alprazolam concentration of approximately 75 ng/ml was necessary to obtain a 50% probability of a patient's being classified as a major responder (greater than 50% reduction) by the main phobia fear score. The numbers of patients classified in each response group are presented in table 2. There were no significant relationships between response classification and plasma alprazolam concentration for the other three phobia ratings.

Physician-Rated and Patient-Rated Global Improvement Scales

There was a significant relationship between plasma alprazolam concentration and treatment response cate-

TABLE 2. Relation of Plasma Alprazolam Concentrations and Response Rates for Patients With Panic Disorder

Plasma Alprazolam Concentration	Response According to Main Phobia Fear Scale Score				Response According to Physician-Rated Global Improvement Scale				Response According to Patient-Rated Global Improvement Scale			
	N	Major	Moderate	None	N	Major	Moderate	None	N	Major	Moderate	None
0 ng/ml	26				29				29			
N		4	8	14		5	3	21		5	8	16
%		15	31	54		17	10	72		17	28	55
1-19 ng/ml	17				19				19			
N		4	3	10		3	9	7		5	8	6
%		24	18	59		16	47	37		26	42	32
20-39 ng/ml	11				14				14			
N		5	2	4		4	4	6		3	7	4
%		46	18	36		29	29	43		21	50	29
40-59 ng/ml	13				14				14			
N		5	3	5		6	5	3		7	4	3
%		39	23	39		43	36	21		50	29	21
60-79 ng/ml	7				7				7			
N		4	1	2		4	2	1		2	4	1
%		57	14	29		57	29	14		29	57	14
≥80 ng/ml	6				6				6			
N		3	0	3		0	5	1		3	2	1
%		50	0	50		0	83	17		50	33	17

gorized according to the physician-rated ($\chi^2=104.3$, $df=90$, $p=0.002$, likelihood ratio=0.143) and patient-rated ($\chi^2=94.46$, $df=90$, $p=0.02$, likelihood ratio=0.353) global improvement scales. Again, actual response frequencies did not consistently increase with increasing alprazolam concentrations. According to the calculated curve, the steady-state alprazolam plasma concentration necessary to result in a 50% probability of a patient being classified as a major responder (markedly improved or back to normal) on the physician-rated global improvement scale was 63 ng/ml. However, this finding should be interpreted cautiously because the actual frequencies of a major response on the physician-rated global scale rating varied widely as shown in table 2. The corresponding levels of response at the same concentration levels for the patient-rated global scale also are shown in table 2.

Treatment-Emergent Side Effects and Symptoms

Treatment-emergent symptoms and side effects were defined as those having a greater severity at the time of evaluation than at baseline. Seven of 42 symptoms were reported more frequently by patients given either 2 mg/day or 6 mg/day of alprazolam: sedation/drowsiness, ataxia/impaired coordination, slurred speech, nasal congestion, constipation, diarrhea, and decreased libido (9). Of these, more patients given 6 mg/day than patients given 2 mg/day reported ataxia/impaired coordination and slurred speech; similar numbers reported the remaining symptoms. Five of 42 symptoms (impaired mentation, insomnia, tachycardia/palpitations, tremor, and excessive sweating) were reported more frequently by patients given placebo (9).

The relationships between alprazolam concentration and the emergence of seven significantly and positively related symptoms and side effects were examined by logistic regression analysis of the data from week 4 and

the last evaluation visit. Similar results were obtained for both evaluations (table 3). At week 4, in addition to the symptoms and side effects listed in table 3, the emergence of tachycardia, a symptom of panic disorder, was significantly related to alprazolam concentrations; however, the probability of its emergence was greatest at low alprazolam concentrations.

DISCUSSION

Patient compliance with the protocol was judged to be good. The majority of patients took the study medications and refrained from medicating themselves with other benzodiazepine agents, and this was confirmed by obtaining plasma drug concentrations (although we did not measure concentration of lorazepam). This finding is essential to the further analyses of correlations between alprazolam concentration and response.

The doses in this study were selected on the basis of preliminary data indicating that doses of 2 mg/day and 6 mg/day of alprazolam should result in a wide range of steady-state plasma alprazolam concentrations and that more patients given 6 mg/day would respond to therapy (3, 4). The expected range and overlap of plasma alprazolam concentrations were achieved, and the two doses resulted in significantly different mean alprazolam concentrations. Thus, the selected doses resulted in data suitable for logistic regression analyses.

The results of our study demonstrate that patient response measured according to multiple dimensions is significantly related to steady-state plasma alprazolam concentrations. For panic attacks, the probability of a subject being classified as having a major, moderate, or no response was significantly and positively related to steady-state plasma alprazolam concentration. In a previous report (11), a pharmacokinetic analysis of the data obtained from the same patients showed that the

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elimination of alprazolam in the patients who had major or moderate responses was approximately 40% less than that in the patients demonstrating no response. Reduced elimination of alprazolam resulted in higher steady-state plasma alprazolam concentrations for a given dose in patients classified as responders. This finding further supports a relationship between concentration and response.

Previous work indicates that there is an approximate relationship of 1 mg/day alprazolam dose to 10 ng/ml alprazolam steady-state concentration (7); our findings agree with this ratio. Therefore, the practitioner could then use our finding of a relationship between response and plasma concentration, based on logistic regression analysis, as a guide to total daily dose. For example, for panic attacks, a 75% probability of having a major response occurred at 48 ng/ml, roughly translating to a dose of 4–5 mg/day of alprazolam (given the caveats about intersubject variability). At approximately 1–2 mg/day alprazolam, there was a 50% probability of a major response for panic attacks. Ciraulo et al. (7) reported that, on global measures of impairment, significant improvement in subjects with panic disorder was achieved at plasma concentrations of 20 ng/ml and maximum response occurred at concentrations of 60 ng/ml and higher. However, Ciraulo et al. did not report information regarding response of specific symptoms. In our study, according to physician-rated and patient-rated global improvement scales, 53% of the subjects achieved a moderate response at 20 ng/ml and 80% at 60 ng/ml, which is similar to the data of Ciraulo et al. (7).

Plasma alprazolam concentration in our patients was not a significant predictor of treatment response based on Hamilton anxiety scores, probably due to the fact that the majority of patients with low alprazolam concentrations were classified as major responders on this outcome measure. In fact, only about 10% of patients with alprazolam concentrations between 20 ng/ml and 80 ng/ml were nonresponders on the Hamilton Rating Scale for Anxiety. Clinically, this means that most of the improvement in Hamilton anxiety scores (indicating more generalized or intercurrent anxiety symptoms) in these patients occurred at low doses and low plasma concentrations of alprazolam.

These results suggest that significant improvement in panic attacks occurs at higher alprazolam concentrations than those required for improvement in concomitant anxiety. Although there was clearly a reduction in panic attacks at lower alprazolam concentrations, higher concentrations increased the likelihood of being panic-free at the end of the trial. This is consistent with our previous finding that, although there were no significant differences in reduction of total panic attacks per week between the patients given 2 mg/day and those given 6 mg/day, there were significantly more patients who were panic-free among the patients given 6 mg/day (9).

Still higher concentrations of alprazolam were necessary to achieve a significant reduction in phobias. For the agoraphobia and main phobia fear scores, concen-

TABLE 3. Alprazolam Concentrations Associated With 50% Probability of Side Effects^a

Side Effect	Alprazolam Concentration (ng/ml) Associated With 50% Probability of Emergence	
	Week 4	Last Evaluation Period
Sedation	40	45
Ataxia	70	70
Memory problems	95	90
Slurred speech	100	90
Fatigue	120	110
Decreased libido	120	110
Sexual dysfunction	180	160

^aThe correlation between alprazolam concentration and emergence of side effects was positive for all side effects.

trations over 70 ng/ml were required to have a 50% probability of a major response. Response of the overall phobia score was not significantly related to alprazolam concentrations. It is difficult to explain these findings. They may be related to the fact that only a small number of patients were in the higher alprazolam concentration ranges and the fact that the actual response frequencies varied inconsistently with increasing alprazolam concentrations. Another explanation might be that phobias are not as responsive to alprazolam as are panic attacks and symptoms of anxiety. It also may be that phobias respond best when panic attacks are under the tightest control, i.e., at the higher plasma concentrations. In any case, this finding might explain why the phobias of some patients do not respond to medication, so that the addition of behavioral psychotherapy is indicated.

Alternatively, the phobias may respond, but only at higher alprazolam concentrations, or it might take longer than 6 weeks of drug treatment for phobias to respond. This possibility is consistent with the results using imipramine reported by Mavissakalian et al. (20). In a fixed-dose study, they observed that although panic attacks responded to lower doses and plasma concentrations of imipramine, higher doses and higher concentrations of imipramine were necessary for a good response in terms of reductions in phobic avoidance behavior.

The treatment-emergent symptoms and side effects we observed were those expected from administration of a benzodiazepine. According to logistic regression analysis, they were positively related to steady-state plasma alprazolam concentrations. The only treatment-emergent symptom or side effect that showed a negative correlation with alprazolam plasma concentration was tachycardia. Since tachycardia is a symptom of panic disorder, we would expect it to be better controlled at higher plasma alprazolam concentrations.

A comparison of the steady-state alprazolam concentrations necessary for a 50% probability of response and the 50% probability of the appearance of treatment-emergent side effects can be used to assess the benefit versus side effect ratio. A major response, defined by a reduction in the number of panic attacks, occurred at lower concentrations than those at which

the treatment-emergent symptom of sedation appeared (concentrations for 50% probability were 15 ng/ml for panic response versus 40 ng/ml for sedation). Other CNS side effects were not evident until much higher plasma alprazolam concentrations were obtained, e.g., ataxia at 70 ng/ml and slurred speech at 100 ng/ml. These results must be interpreted in the context of the study, because they are dependent on the rating scales used and the criteria for response classification. They also do not take into account very early treatment-emergent symptoms that may have developed within a few days but subsided as the patient became tolerant to the side effect or the fact that more patients dropped out of the higher alprazolam dose group.

The results of these analyses must not be interpreted as establishing a minimum effective alprazolam concentration or window of effective concentrations for the treatment of panic disorder. Rather, they should be interpreted as demonstrating that there is considerable between-patient variability in alprazolam pharmacokinetics, which explains some, but not all, of the variability in patient response at a given dose. They also offer an understanding that there exist different alprazolam concentrations (and, by inference, doses) which may be necessary to achieve improvement in specific symptoms of the panic syndrome. These findings further support the necessity for careful, individualized alprazolam dose adjustments to achieve the desired treatment response while minimizing undesirable side effects.

A final note of caution: we are not recommending the routine use of benzodiazepine plasma levels for clinical practice. Plasma level determinations of benzodiazepines have not been well standardized and are not readily available in many clinical laboratories. We believe that the use of benzodiazepine plasma levels requires further study in research settings before they can be considered for general practice.

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ANEXO 5

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The Relationship of Alprazolam Dose to Steady-State Plasma Concentrations

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Alprazolam is a widely used antianxiety agent, yet relatively little is known about the relationship between chronic oral doses and steady-state plasma levels. This study examines the relationship over a wide range of therapeutic doses. We conducted a parallel, double-blind, placebo-controlled study in 36 patients with agoraphobia with panic attacks, or panic disorder with limited phobic avoidance based on DSM-III criteria. Patients received alprazolam (N = 25) or placebo (N = 11) beginning at 1 mg/day and increased weekly until either a maximum tolerated dose or 10 mg/day was achieved. Dosages were then gradually tapered according to a predetermined schedule. The entire study period lasted 14 weeks. Laboratory and clinical assessments were conducted weekly. Doses up to 6 mg/day were tolerated by 80% of patients on alprazolam and doses of 10 mg/day were tolerated by 40% of patients. Twenty-seven percent of the placebo patients reached 10 tablets/day. In the alprazolam group, the principal cause of intolerance was sedation. Throughout the study no significant changes in vital signs or laboratory parameters were observed. Steady state alprazolam, 4-hydroxy alprazolam, and α -hydroxy alprazolam plasma levels were linearly related to dose. A 1 mg dosage increment produced, on the average, a corresponding 10 ng/ml increase in steady state level of the parent drug. Significant response was observed in subjects who achieved concentrations greater than 20 ng/ml, with a maximum of 81% of the samples classified as responders within the 60 ng/ml and above group. During taper, no severe withdrawal symptoms were noted; 12 of 25 alprazolam patients, however, reported rebound anxiety (anxiety rating scale scores higher than baseline), which occurred most commonly at the reduction from 1 mg to 0 mg.

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ALPRAZOLAM is a triazolobenzodiazepine with established efficacy in the treatment of panic disorder and panic attacks.¹⁻⁶ Relatively little is known regarding the relationship of chronic oral doses to steady state plasma levels of alprazolam. An inpatient study of six males with panic disorder or agoraphobia with panic attacks given single and multiple alprazolam doses found a linear relationship between oral dose and plasma level at doses of 3, 6, and 9 mg daily.⁶ The mean steady state concentration at 3 mg daily was 29.6 ng/ml, at 6 mg, 61.5 ng/ml, and at 9 mg, 102.9 ng/ml. In that carefully controlled inpatient study, there appeared to be an approximate 1 mg to 10 ng/ml relationship between oral dose and steady state alprazolam serum level. It was important to determine whether such a relationship would also be found in an outpatient study, the setting in which most patients with panic disorder are treated.

The current study evaluated the relationship of alprazolam doses to steady state plasma levels of parent compound and metabolites for doses up to 10 mg daily. Clinical and adverse effects were also assessed. A double-blind, placebo-controlled, fixed-dosage regimen was followed unless unacceptable side effects developed. Dosage adjustments were not made on the basis of clinical response, and doses were increased even if investigators felt the maximum clinical effect had been achieved. We assessed the relationships between dosage and plasma levels in a 14-week outpatient program for patients with agoraphobia with panic attacks or panic disorder with limited phobic avoidance. The study was not designed to determine the relationship between response and plasma levels; however, some preliminary data are presented.

Methods

Patient selection

After giving written informed consent, 37 patients en-

rolled in the study. The clinical sites for the study were the University of Connecticut Health Center in Farmington (site 1) and Lutheran General Hospital in Chicago (site 2). All patients had agoraphobia with panic attacks, or panic disorder with limited phobic avoidance, based on DSM-III criteria. The operational criteria required a minimum of one spontaneous panic attack and/or one situational panic attack per week for the 3 weeks prior to entering the study. Patients were not entered in the study if they had current or prior psychoactive substance dependence; major affective illness; psychotic disorder or drug-induced psychosis occurring within 6 months preceding the study; acute suicidal ideation; current or lifetime history of bipolar disorder; or history of dementia, epilepsy, or seizures. Patients were also excluded if they had clinically important abnormal laboratory values or uncontrolled renal, hepatic, cardiac, pulmonary, endocrinological, or autoimmune disease as determined by history, physical examination, vital signs, and clinical laboratory determinations. During the study period psychotherapy and behavioral therapy were withheld.

Study design

A parallel, double-blind, placebo-controlled study design was used. Patients were randomly assigned to either alprazolam or placebo treatments in a 2:1 ratio and remained in their treatment cell for the 14-week study period. Weekly dose adjustments were made as shown in Table 1 unless intolerable side effects were experienced, in which case the dose was lowered to the previous level for 7 days, and then another attempt was made to increase it. If the patient still did not tolerate the higher dose, the dose was lowered back to the last tolerated, and this was recorded as the maximum for that patient. Clinicians were instructed to follow the dosing schedule even if, in their opinion, maximal clinical improvement had occurred. After the patient had completed the entire dose range or had reached the maximum tolerated dose, medication was discontinued by tapering as indicated in Table 1.

The study was conducted on an outpatient basis with the patients receiving a 1-week supply of medication after each weekly evaluation. At the end of each week, the patient was evaluated, and vital signs, side effects, and behavioral assessments were obtained. Side effects were evaluated using the SAFTEE-UP brief structured interview, which is a modification of the more comprehensive SAFTEE rating system.⁷ Behavioral assessments included the Clinician Rated Anxiety Scale (CRAS), Patient Rated Anxiety Scale (PRAS), and Physician Global Rating. Blood chemistries were obtained at screening and weeks 1, 4, 8, 11, and 13.

TABLE Table 1. Protocol dosage schedule

Week	Days	Total tablets/day	Dosage Regimen				24 hour total (mg/day)
			a.m.	Noon	p.m.	Bedtime	
1	1-7	1	0	0	0	1	1
2	8-14	2	1	0	0	1	2
3	15-21	4	1	1	1	1	4
4	22-28	6	1	2	1	2	6
5	29-35	7	1	2	2	2	7
6	36-42	8	2	2	2	2	8
7	43-49	9	2	2	2	3	9
8	50-56	10	2	3	2	3	10
9	57-60	9	2	2	2	3	9
9	61-68	8	2	2	2	2	8
10	64-66	7	1	2	2	2	7
10	67-70	6	1	2	1	2	6
11	71-73	5	1	1	1	2	5
11	74-77	4	1	1	1	1	4
12	78-80	3	1	0	1	1	3
12	81-84	2	1	0	0	1	2
13	85-87	1	0	0	0	1	1
13	88-91	0	0	0	0	0	0

Blood samples and assay

Two 10 ml blood samples were obtained at each weekly evaluation 8-10 hours after the last dose. The samples were immediately centrifuged; the plasma was harvested and frozen until assay was performed. One plasma sample was analyzed for concentrations of two alprazolam metabolites (4-hydroxy alprazolam and α -hydroxy alprazolam) using high performance liquid chromatography.⁸ The other sample was analyzed by gas liquid chromatography with electron capture detection to determine concentrations of intact alprazolam⁹ and of diazepam and desmethyldiazepam¹⁰ (if present). Weekly CBC, SMA-12, temperature, blood pressure, and pulse were assessed.

The regression analysis of the reported variables of interest with alprazolam and metabolite concentration was performed using the General Linear Model Procedure (SAS Institute Inc., SAS Users Guide, Version 5 Edition, Cary, NC).

Results

Patient demographics

Twenty-six patients were enrolled in the alprazolam treatment group and 11 patients were enrolled in the placebo group. One alprazolam treatment patient refused therapy after enrollment and never received medication. The patients ranged in age from 20-57 years and 72% were women. The age and weight ranges were similar for both study sites and for both treatment groups and are contained in Table 2.

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TABLE 2. Patient demographics by site and treatment group (Means $\pm$ SD)

	Site 1		Site 2	
	Alprazolam	Placebo	Alprazolam	Placebo
N	16	7	9	4
Age (yrs)	36.5 $\pm$ 9.2	41.9 $\pm$ 9.4	34.7 $\pm$ 10.2	33.0 $\pm$ 7.8
Weight (kg)	72.8 $\pm$ 19.4	68.8 $\pm$ 19.3	65.5 $\pm$ 19.2	82.5 $\pm$ 13.8
Gender (M/F)	5/11	1/6	2/7	2/2

Maximum daily dose

Dosing records were validated versus drug distribution forms (actual returned tablet counts). Only two discrepancies were noted. In these cases, fewer tablets were returned than expected. Therefore, doses for these sequences in question were recorded as missing. In the alprazolam group, doses up to 6 tablets/day were well tolerated by the majority of the subjects (80%). Forty percent of the patients (10 of 25) in the alprazolam group and 27% (three of 11) in the placebo groups reached the maximum dose of 10 tablets/day (Fig. 1). In the alprazolam treatment group the principal cause of intolerance was sedation.

Alprazolam and metabolite plasma levels

Mean plasma concentrations of alprazolam, 4-hydroxy alprazolam, and α -hydroxy alprazolam plasma concentrations in relation to alprazolam dose for the alprazolam treatment group are shown in Figure 2. The total plasma concentrations of hydroxylated metabolites on average were less than 15% of the unchanged alprazolam plasma concentration. Linear regression of alprazolam or metabolite plasma concentrations as a function of dose were conducted for samples obtained during dose escalation, during dose tapering, and by combining the escalation and taper data. Steady state alprazolam plasma concentrations were directly proportional to the dose administered during dose escalation and dose tapering; data from

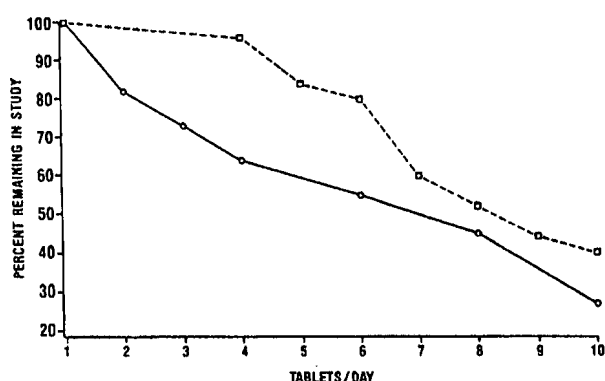


FIG. 1. Percent of total patients achieving daily dose for alprazolam (□) and placebo treatment (○).

each study site gave similar slopes. The regression of combined data from both study sites resulted in a slope of 10.7 ($R^2 = 0.636$, $p < 0.0001$), which indicates that on average for each 1 mg increment in dose there is a corresponding 10 ng/ml change in alprazolam steady state concentration (Fig. 2a). The steady state metabolite concentrations were also linearly related to the alprazolam dose, $R^2 = 0.526$ and $R^2 = 0.517$ for the 4-hydroxy and α -hydroxy metabolites, respectively (Figures 2b, c). Monitoring of diazepam/desmethyl-diazepam plasma levels confirmed that all patients except one had discontinued prior drug therapy according to protocol. Because the levels were subtherapeutic by the third treatment week and not present at all at the time of clinical improvement, this subject was included in the data analysis.

Behavioral effect ratings

Significant correlations (negative for CRAS and PRAS, positive for Physician Global) were observed between the rating scores and alprazolam concentrations during both ascending and descending dose sequences. These trends between response and concentration are also depicted in Figure 3, which presents the percent of responding/non-responding alprazolam patients (defined as having a 50% decrease in CRAS score from baseline) as a function of the patient's alprazolam concentration at the time of evaluation. Significant levels of response were observed in subjects who achieved concentrations above 20 ng/ml, with a maximum of 81% of the samples classified as responders within the 60 ng/ml and above group. The percent of responders was found to be significantly related to concentration by chi-square analysis ($p < 0.001$). During dose escalation, the alprazolam patients displayed mean ($\pm$ SD) minimum CRAS scores of 10.9 ± 8.3 at a mean dose of 5.8 ± 3.2 mg/day and mean minimum PRAS scores of 20.5 ± 16.1 at a mean dose of 5.9 ± 2.8 mg/day. The placebo patients displayed mean minimum CRAS scores of 30.3 ± 21.7 occurring at a mean dose of 5.2 ± 3.7 tablets/day and mean minimum PRAS scores of 46.1 ± 34.7 occurring at a mean dose of 4.7 ± 4.3 tablets/day. Alprazolam patients received the drug for a mean 47.3 ± 15.6 days prior to taper. Within the descending dose sequences, 15 of 36 patients had greater CRAS scores than at baseline; of these, three were placebo and 12 were alprazolam subjects. Eleven of the 15 experienced the first higher anxiety rating at the last dosage reduction (i.e., reduction to 0 mg, including two placebo patients). The evaluating physicians rated the study treatment more or much more effective compared with previous treatment experiences in 15 alprazolam patients and in none of the placebo patients.

Adverse effects

Within the structured side effect evaluation, the oc-

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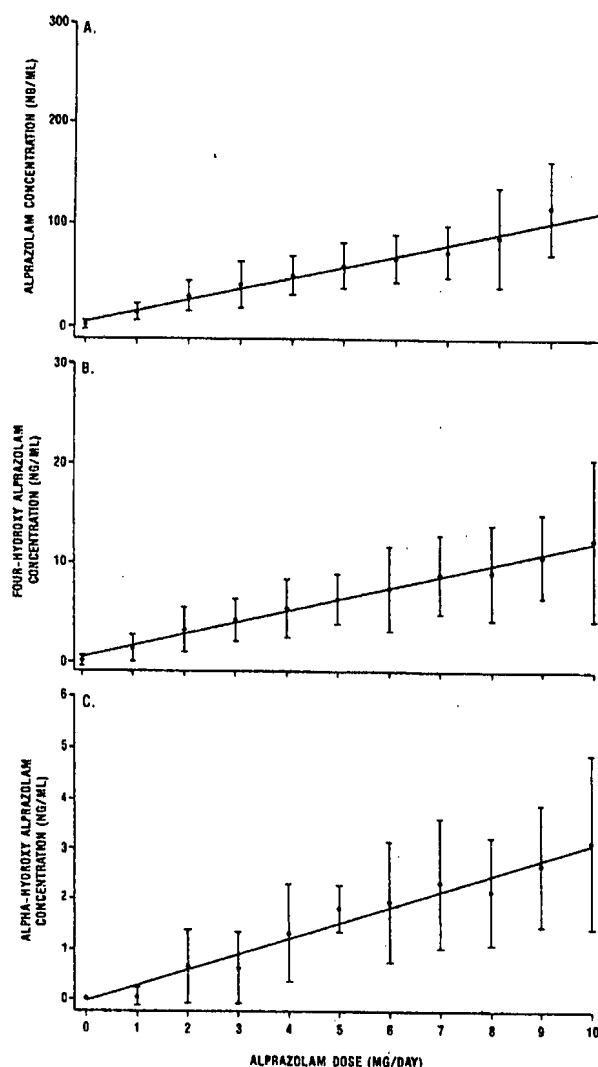


FIG. 2. Mean ($\pm$ SD) plasma concentrations of alprazolam (top), 4-hydroxy alprazolam (middle), and α -hydroxy alprazolam (bottom) in relation to daily dose of alprazolam. Mean value ($\circ$), predicted regression line (—).

currences of 42 potential adverse effects were determined; drug relation, severity (0 = none through 3 = severe), and action taken were recorded. Side effect severity scores were also totaled to provide an indication of overall patient status. No significant correlations were observed between total severity scores and alprazolam

concentrations during ascending dose sequences. A statistically significant but small negative correlation was observed between total severity score and alprazolam concentration during descending dose sequences ($R^2 = 0.078$, slope = 0.063, $p = 0.0026$). In evaluating ascending doses only, the alprazolam patients displayed mean maximum severity scores of 19.1 ± 10.6 occurring at a mean dose of 3.0 ± 3.6 mg/day. The placebo patients displayed mean maximum severity scores (23.7 ± 13.9) occurring at a mean dose of 0.18 ± 0.40 tablets/day. The most frequently reported side effects in the alprazolam patients during ascending dose sequence were sedation, depression, irritability, dry mouth, excitement/nervousness, and increased appetite. During descending dose sequences these alprazolam patients most frequently reported sedation, irritability, excitement/nervousness, insomnia, depression, dry mouth, fatigue, and headache. The most frequently reported side effects in the placebo patients during ascending dose sequences were excitement/nervousness, depression, insomnia, tachycardia/palpitation, excessive sweating, and fatigue. During descending dose sequences the placebo patients most frequently reported excitement/nervousness, dry mouth, depression, and irritability. Physicians assessed the sedation and dry mouth as drug related in the alprazolam group.

Significant but small negative correlations were observed between systolic pressure and alprazolam concentrations ($R^2 = 0.027$, slope = 0.061, $p = 0.0054$), and diastolic pressure and alprazolam concentration ($R^2 = 0.015$, slope = 0.033, $p = 0.0381$). No consistent changes in pulse rate or laboratory parameters were observed.

Termination from study

Study completion was judged by the achievement of the maximum tolerated dose, not necessarily 10 mg/day. Within the alprazolam group three patients did not complete the study because they refused treatment and one did not because of side effects. Five placebo patients did not complete the study because of ineffectiveness of therapy (three patients), refused treatment (one patient), and other reasons (one patient left the study because of finding a job). The patient with diazepam levels was not terminated because levels were subtherapeutic and declining by the third visit.

Discussion

The current study confirms our previous finding that the pharmacokinetics of alprazolam are dose independent for clinically relevant dosages (up to 10 mg daily). On average for each 1 mg increment in dose there was a corresponding 10 ng/ml change in steady state concen-

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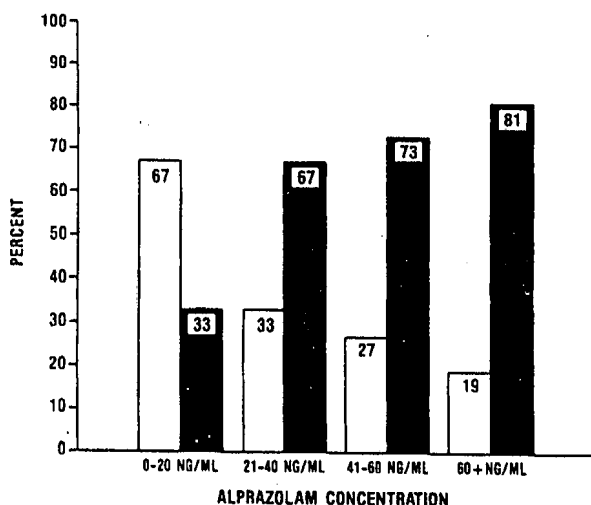


FIG. 3. Shaded bar graphs represent percent of patients responding (defined as a 50% improvement in CRAS score from baseline) and open bar graphs represent percentage on nonresponders as a function of the plasma alprazolam concentration at the time of evaluation.

tration. Concentrations of the two hydroxylated metabolites of alprazolam were also linearly related to dose and are considerably lower than those of the parent drug.⁸ Furthermore, the metabolic products have considerably less pharmacologic activity than the parent drug.¹¹ Therefore, clinical effects attributable to alprazolam administration are likely to be almost entirely due to the parent compound.

We wish to emphasize that the current study was not designed to determine clinical outcome/plasma level relationships, because the effects of time were not controlled. The following data should thus be considered preliminary. There appears to be a relationship between steady state plasma level and clinical response, with significant response levels observed in patients who achieved 20 ng/ml and a maximum response observed in the 60 ng/ml and above group. All rating scales as well as physician's global assessment favored the alprazolam group. There was a positive correlation between plasma level and clinical response, although a strict fixed-dose protocol will be needed to further assess this relationship. Again, the effects of duration of treatment on outcome were not controlled in this study.

The principal dose-limiting side effect was sedation; for most subjects, however, slower escalation of dosage obviated this problem. Because the design was not fixed-dose (i.e., dose escalation was interrupted and taper begun when side effects developed), no relationship between plasma level and side effects was evident using the SAFTEE-UP structured interview. Additionally, for

adverse effects, rating scales may provide a better pharmacodynamic measure than structured interviews.⁶ No sustained significant changes were observed in either vital signs or laboratory measures. Eighty percent of subjects achieved a dose of 6 mg daily, and 40% reached a dose of 10 mg daily.

Alprazolam was discontinued without severe withdrawal symptoms in any patient, suggesting that the risk of a severe withdrawal syndrome can be minimized by using a rate of taper of 1 mg/week in patients on the drug for short periods (mean length of treatment prior to taper was 47.3 ± 15.6 days). The only new symptom to emerge during withdrawal was headache. Rebound anxiety (anxiety scale scores higher than baseline values), however, was commonly seen, as 12 of 25 alprazolam patients had higher CRAS scores during taper than at baseline. Rebound anxiety, in itself, is not sufficient evidence of a withdrawal syndrome, which is usually characterized by autonomic symptoms. Furthermore, most of the symptoms observed were likely a recurrence of the original anxiety symptoms.

Although rapid or abrupt discontinuation of alprazolam may lead to seizures and somatosensory disturbances,¹² slow taper does not usually result in severe withdrawal syndromes.¹³ In a study of alprazolam withdrawal in 10 patients, Mellman and Uhde found increased anxiety ratings, pulse, and plasma cortisol levels when a mean rate of taper of 1.33 mg/week was used.¹⁴ Their patient population and length of drug treatment are not directly comparable to the present study's.

In another study, 17 patients with panic disorder or agoraphobia with panic attacks were tapered from alprazolam at a rate of 10% of starting dose every 3 days.¹⁵ Mean starting dose was approximately 5 mg/day and mean length of treatment prior to discontinuation was 28 weeks. Only four of 17 patients were able to complete withdrawal on schedule. No patient had severe withdrawal symptoms, although 15 had return of panic symptoms of equal or greater intensity than prior to treatment. Nine patients had new symptoms, most commonly malaise, weakness, insomnia, tachycardia, lightheadedness, and dizziness. The return of anxiety observed by these investigators is consistent with our findings; however, the development of several new symptoms is in contrast to our finding that headache was the only new symptom reported during withdrawal. This apparent discrepancy may be due to that study's lack of a placebo control, use of a more sensitive withdrawal scale rather than our structured interview, and/or a much longer mean length of treatment.

In the largest study of alprazolam withdrawal to date, 60 alprazolam-treated patients received a mean dose of 4.8 mg/day over an 8-week period followed by a 4-week taper.¹⁶ Although the suggested rate of taper was 1 mg

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every 3 days (2 mg/week), variability in taper rates across sites occurred. During the first taper week the alprazolam group was receiving a mean 4.0 mg/day. Mean daily dose beginning the last taper week was 1.5 mg/day. Twenty-seven percent of the alprazolam-treated groups reported a rebound of panic attacks during taper, 13% reported anxiety, and 35% reported a mild-to-moderate withdrawal syndrome. That study found a lower rebound anxiety rate than the present one because their definition was more stringent (a 50% increase in Hamilton Anxiety Score in that study compared with any elevation from baseline in ours).

Our findings suggest that for patients receiving higher doses of alprazolam for much shorter periods of time, a rate of taper of 1 mg/week is appropriate until the reduction to 0 mg is made. A slow taper from 1 mg to 0 mg over several weeks may be necessary to avoid rebound anxiety. During taper, the original anxiety symptoms commonly returned at an intensity equal to or transiently greater than baseline, and almost all alprazolam subjects experienced subjective discomfort during the tapering phase of the study. The applicability of our findings to longer periods of treatment is unknown.

In summary, plasma alprazolam levels are linearly related to dose, with a 1 mg increment in dose producing a corresponding 10 ng/ml increase in steady state levels. There is a positive correlation between response and plasma level up to about 60 ng/ml (corresponding to dosages of about 6 mg daily). Severe withdrawal symptoms did not occur during dosage taper, although symptomatology consistent with rebound anxiety was common. Withdrawal symptoms and rebound anxiety following therapeutic doses of benzodiazepines have been reported for several different drugs of the class.¹⁴⁻²⁵ Future studies should address whether the character and intensity of the alprazolam withdrawal syndrome differ from other drugs of this class and should explore therapeutic strategies of clinical management.

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ANEXO 6

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Drug Treatment of Panic Disorder Comparative Efficacy of Alprazolam, Imipramine, and Placebo

CROSS-NATIONAL COLLABORATIVE PANIC STUDY, SECOND PHASE INVESTIGATORS

The Cross-National Collaborative Panic Study, Phase Two, compared alprazolam with imipramine and with placebo in a sample of 1168 randomly assigned subjects. The study, conducted at 12 centres, assessed clinical change over eight weeks of double-blind drug treatment. Improvement occurred with alprazolam by week 1 and 2, and with imipramine by week 4. By the end of week 8, however, the effects of the two active drugs were similar to each other, and both were superior to placebo for most outcome measures.

Clinical descriptions of conditions that would be called 'panic anxiety' by current criteria were reported as early as the 19th century (Westphal, 1872). For many decades, most psychiatrists accepted a model of anxiety that postulated a continuum of severity, ranging from normal fears and tensions, through mild to moderate levels of responses to stress and anxiety neurosis and, finally, to severe states, such as agoraphobia (Marks, 1987a). This continuum model was challenged in the 1960s by clinicians and investigators in Western Europe and the US (Klein, 1964; McNair & Fisher, 1978). Clinical states of 'phobic-depersonalisation syndrome' (Roth, 1959) and 'vital anxiety' were described (Lopez-Ibor, 1950, 1970). In the 1970s, Klein proposed that the diagnostic class of 'anxiety neurosis' be reformulated to separate panic anxiety from other forms of anxiety (Klein, 1981).

This separation was first embodied in the Research Diagnostic Criteria (RDC; Spitzer *et al.*, 1978), developed for the Collaborative Program on the Psychobiology of Depression: Clinical Studies (Katz & Klerman, 1979) of the National Institute of Mental Health (NIMH). It was later incorporated in DSM-III (American Psychiatric Association, 1980). DSM-III eliminated the general category of 'neuroses', and a new category of anxiety disorders was included, within which panic anxiety was separated from generalised anxiety.

Although the validity of the diagnosis of 'panic disorder' has been the subject of controversy (Tyrer, 1984, 1986; Marks, 1987b; Ashcroft *et al.*, 1987; Roth & Argyle, 1988; Gelder, 1989), it has become increasingly accepted in clinical and research settings, particularly in North America (Sheehan, 1982; Barlow, 1988). The effects of panic anxiety on quality of life (Markowitz *et al.*, 1989) and on

increasing risk for suicidal ideation and attempts has been reported (Weissman *et al.*, 1989). Acceptance increased with the modifications of hierarchical rules in DSM-III-R (American Psychiatric Association, 1987), allowing for the diagnosis of panic disorder in conjunction with depression and other conditions. Panic disorder appears as a category in the draft of ICD-10 (World Health Organization, 1988).

Concurrent with these diagnostic changes, the treatments for panic disorder have diversified (Sheehan *et al.*, 1980; Roy-Byrne & Katon, 1987). Behavioural psychotherapies developed for agoraphobia have been modified for treatment of panic anxiety (Marks, 1987a,b; Barlow, 1988). Members of three classes of drugs have been shown to have efficacy - tricyclic antidepressants, monoamine oxidase (MAO) inhibitors, and benzodiazepines. Imipramine has been the tricyclic antidepressant most extensively studied, although there have also been controlled trials with chlormipramine. Phenelzine has been the MAO inhibitor most studied.

Until recently, the conventional wisdom among experienced psychopharmacologists was that standard benzodiazepines were relatively ineffective against panic anxiety. However, in the 1980s, a new class of benzodiazepines, the triazolobenzodiazepines, became available. One member of that class, alprazolam, was reported to be effective in the treatment of panic disorder and agoraphobia with panic disorder (Chouinard *et al.*, 1982; Alexander & Alexander, 1986).

In 1982, the Cross-National Collaborative Panic Study was initiated. The aims and design of the study have been reported in detail (Klerman *et al.*, 1986; Klerman, 1988). Phase One (of two phases) compared alprazolam with placebo in eight centres. Findings were reported by Ballenger *et al.* (1988),

A detailed listing of participants appears at the end of the article.

Noyes *et al* (1988), and Pecknold *et al* (1988). The findings from Phase One have been the subject of extensive discussion (Marks *et al*, 1989; Klerman *et al*, 1989).

Given the growing evidence for the efficacy of alprazolam over placebo in the treatment of panic attacks and agoraphobia, it was decided that, for Phase Two, alprazolam would be compared with a compound from another drug class shown to have antipanic effects. Imipramine was chosen because of the literature supporting its efficacy and the extent of its use among experienced psychopharmacologists. Preliminary findings have been reported at professional meetings (e.g. Klerman, 1990). The purpose of this paper is to report the major findings regarding short-term efficacy from Phase Two of the Cross-National Collaborative Panic Study, which compared alprazolam, imipramine, and placebo in the treatment of patients with panic disorder.

Method

The study employed a randomly assigned, placebo-controlled, double-blind design. Before randomisation to drug group and completion of the baseline measures, screening and diagnostic assessments were done. This was followed by a one- to two-week drug washout period, during which the patient was withdrawn from all drugs acting on the central nervous system (CNS). Patients were then randomly assigned to alprazolam, imipramine, or placebo in a double-blind treatment that was to last eight weeks. The study protocol attempted to achieve the effective dose by the end of week 3, but dose adjustments of individual patient's medication were permitted based upon clinical status after an initial period of pre-established dose increases. Assessment of efficacy and safety of the substances was via a set of rating scales and clinical interviews. Patients completing three weeks of treatment comprised the sample to be evaluated.

To be eligible for the study, patients had to satisfy modified DSM-III criteria for lifetime diagnosis of panic disorder, including at least one episode of a spontaneous panic attack with four symptoms. In addition, in order to ensure that the patient had current symptoms, the patient must have experienced at least one panic attack with three or more symptoms per week for the previous three weeks. A panic attack was defined as a discrete period of intense discomfort or fear in which at least four of the following 14 symptoms occurred: shortness of breath; choking or smothering sensation; palpitation or tachycardia; chest pain or discomfort; sweating; faintness; dizziness, lightheadedness or feelings of unsteadiness; nausea or abdominal distress; depersonalisation or derealisation; paraesthesia; hot flushes or chills; trembling or shaking; fear of dying; fear of 'going crazy' or fear of 'loss of control'.

To be included in the study the patients had to satisfy modified study criteria for: uncomplicated panic disorder, panic disorder with limited phobic avoidance, or panic

disorder with extensive phobic avoidance (panic attacks with agoraphobia). In addition, patients had to participate voluntarily; be able to take oral medication; be of non-child-bearing potential or use contraceptive precautions; and be between 18 and 65 years of age.

Patients with any of the following conditions were excluded: acute suicidal ideation; pregnant or lactating; a history of epilepsy or seizures; distinctly abnormal laboratory values; uncontrolled renal, hepatic, cardiac, pulmonary, endocrinological or collagen disease as determined by physical examination, vital signs, and clinical laboratory test; psychotic disorder (either past or current); a drug-induced psychosis within the last six months as defined by the DSM-III criteria for substance abuse; dementia; a current or lifetime history of bipolar disorder; cyclothymic disorders; a history of alcoholism or drug abuse within the last six months (as defined by DSM-III); not available for weekly follow-up visits; currently taking medication containing α - or β -blockers; undergoing concurrent psychotherapy or behavioural therapy; evidence of hypersensitivity to benzodiazepines; intolerance or contraindication to tricyclics; a history of obsessive-compulsive disorder as defined by DSM-III. Patients with current major depression were excluded unless the depression was judged to be secondary to the anxiety disorder and did not have melancholic or psychotic features. Patients taking CNS drugs, including benzodiazepines, were excluded from the study. During the washout period, blood was drawn for benzodiazepine screening.

Study procedures

Patients were recruited from multiple sources, including articles in the local media and referrals from general practitioners, psychiatrists, psychiatric clinics and other health-care facilities. At the end of the eight weeks, their clinical management and treatment were no longer under the control of the research protocol, but at the discretion of the psychiatrists at the local centres.

The study was conducted at 12 study centres (see under 'Participants', below). The design may be regarded as a replication across 12 randomisation blocks of the basic three-cell, random-assignment, parallel-treatment design. At two centres, two clinical sites were used: thus 14 sites comprised the 12 centres. At each centre, patients were blindly and randomly assigned to alprazolam, imipramine, or placebo treatment based on a table of random numbers. The study protocol called for 90 patients to be evaluated at each of the 12 centres. Patients removed from the protocol before three weeks had to be replaced; after three weeks, non-completers were not replaced.

The unit dosage was 25 mg of imipramine and 1 mg of alprazolam. Dosage was increased steadily according to a pre-determined schedule that specified a dosage of 6 mg of alprazolam, 150 mg of imipramine, or 6 placebo tablets by day 19. Subsequently, doctors could raise or lower the dose, depending upon the individual patient's clinical state or adverse effects, to a total of 10 identical capsules (10 mg alprazolam, 250 mg imipramine, 10 placebo capsules). The dose of alprazolam was based on previous experience (Chouinard *et al*, 1982; Sheehan, 1982; Alexander & Alexander, 1986).

Patients were evaluated on the local standard clinical procedures and by Structured Clinical Interview for DSM-III Diagnosis, Upjohn (SCID-UP; Spitzer & Williams, 1983, 1988). Reliability studies on the SCID-UP have been conducted and the results indicate very good inter-rater reliability ($\kappa = 0.86$) for the diagnosis of panic disorder (Williams & Spitzer, 1992).

Efficacy of drug treatment was assessed using eight measures.

(a) *Overall clinical efficacy and improvement.* Overall change was measured by the rate of patients' completion of the eight-week protocol, a physician's global improvement scale, and a patient's global improvement scale (10-point scales).

(b) *Panic anxiety.* Panic anxiety was assessed by the patient's weekly diary and by the Panic Attack Scale developed by Sheehan & Sheehan (1982) and modified for this study. This scale recorded the total number of panic attacks per week and divided them into spontaneous and situational types. In addition, there were patient judgements as to the duration and intensity of average attacks per week. Attacks of lesser severity, called 'limited symptom attacks', were assessed separately from full panic attacks.

(c) *Phobias.* The Overall Phobia Scale was modified from the scale developed by Marks & Matthews (1979). Patients identified their main, or most troublesome, phobia for which they most wanted treatment. Also, fear of the phobic stimulus was rated on a 10-point scale. The Phobic Anxiety Factor of the Symptom Check List-90 (SCL-90) also measured the severity of phobic fears (Derogatis *et al.*, 1973).

(d) *Anticipatory anxiety.* Anticipatory anxiety referred to the percentage of time spent in anticipation of a panic episode.

(e) *Anxiety.* Anxiety was rated by the 14-item Hamilton Rating Scale for Anxiety (HRSA; Hamilton, 1959).

(f) *Social functioning.* Overall impairment in work and social disability was rated on a five-point scale. In addition, there were three ratings specifying work-related disability, impairment of social life and leisure activities, and impairment of family life and home responsibilities.

(g) *Depression.* The 21-item Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960) was used to assess the severity of depression.

(h) *Patient's self-report.* The Hopkins SCL-90 patient self-rating scale measures the presence and intensity of symptoms over many categories. The total score was used in this report (Derogatis *et al.*, 1973).

Adverse effects were assessed by three methods. Firstly, whenever a serious adverse effect occurred, an adverse drug report (ADR) was completed, following the requirements of the US Food and Drug Administration. These reports provided an inventory of events with potentially serious medical consequences. Secondly, for those patients who were withdrawn from the study protocol before the eight-week completion ('drop-outs' or 'non-completers'), symptoms indicative of adverse drug effects were assessed at the last week by treatment, recorded blindly, and analysed according to drug group and week of treatment. Thirdly, all patients were rated weekly for adverse effects using a modification of SAFTEE, which was developed by Levine and Schooler at the NIMH Psychopharmacology Research Branch (Levine & Schooler, 1986).

Every effort was made to limit the extent to which patients received formal psychotherapy or behavioural treatment during the eight-week treatment phase. The protocol stipulated that they were not to be scheduled in individual, family, group, or behavioural psychotherapy. Direct instructions for exposure and other behavioural techniques - particularly relaxation, desensitisation, and exposure in imagery - were contraindicated.

Supervision

The study was directed by a steering committee of the investigators, co-chaired by James H. Coleman, the Upjohn Company, and Gerald L. Klerman, MD, consultant. In 1983, before the study began, the investigators and their staff participated in a training workshop at Pisa, Italy, in the use of SCID-UP and other assessments in order to standardise procedure. During the study, an extensive procedure manual was used at each study site. Monthly telephone conferences and six-monthly meetings of the investigators were held throughout the three years of data collection (1984-87) and throughout data analysis and manuscript preparation (1984-88).

Quality-assurance teams visited each research centre twice during the study. These teams were composed of two experienced psychiatric research consultants not otherwise involved in the study and were accompanied by a medical monitoring team from the Upjohn Company. Each visit lasted two days, during which time the diagnostic procedures were reviewed for accuracy, and the consistency of methods for assessment of outcome measures were observed directly. Safety and ethical concerns were monitored by a study review committee of independent psychiatric and psychopharmacological researchers and statisticians who met approximately every six months to review patient flow and adverse effects.

Data analysis and statistical methods

For each outcome variable, the distribution of baseline and subsequent values was examined for possible marked non-Gaussian distributions that might invalidate the standard analysis of variance (ANOVA). Non-Gaussian distributions were found for the highly skewed counts of panic attacks (spontaneous, situational, and total), but for other variables the distributions were not deviant. In the non-Gaussian cases, non-parametric analysis of variance was used based on ranks (the Kruskal-Wallis test computed by SAS procedure NPARIWAY) to check the results of normal-theory *F*-tests from ANOVA.

Each ANOVA was calculated for the two-way layout of 12 centres by three medications in the procedure suggested by Fleiss *et al.* (1972) (SAS procedure GLM). The main effects and interactions were calculated, and the centre-by-medication term tested for statistical significance using a liberal cut-off ($P=0.10$) to avoid the chance that interactions might be missed. With this sample size, no clinically important medication-by-centre interaction would escape notice. In the absence of a significant interaction, one may interpret the observed differences across centres in the treatment effects (contrasts among treatments) as due to chance,

and, therefore, calculate an overall main treatment effect, adjusting for a main centre effect. This was done in the standard manner and all ANOVA main effects of medication were tested for significance by this procedure. In the rare event that an interaction was indicated, the SAS type III procedure was used to estimate and test a pooled treatment effect. The type III procedure is a widely recommended way to test such 'composite' effects and is based on an average treatment effect (over sites).

The 'multiple comparisons' problem was addressed in two ways. Within each outcome there are three possible pairs of treatment contrasts making up the main-effects analysis. We used Tukey's standardised range procedure (SAS option Tukey to means statement in PROC GLM) to avoid increasing the type I error rate for each individual outcome. Tukey's procedure is widely recommended to control the error rate, while all possible pairs of group comparisons are made. Multiple outcome variables create the second kind of multiple-comparisons problem. This problem has been dealt with by pre-specifying the central outcome variables (a-h, above). The *P* values associated with these outcomes can be taken at face value. Other contrasts have been included for descriptive purposes and for the sake of completeness; their significance tests should be regarded as indicative rather than confirmatory. As we expected, a general effect was found across most outcomes, so that the problem of multiple comparisons is not acute.

Results

Of 1168 patients randomised, 1122 met criteria for 'intent to treat' (ITT): i.e. 46 patients did not complete the baseline measures.

The sample was predominantly female (62%). The mean age of the patients was 34 years. The majority of the sample was married (63%), although 10% were separated, divorced or had annulled marriages. This was a relatively young adult sample - only 1% were widowed and 24% never married. The average patient had had panic disorder for five years; thus the mean age at onset of the panic disorder was 29 years.

Among patients with agoraphobic symptoms, using a three-part classification, 22% had uncomplicated panic, 40% had panic with limited phobic avoidance, and 36% had panic with extensive phobic avoidance, and 2% were unclassifiable. Patients with comorbid anxiety included 28% of patients with simple phobia and 15% with social phobia. In addition, 3% reported obsessive symptoms, and 3% had mild compulsive symptoms but did not meet criteria for obsessive-compulsive disorder.

Comorbidity with depression was assessed in a number of ways. The mean HRSD score was 14.1 at baseline. Using DSM-III criteria for major depression, 16% of the sample were currently depressed, 16% met criteria for major depressive episode in the past, and 69% were not depressed currently or in the past. Eleven per cent of the sample met DSM-III criteria for dysthymia.

The patients randomised in the three drug groups did not significantly differ on demographic and clinical variables, nor did they differ on the baseline levels of the outcome measures. The range of demographic and clinical

Table 1
Percentage completion rates: reason for early termination by treatment group

	Alprazolam (n = 386)	Imipramine (n = 391)	Placebo (n = 391)
Completed study	82.6	69.8	56.3
Did not complete study	17.4	30.2	43.7
side-effects	3.4	5.9	3.1
ineffective	3.1	2.6	12.8
refused treatment	3.9	14.1	16.1
other	7.0	7.7	11.8

characteristics were also relatively comparable across sites; however, cross-national differences will be the subject of a separate report.

Of the 1168 randomised patients, 1010 (86%) completed week 3 (i.e. could be evaluated for the purposes of the study), and 812 (69%, 80% of those evaluated) completed week 8 (Table 1). There were significant differences in the completion rate among the three drug groups. Of those patients randomised, 83% of alprazolam patients completed the protocol, compared with 70% of imipramine patients and 56% of placebo patients. The reasons for patients' non-completion of the eight-week protocol differed statistically among the drug groups (Table 1). Ineffectiveness of treatment was most often attributed to placebo drop-out (12.8%). Adverse effects were relatively infrequent causes of failure to complete the protocol and were more common with the imipramine-treated patients (5.9%). Of note is the category of 'patient refusal', which accounted for 3.9% of the alprazolam, 14.1% of the imipramine, and 16.1% of the placebo groups. Lacking further information, it is difficult to ascertain the extent to which this category refers to patients experiencing adverse effects, to worsening of the patient's condition, or to other reasons.

The physicians' double-blind prescriptions were as follows: the largest weekly average number of tablets prescribed were of placebo, followed by imipramine and alprazolam. At the end of eight weeks, the average daily dose for imipramine was 6.2 tablets (155 mg); alprazolam, 5.7 tablets (5.7 mg); placebo, 6.8 tablets per day.

Blood was drawn at entrance into the study and periodically throughout the study for determination of levels of alprazolam, imipramine, and other benzodiazepines. Moderate degrees of non-compliance in the placebo group were manifested by approximately 9% of the placebo patients having benzodiazepine in their blood at week 8. Comparable figures for alprazolam and imipramine were 2% and 4%, respectively. It is not possible to draw conclusions concerning the blood level and symptom improvement since there were a large number of missing samples across the three drug groups.

Measures of drug efficacy

On most measures, improvement occurred with alprazolam by week 1 and 2; and with imipramine by week 4. By the end of the study, however, the two active drugs were

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Table 2
Physician's global improvement rating¹ and HRSD score² by week and treatment group

	Treatment group			P value	Tukey's ranges	Interaction P value
	alprazolam (A)	imipramine (I)	placebo (P)			
<i>Physician's rating</i>						
Baseline	5.0	5.0	5.0	NS	AIP	NS
Week 1	6.7	5.4	5.4	***	A-P-I	NS
Week 2	7.2	6.3	6.0	***	A-P-I	NS
Week 3	7.4	6.9	6.4	***	A-P-I	*/***
Week 4	7.7	7.3	6.8	***	A-P-I	**/*
Week 6	7.9	8.0	7.1	***	AI-P	**/*
Week 8	8.3	8.4	7.5	***	AI-P	**/*
End-point	8.0	7.9	6.7	***	AI-P	**/*
<i>HRSD score</i>						
Baseline	13.9	14.0	14.4	NS	AIP	
Week 3	8.0	8.8	10.4	***	AI-P	
Week 8	6.2	5.8	7.1	**	IA-P	
End-point	7.4	6.8	9.4	***	IA-P	

1. Scale range 1-10, with higher score indicating more illness.
2. Scale range 0-42, with higher score indicating more depression.
NS not significant, * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$.

similar to each other and superior to placebo for most outcome measures by both completer and end-point analyses.

The pattern of improvement on most of the specific variables was very similar to that shown in Table 2, namely that there was an initial improvement in the alprazolam group. By week 8, the imipramine group had improved, so that both active drugs were more efficacious than placebo. There were some differences between end-point and completer analyses, and these are identified below.

Drug-related improvement of panic anxiety was evident in weekly measures of panic attacks. Alprazolam patients showed improvement after week 1, and the imipramine group improved by week 6. By the end of the study, the two drug groups improved more than the placebo group in the number of panic attacks on both completer analysis and end-point analysis. In the end-point analysis, 70% of the alprazolam and imipramine patients had no panic

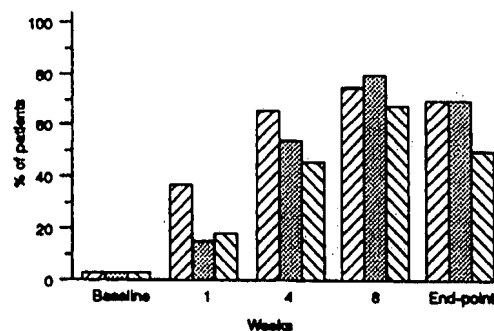


Fig. 1 Percentage of patients experiencing no panic attacks each week.

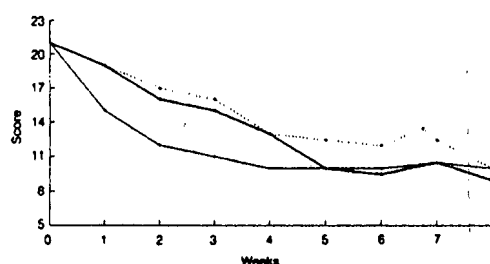


Fig. 2 Completer analysis of scores on the HRSA (—●— alprazolam, —■— imipramine, --- placebo).

attacks, compared with 50% of those taking placebo (Fig. 1). End-point analysis of spontaneous panic attacks and situational panic attacks favoured the two active drugs over placebo, but on the completer analysis there were no statistically significant differences among the three treatment groups at week 8.

On percentage of time spent in anticipation of a panic attack and on the total score on the HRSA, alprazolam produced improvement within the first two weeks, while improvement with imipramine did not occur until the fourth week (Fig. 2). On end-point analysis the two active compounds showed statistically greater effect than placebo on HRSA scores.

The patient's major phobia, rated as to fear and avoidance, showed improvement in the alprazolam group early in the trial; imipramine effects appeared later. Both end-point and completer analyses showed greater improvement with the two active drugs than with the placebo except at week 8.

The pattern for the drug groups on the (self-reported) SCL-90 total score differs from that of other outcome

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Table 3
Adverse effects

	% patients in drug-treatment group ¹ reporting effect								
	Week 1			Week 4			Week 8		
	A	I	P	A	I	P	A	I	P
Ataxia/impaired co-ordination	16	6	8	9	2	4	8	1	2
Blurred vision	9	16	10	5	15	10	5	5	6
Constipation	12	18	7	13	31	6	12	23	5
Decrease appetite	6	17	11	9	7	8	7	5	6
Difficulty urinating	1	6	2	3	9	1	4	9	1
Dizziness/faintness	15	22	18	10	11	14	6	7	9
Dry mouth	16	52	22	17	51	15	11	40	7
Excessive sweating	9	27	12	6	24	11	4	19	7
Excitement/nervousness	6	16	11	9	8	10	7	4	12
Fatigue/weakness	20	19	16	18	13	12	13	7	9
Headache	13	21	18	15	13	13	11	7	11
Increase appetite	21	6	12	13	15	11	8	14	7
Insomnia	3	22	12	5	11	12	4	7	8
Irritability	12	13	11	14	7	8	11	7	8
Malaise	6	12	11	6	6	8	5	4	5
Memory problems	16	13	11	15	9	9	14	7	5
Nausea/vomiting	7	17	13	5	8	10	6	3	6
Other sleep disturbances	4	14	11	5	10	8	5	6	6
Sedation	58	31	21	44	20	16	29	10	11
Slurred speech	7	4	4	7	1	3	4	1	1
Tachycardia	6	18	11	3	7	8	3	4	6
Tremor	8	14	12	5	13	9	6	10	5
Weight gain	11	2	2	12	12	6	8	14	11

1. A, alprazolam; I, imipramine; P, placebo.

measures; imipramine was significantly more effective than both alprazolam and placebo at week 8 by completer analysis and by end-point analysis. Alprazolam was more effective than placebo in the end-point analysis.

Approximately 25% of the patients in the sample had current or past major depression according to DSM-III criteria. The drug effects on HRSD scores is shown in Table 2. No difference between alprazolam and imipramine was found. A more detailed analysis of the drug effects in relationship to depressive symptoms has been undertaken (Deltito *et al*, 1990; Klerman, 1990).

The pattern of drug effect on four measures of social functioning is similar to the effects on symptom patterns: alprazolam produced beneficial effects sooner than imipramine; in the end-point analysis the two active drugs were better than placebo (Fig. 3).

Adverse effects and effects of drug discontinuation

Overall, the drugs were well tolerated. There were no deaths or episodes of cardiac, hepatic, or renal illness. There were no suicides, murders, or serious car or industrial accidents. About 4% of the patients did not complete the eight-week protocol because of discomforting adverse effects. Among those patients completing the eight-week protocol, many reported adverse effects which were uncomfortable, but seldom disabling or medically life-threatening. Patients on alprazolam experienced more sedation, fatigue, memory problems, and ataxia. Patients receiving imipramine

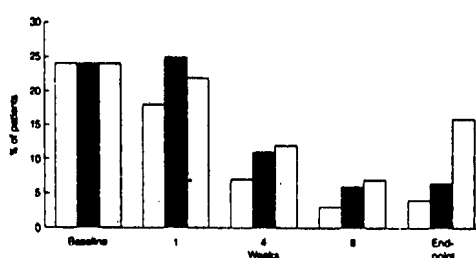


Fig. 3 Ratings of work and social disability: percentage of patients requiring change in activity (□ alprazolam, ■ imipramine, ▒ placebo).

experienced dry mouth, constipation, difficulty in urinating, excessive sweating, tremor, and sleep disturbances. Patients receiving placebo experienced more insomnia, excitement, headache, tachycardia, hyperventilation, chest pain, sleep disturbances, irritability, dizziness, and tremor.

The effects of discontinuation were not consistent because of variations in procedures across centres. After the eight-week control phase, the following percentages of patients discontinued the study medication: 78% alprazolam, 92% imipramine, and 94% placebo.

After discontinuation, many of the alprazolam patients experienced symptoms of discomfort, including increase in anxiety levels reflecting a likely combination of relapse

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of anxiety symptoms and benzodiazepine withdrawal. The symptoms, while discomforting, were not serious. There were no convulsions, no delirium, and none of the patients required admission to hospital.

Inter-centre variation

Although there were small differences in frequency of individual symptoms, sex ratio, or age at onset, the pattern of symptoms and associated anxiety and depressive comorbidity were generally similar across centres.

In regard to the possibility of inter-centre variation in drug outcome, statistical analyses (ANOVA) of the main effects at each centre revealed significant differences among the sites in the level of most outcome measures at baseline and at subsequent assessment points through the eight weeks. However, there were very few centre-by-medication interaction effects on the outcome measures. The timing of drug effects and the relative advantage of the two active drugs over placebo did not vary significantly from site to site.

Discussion

The analyses reported were done on the sample of patients who could be evaluated – that is, those who completed three weeks of medication, as specified in the initial protocol. We also did an ITT analysis; this produced greater drug/placebo differences, since a proportion of patients in the placebo group dropped out of the study before week 3 owing to their adverse clinical condition. These patients were not included in the end-point analysis, but they were included in the week 1 and 2 completer analysis. A small number of imipramine patients did not complete week 3 because of adverse effects. The end-point analysis of the ITT sample shows greater efficacy for the two active drugs.

Two other analytic strategies might have been applied to these data: multivariate outcome repeated measure (MVRM) and covariance adjustment for baseline values. A multivariate longitudinal approach was not used for several reasons. Firstly, the pattern of missing data violates the basic assumptions of all MVRM procedures and no standard imputation strategy is suitable to 'fill the gaps'. Secondly, the usual MVRM analysis makes assumptions about the error covariance (at all lags the correlations are the same) that are not fulfilled in the data. Finally, the large increase in complexity of explication and interpretation that would be required was not justified by the modest increase in power. ANOVA was not used because the randomisation of such large numbers makes it unnecessary to adjust for imbalance (Lavori *et al*, 1983), and the modest increase in power is unnecessary and not worth the extra assumptions and complexity of exposition.

The principal statistical problem arising in this study relates to the large number of placebo patients who dropped out before the end of the trial. This issue was addressed using several methods. Firstly, as planned from the outset, efficacy analyses were restricted to patients who completed week 3. Secondly, since a significant proportion of placebo patients who dropped out gave 'lack of improvement' as their reason for leaving the study, the drop-out rate among patients evaluated was analysed as an outcome in itself. Thirdly, outcomes at all weeks were examined to compare the efficacy of the treatments among patients who remained in the study at these time points (i.e. completer analyses). The week-4 comparisons were not influenced significantly by drop-outs, since few of the patients evaluated dropped out before week 4. The week-8 comparison of study completers had a bias favouring placebo, since at that point nearly half of those on placebo who could be evaluated had dropped out. Patients remaining in the placebo group at week 8, therefore, included a high percentage of those who responded to placebo.

The end-point values from all patients who were evaluated were also analysed, using the week-8 data when they were present, and the last available value carried forward when they were not. This end-point analysis, which is a standard method in psychopharmacological trials, may favour the two active drugs, since carrying forward the last value of placebo patients who drop out because of inefficiency may underestimate their improvement if they had remained in the study for eight weeks. This possibility may also apply to alprazolam/imipramine comparisons, since on many scales alprazolam became efficacious at a faster rate than did imipramine.

It is also possible that the placebo patients who dropped out would have further worsened clinically had they remained in the trial, thus invalidating our hypothesis about the direction of end-point analysis. However, this possibility would increase the already significant drug/placebo differences observed with the end-point analysis.

It was presumed that the range of values from the two analyses of week-8 outcomes – the completer and end-point analyses – would contain the (statistical) 'true' treatment effects of the three drugs, defined as the expected differences that would have been observed if all patients had remained in the study until week 8. This opinion is based on the reported reasons for drop-out (especially for inefficacy), the observed associations of drop-out status with poor clinical state in placebo patients, and the general trend to improvement seen in all treatment groups.

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We conclude that the week-4 comparisons are the most statistically valid reflections of the short-term relative efficacy of placebo, alprazolam, and imipramine, and are less influenced than are the week-8 comparisons by the differential drop-out rate. Also since relatively few alprazolam patients (17%) failed to complete the eight weeks of the trial, week-8 alprazolam results provide an accurate estimate of the efficacy of alprazolam over that time period when categories of response are reported (e.g. percentage of patients no longer having panic attacks). The true magnitude of the drug/placebo comparisons are uncertain in the week-8 analyses. Percentages reported in various response categories for the placebo group at week 8 are overestimates because of the high proportion of positive placebo responders remaining in study at week 8.

All the differences reported in this paper are statistically significant. The clinical importance of these differences requires clinical judgement and clinical assessment, which are independent of statistical considerations.

Patient's responses

Rate of completion by patients of the eight-week protocol varied across the three compounds. The lowest rate of patient non-completion was for alprazolam; the highest rate was for placebo, with imipramine in between. The significant high non-completion rate of the placebo group, attributed to patients' clinical condition worsening, or to no improvement being recorded, may be regarded as an indirect measure of the efficacy of the active compounds. A number of patients dropped out because of adverse effects, with the highest rate occurring in the imipramine group.

Blood level determinations showed that some patients in the imipramine and placebo group continued to take benzodiazepines, probably resulting in an overestimate of the efficacy of placebo.

The statistically significant and clinically important overall trend towards improvement in all three groups during the eight-week trial is of note. Clinical and research experience suggests that some portion of this general improvement was due to non-pharmacological factors such as patients' expectations on taking medication. It is likely that patients also benefited from the effects of clinical and research procedures, accurate diagnosis, the extensive attention given to them during the initial screening and evaluation, and the frequency and intensity of subsequent outcome assessments. This schedule of assessments resulted in patients being seen for longer than usual clinical practice.

Although every attempt was made to minimise inadvertent instructions for exposure *in vivo* and other techniques of behavioural or dynamic psychotherapy, considerable supportive psychotherapy was likely to have been provided in the course of the frequent visits and the time devoted to assessment. At each subsequent visit, one hour or more was spent in the assessment of symptoms and intercurrent social functioning, physical examination, and drawing of blood. The possible therapeutic impact of reassurance, encouragement, and other non-specific psychotherapeutic interactions as an inadvertent concomitant of the assessment schedule cannot be measured, nor can the effects of social interactions with other patients, but we believe they contributed to the general improvement. Also, other factors inherent to the patients and their anxiety disorder (such as level of symptoms) may have contributed to improvement on placebo.

It should be stressed, however, that despite the general trend of improvement over time with all three treatments, the two active drugs were clearly differentiated from the placebo treatment condition. The clinical significance of these differences cannot be ascertained with precision in a trial such as this due to the complex research procedures employed.

A minority of the patients in the study met DSM-III criteria for major depression, although suicidal, bipolar, psychotic, and melancholic patients were excluded. Nevertheless, for these patients, both active drugs were significantly better than placebo on the SCL-90 measure of depression and on the HRSD. The therapeutic value of benzodiazepine for depression has been the subject of investigation, but the results are controversial.

Comorbidity of depression and panic disorder occurs frequently. Estimates as high as 40-50% have been reported in treatment settings (Breier *et al.*, 1985; Buller *et al.*, 1986; Lesser & Rubin, 1988; Klerman, 1990). There is disagreement as to the nature of these depressive symptoms. At times they have been called 'secondary depression' (Lesser & Rubin, 1988) or 'demoralisation' (Klein, 1981).

There were few serious problems due to adverse effects, even among those whose drop-out was attributed to adverse effects. Levels of sedation with alprazolam were easily managed by dose adjustment. Patients tolerated the tricyclic, imipramine, less well: more patients on imipramine left the study because of adverse effects.

Drug discontinuation and benzodiazepine dependence

This paper has focused on findings relevant to short-term clinical efficacy, the major goal of the

Cross-National Panic Study. The use of a benzodiazepine, alprazolam, at a relatively high dose represented a departure from standard clinical practice, and its effects at various dosages has been investigated (Ulenhuth *et al*, 1989; Lydiard *et al*, 1990).

When patients with panic disorder discontinue drug treatment, a moderate proportion become symptomatic and distressed. The clinician must distinguish whether these symptoms are due to the relapse of the anxiety disorder or are discontinuation reactions due to pharmacological dependence, as may occur with alprazolam and other benzodiazepines (Busto *et al*, 1984; Roy-Byrne & Katon, 1987). A mild withdrawal syndrome has been reported with alprazolam that may be severe if the drug is discontinued abruptly (Pecknold, 1988; Fyer & Sandberg, 1988); if dose reduction is gradual, patient discomfort is reduced, and serious effects rarely occur (Pecknold *et al*, 1988). The design of this study was not sufficiently controlled to estimate the possible risk of withdrawal and dependence associated with alprazolam. Additional studies are needed for a benefit/risk assessment of the value of short-term and long-term treatment with alprazolam in panic disorder. A withdrawal syndrome has also been observed following the discontinuation of tricyclics (Baldessarini, 1985).

Pharmacological mechanisms of clinical effects

The CNS mechanism of action of the antianxiety, antiphobic and antipanic effects of alprazolam and imipramine is unknown. Some investigators have implicated the noradrenergic system, particularly in view of the action of antidepressant drugs on the locus coeruleus (Charney & Heninger, 1985). A common mode of action on CNS noradrenergic systems for alprazolam, tricyclic, and MAO inhibitor has been hypothesised. Pharmacological evidence suggests that alprazolam reduces noradrenergic function (Sethy & Hodges, 1982; Lahti *et al*, 1983; O'Connor *et al*, 1985). The clinical effectiveness of alprazolam may also result from its high affinity for the benzodiazepine receptor and its high-dose potency (1 mg of alprazolam is clinically equivalent to 10–15 mg of diazepam). Panic patients tolerated doses of alprazolam equivalent to 50–60 mg of diazepam, far higher than is usually prescribed for ambulatory patients. The extent to which alprazolam may differ from other benzodiazepines is not clear (Carr *et al*, 1986). Further research is needed to clarify whether or not alprazolam has pharmacokinetic or pharmacodynamic properties different from other benzodiazepines and to determine how

these properties may contribute to alprazolam's clinical efficacy in panic disorder and dependence potential.

Comparative efficacy of drug treatments

Currently, there are compounds from three classes of drugs that have demonstrated efficacy for panic disorder in controlled clinical trials (Ballenger, 1986; Fyer & Sandberg, 1988). These compounds are imipramine, the prototypic tricyclic antidepressant; phenelzine, the MAO inhibitor (Kelley *et al*, 1970; Tyrer & Shawcross, 1988); and alprazolam, a triazolobenzodiazepine (Ballenger, 1986).

Other tricyclic antidepressants are also in clinical use for panic disorder. Clomipramine is the best-studied tricyclic compound other than imipramine (Escobar & Landbloom, 1976; Amin *et al*, 1977; Cassano *et al*, 1988). Bupropion, a new non-tricyclic antidepressant, lacks efficacy for panic disorder, and trazodone has only minimal efficacy (Sheehan *et al*, 1983; Charney *et al*, 1986; Cassano *et al*, 1988).

Anxiolytics have been widely used for anxiety states, including panic disorder. The efficacy of benzodiazepines other than alprazolam for panic disorder is under investigation. Conventional wisdom has been that standard benzodiazepines are not effective against panic disorder (Sheehan, 1982). However, this view is now being re-evaluated. Placebo-controlled clinical trials of diazepam, clonazepam, lorazepam, and bromazepam have been completed (Noyes *et al*, 1984; Beaudry *et al*, 1984; Tesar *et al*, 1984; Dunner *et al*, 1986; Liebowitz *et al*, 1986; Rosenbaum, 1987; Charney & Woods, 1989). The non-benzodiazepine anxiolytic buspirone has been reported as ineffective for panic anxiety (Sheehan *et al*, 1988).

Although there have been a number of studies evaluating individual compounds for the treatment of panic disorder, there have been relatively few studies comparing more than one active compound (Sheehan *et al*, 1980; Versiani *et al*, 1985; Charney *et al*, 1986). The Cross-National Collaborative Panic Study on drug treatment is the largest comparative study reported to date. More large studies directly comparing two or more compounds with each other and with placebo are needed.

Conclusions

The range of treatments for panic disorder is now widening. With the publication of results on the short-term efficacy and safety of treatment by alprazolam compared with imipramine and placebo for patients with panic disorder, the clinician now

has available representatives of three classes of drugs with demonstrated efficacy. In addition, behavioural psychotherapies have also proved effective.

Two unresolved issues remain.

(a) What is the optimum duration of drug treatment? This drug study assessed clinical change over eight weeks. However, this is a relatively brief period in the natural history of panic disorder. Although there was a significant reduction in the number and intensity of panic attacks, a proportion of patients will experience recurrence of their anxiety symptoms if medication is discontinued after eight weeks (Versiani *et al*, 1985; Pecknold *et al*, 1988). It is not yet clear how long treatment should continue; therefore, research on the optimum duration of long-term continuation and maintenance treatment is of clinical importance. Studies involving varying durations of drug treatment of panic disorder to prevent relapse and recurrence are underway (Versiani *et al*, 1985). This aspect of the drug treatment of panic disorder is very similar to the issues that emerged about two decades ago with regard to the value of continuation and maintenance drug treatment of depressive disorders.

(b) What is the comparative efficacy of drugs and psychotherapy alone and in combination? The best-studied psychotherapies are the behavioural techniques, including exposure *in vivo*. Only a few comparative and combined studies have been undertaken. New forms of behavioural psychotherapies have been developed for panic anxiety, for which preliminary reports suggest antipanic effects (Clark *et al*, 1985; Shear & Barlow, 1988). There are unresolved questions as to the sequence and duration of improvement with drugs and with psychotherapy, as well as to the clinical value of combining treatments.

In conclusion, the results of this study establish alprazolam as effective in the short-term treatment of panic attacks and comparable with imipramine, for which efficacy has been previously demonstrated. Currently, alprazolam, imipramine, or any other compound or class of compounds has not established itself as the single drug of choice in the treatment of panic disorder.

Participants

This is a report from the Cross-National Collaborative Panic Study, Second Phase. This study consists of a Steering Committee: James H. Coleman, Chairman (Kalamazoo, Michigan, USA); Gerald L. Klerman, Co-Chairman (New York, USA); and project directors: Jose Luis Ayuso (Madrid, Spain), Per Bech (Hillerød, Denmark), Otto Benkert (Mainz, Germany), Sydney Brandon (Leicester, England), Giovanni B. Cassano (Pisa, Italy), Jorge A. Costa e Silva (Rio de Janeiro, Brazil), George C. Curtis (Ann Arbor, Michigan, USA), Juan R. de la Fuente (Mexico City, Mexico), Jose Guimon

(Bilbao, Spain), Hanns Hippus (Munich, Germany), Yves Lecrubier (Paris, France), Carlos A. Leon (Cali, Colombia), Juan J. Lopez-Ibor, Jr (Madrid, Spain), Heinz Katschnig (Vienna, Austria), Juan Massana (Barcelona, Spain), Mogens Møllergaard (Glostrup, Denmark), Jan-Otto Ottoson (Göteborg, Sweden), Ole J. Rafaelsen (deceased), Raben Rosenberg (Copenhagen, Denmark), Martin Roth (Cambridge, England), Javier Sepúlveda (Mexico City, Mexico), Leslie Solyom (Vancouver, British Columbia, Canada), Marco Versiani (Rio de Janeiro, Brazil), Jean Willemotte (Marchienne-au-Point, Belgium).

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British Journal of Psychiatry (1992), 160, 202-205

Comment on the Second Phase of the Cross-National Collaborative Panic Study

ISAAC MARKS, JOHN GREIST, METIN BASOGLU, HOMA NOSHIRVANI and GERALDINE O'SULLIVAN

"Phase Two compared alprazolam with imipramine and with placebo in a sample of 1168 randomly assigned subjects. . . . By the end of week 8 . . . the effects of the two active drugs were similar to each other, and both were superior to placebo for most outcome measures."

In our view, this study's elephantine labour has resulted in the delivery of a mouse. Despite its

randomised design, with many patients and measures, its analysis is scanty and biased towards alprazolam. It fails to note that other benzodiazepines act like alprazolam (Noyes *et al*, 1991).

Careful reading shows that the 'Phase Two' drug 'effects' are reported on only a small minority of measures, and are tiny even then. The gloss is

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ANEXO 7

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ALPRAZOLAM

THERAPEUTICS

Brands • Xanax, Xanax XR
see index for additional brand names

Generic? Yes (not for XR)



Class

• Benzodiazepine (anxiolytic)

Commonly Prescribed For

(bold for FDA approved)

- **Generalized anxiety disorder (IR)**
- **Panic disorder (IR and XR)**
- Other anxiety disorders
- Anxiety associated with depression
- Premenstrual dysphoric disorder
- Irritable bowel syndrome and other somatic symptoms associated with anxiety disorders
- Insomnia
- Acute mania (adjunctive)
- Acute psychosis (adjunctive)



How The Drug Works

- Binds to benzodiazepine receptors at the GABA-A ligand-gated chloride channel complex
- Enhances the inhibitory effects of GABA
- Boosts chloride conductance through GABA-regulated channels
- Inhibits neuronal activity presumably in amygdala-centered fear circuits to provide therapeutic benefits in anxiety disorders

How Long Until It Works

- Some immediate relief with first dosing is common; can take several weeks with daily dosing for maximal therapeutic benefit

If It Works

- For short-term symptoms of anxiety – after a few weeks, discontinue use or use on an “as-needed” basis
- For chronic anxiety disorders, the goal of treatment is complete remission of symptoms as well as prevention of future relapses
- For chronic anxiety disorders, treatment most often reduces or even eliminates symptoms, but not a cure since symptoms can recur after medicine stopped

- For long-term symptoms of anxiety, consider switching to an SSRI or SNRI for long-term maintenance
- If long-term maintenance with a benzodiazepine is necessary, continue treatment for 6 months after symptoms resolve, and then taper dose slowly
- If symptoms reemerge, consider treatment with an SSRI or SNRI, or consider restarting the benzodiazepine; sometimes benzodiazepines have to be used in combination with SSRIs or SNRIs for best results

If It Doesn't Work

- Consider switching to another agent or adding an appropriate augmenting agent
- Consider psychotherapy, especially cognitive behavioral psychotherapy
- Consider presence of concomitant substance abuse
- Consider presence of alprazolam abuse
- Consider another diagnosis, such as a comorbid medical condition



Best Augmenting Combos for Partial Response or Treatment-Resistance

- Benzodiazepines are frequently used as augmenting agents for antipsychotics and mood stabilizers in the treatment of psychotic and bipolar disorders
- Benzodiazepines are frequently used as augmenting agents for SSRIs and SNRIs in the treatment of anxiety disorders
- Not generally rational to combine with other benzodiazepines
- Caution if using as an anxiolytic concomitantly with other sedative hypnotics for sleep

Tests

- In patients with seizure disorders, concomitant medical illness, and/or those with multiple concomitant long-term medications, periodic liver tests and blood counts may be prudent

SIDE EFFECTS

How Drug Causes Side Effects

- Same mechanism for side effects as for therapeutic effects – namely due to

ALPRAZOLAM (continued)

excessive actions at benzodiazepine receptors

- Long-term adaptations in benzodiazepine receptors may explain the development of dependence, tolerance, and withdrawal
- Side effects are generally immediate, but immediate side effects often disappear in time

Notable Side Effects

- * Sedation, fatigue, depression
- * Dizziness, ataxia, slurred speech, weakness
- * Forgetfulness, confusion
- * Hyper-excitability, nervousness
- Rare hallucinations, mania
- Rare hypotension
- Hypersalivation, dry mouth



Life Threatening or Dangerous Side Effects

- Respiratory depression, especially when taken with CNS depressants in overdose
- Rare hepatic dysfunction, renal dysfunction, blood dyscrasias

Weight Gain



- Reported but not expected

Sedation



- Occurs in significant minority
- Especially at initiation of treatment or when dose increases
- Tolerance often develops over time

What To Do About Side Effects

- Wait
- Wait
- Wait
- Lower the dose
- Switch to alprazolam XR
- Take largest dose at bedtime to avoid sedative effects during the day
- Switch to another agent
- Administer flumazenil if side effects are severe or life-threatening

Best Augmenting Agents for Side Effects

- Many side effects cannot be improved with an augmenting agent

DOSING AND USE

Usual Dosage Range

- Anxiety: alprazolam IR: 1–4 mg/day
- Panic: alprazolam IR: 5–6 mg/day
- Panic: alprazolam XR: 3–6 mg/day

Dosage Forms

- Alprazolam IR tablet 0.25 mg scored, 0.5 mg scored, 1 mg scored, 2 mg multi-scored
- Alprazolam IR solution, concentrate 1 mg/mL
- Alprazolam XR (extended-release) tablet 0.5 mg, 1 mg, 2 mg, 3 mg

How to Dose

- For anxiety, alprazolam IR should be started at 0.75–1.5 mg/day divided into 3 doses; increase dose every 3–4 days until desired efficacy is reached; maximum dose generally 4 mg/day
- For panic, alprazolam IR should be started at 1.5 mg/day divided into 3 doses; increase 1 mg or less every 3–4 days until desired efficacy is reached, increasing by smaller amounts for dosage over 4 mg/day; may require as much as 10 mg/day for desired efficacy in difficult cases
- For panic, alprazolam XR should be started at 0.5–1 mg/day once daily in the morning; dose may be increased by 1 mg/day every 3–4 days until desired efficacy is reached; maximum dose generally 10 mg/day



Dosing Tips

- Use lowest possible effective dose for the shortest possible period of time (a benzodiazepine-sparing strategy)
- Assess need for continued treatment regularly
- Risk of dependence may increase with dose and duration of treatment
- For inter-dose symptoms of anxiety, can either increase dose or maintain same total daily dose but divide into more frequent

doses, or give as extended-release formulation

- Can also use an as-needed occasional "top up" dose for inter-dose anxiety
- Because panic disorder can require doses higher than 4 mg/day, the risk of dependence may be greater in these patients
- Some severely ill patients may require 8 mg/day or more
- Extended release formulation only needs to be taken once or twice daily
- Do not break or chew XR tablets as this will alter controlled release properties
- Frequency of dosing in practice is often greater than predicted from half-life, as duration of biological activity is often shorter than pharmacokinetic terminal half-life
- Alprazolam and alprazolam XR generally dosed about one tenth the dosage of diazepam
- ✱ Alprazolam and alprazolam XR generally dosed about twice the dosage of clonazepam

Overdose

- Fatalities have been reported both in monotherapy and in conjunction with alcohol; sedation, confusion, poor coordination, diminished reflexes, coma

Long-Term Use

- Risk of dependence, particularly for treatment periods longer than 12 weeks and especially in patients with past or current polysubstance abuse

Habit Forming

- Alprazolam is a Schedule IV drug
- Patients may develop dependence and/or tolerance with long-term use

How to Stop

- Seizures may rarely occur on withdrawal, especially if withdrawal is abrupt; greater risk for doses above 4 mg and in those with additional risks for seizures, including those with a history of seizures
- Taper by 0.5 mg every 3 days to reduce chances of withdrawal effects
- For difficult to taper cases, consider reducing dose much more slowly after reaching 3 mg/day; perhaps by as little as 0.25 mg per week or less

- For other patients with severe problems discontinuing a benzodiazepine, dosing may need to be tapered over many months (i.e., reduce dose by 1% every 3 days by crushing tablet and suspending or dissolving in 100 ml of fruit juice and then disposing of 1 ml while drinking the rest; 3–7 days later, dispose of 2 ml, and so on). This is both a form of very slow biological tapering and a form of behavioral desensitization
- Be sure to differentiate reemergence of symptoms requiring reinstitution of treatment from withdrawal symptoms
- Benzodiazepine-dependent anxiety patients and insulin-dependent diabetics are not addicted to their medications. When benzodiazepine-dependent patients stop their medication, disease symptoms can reemerge, disease symptoms can worsen (rebound), and/or withdrawal symptoms can emerge

Pharmacokinetics

- Metabolized by CYP450 3A4
- Inactive metabolites
- Elimination half-life 12–15 hours



Drug Interactions

- Increased depressive effects when taken with other CNS depressants
- Inhibitors of CYP450 3A, such as nefazodone, fluvoxamine, fluoxetine, and even grapefruit juice, may decrease clearance of alprazolam and thereby raise alprazolam plasma levels and enhance sedative side effects; alprazolam dose may need to be lowered
- Thus,azole antifungal agents (such as ketoconazole and itraconazole), macrolide antibiotics, and protease inhibitors may also raise alprazolam plasma levels
- Inducers of CYP450 3A, such as carbamazepine, may increase clearance of alprazolam and lower alprazolam plasma levels and possibly reduce therapeutic effects



Other Warnings/Precautions

- Dosage changes should be made in collaboration with prescriber

ALPRAZOLAM (continued)

- Use with caution in patients with pulmonary disease; rare reports of death after initiation of benzodiazepines in patients with severe pulmonary impairment
- History of drug or alcohol abuse often creates greater risk for dependency
- Hypomania and mania have occurred in depressed patients taking alprazolam
- Use only with extreme caution if patient has obstructive sleep apnea
- Some depressed patients may experience a worsening of suicidal ideation
- Some patients may exhibit abnormal thinking or behavioral changes similar to those caused by other CNS depressants (i.e., either depressant actions or disinhibiting actions)

Do Not Use

- If patient has narrow angle-closure glaucoma
- If patient is taking ketoconazole or itraconazole (azole antifungal agents)
- If there is a proven allergy to alprazolam or any benzodiazepine

SPECIAL POPULATIONS

Renal Impairment

- Drug should be used with caution

Hepatic Impairment

- Should begin with lower starting dose (0.5–0.75 mg/day in 2 or 3 divided doses)

Cardiac Impairment

- Benzodiazepines have been used to treat anxiety associated with acute myocardial infarction

Elderly

- Should begin with lower starting dose (0.5–0.75 mg/day in 2 or 3 divided doses) and be monitored closely



Children and Adolescents

- Safety and efficacy not established but often used, especially short-term and at the lower end of the dosing scale
- Long-term effects of alprazolam in children/adolescents are unknown

- Should generally receive lower doses and be more closely monitored



Pregnancy

- Risk Category D [positive evidence of risk to human fetus; potential benefits may still justify its use during pregnancy]
- Possible increased risk of birth defects when benzodiazepines taken during pregnancy
- Because of the potential risks, alprazolam is not generally recommended as treatment for anxiety during pregnancy, especially during the first trimester
- Drug should be tapered if discontinued
- Infants whose mothers received a benzodiazepine late in pregnancy may experience withdrawal effects
- Neonatal flaccidity has been reported in infants whose mothers took a benzodiazepine during pregnancy
- Seizures, even mild seizures, may cause harm to the embryo/fetus

Breast Feeding

- Some drug is found in mother's breast milk
- ✱ Recommended either to discontinue drug or bottle feed
- Effects on infant have been observed and include feeding difficulties, sedation, and weight loss

THE ART OF PSYCHOPHARMACOLOGY

Potential Advantages

- Rapid onset of action
- Less sedation than some other benzodiazepines
- Availability of an XR formulation with longer duration of action

Potential Disadvantages

- Euphoria may lead to abuse
- Abuse especially risky in past or present substance abusers

Primary Target Symptoms

- Panic attacks
- Anxiety

**Pearls**

- * One of the most popular benzodiazepines for anxiety, especially among primary care physicians and psychiatrists
- Is a very useful adjunct to SSRIs and SNRIs in the treatment of numerous anxiety disorders
- Not effective for treating psychosis as a monotherapy, but can be used as an adjunct to antipsychotics
- Not effective for treating bipolar disorder as a monotherapy, but can be used as an adjunct to mood stabilizers and antipsychotics
- May both cause depression and treat depression in different patients
- Risk of seizure is greatest during the first 3 days after discontinuation of alprazolam, especially in those with prior seizures, head injuries, or withdrawal from drugs of abuse
- Clinical duration of action may be shorter than plasma half-life, leading to dosing more frequently than 2–3 times daily in some patients, especially for immediate release alprazolam
- Adding fluvoxamine, fluoxetine, or nefazodone can increase alprazolam levels and make the patient very sleepy unless the alprazolam dose is lowered by half or more

- When using to treat insomnia, remember that insomnia may be a symptom of some other primary disorder itself, and thus warrant evaluation for comorbid psychiatric and/or medical conditions
- * Alprazolam XR may be less sedating than immediate release alprazolam
- * Alprazolam XR may be dosed less frequently than immediate release alprazolam, and lead to less inter-dose breakthrough symptoms and less "clock-watching" in anxious patients
- Slower rises in plasma drug levels for alprazolam XR have the potential to reduce euphoria/abuse liability, but this has not been proven
- Slower falls in plasma drug levels for alprazolam XR have the potential to facilitate drug discontinuation by reducing withdrawal symptoms, but this has not been proven
- * Alprazolam XR generally has longer biological duration of action than clonazepam
- * If clonazepam can be considered a "long-acting alprazolam-like anxiolytic", then alprazolam XR can be considered "an even longer-acting clonazepam-like anxiolytic" with the potential of improved tolerability features in terms of less euphoria, abuse, dependence, and withdrawal problems, but this has not been proven

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AMISULPRIDE

THERAPEUTICS

Brands • Solian
see index for additional brand names

Generic? No



Class

- Atypical antipsychotic (benzamide; possibly a dopamine stabilizer and dopamine partial agonist)

Commonly Prescribed For

(bold for FDA approved)

- Schizophrenia, acute and chronic (outside of U.S., especially Europe)
- Dysthymia



How The Drug Works

- Theoretically blocks presynaptic dopamine 2 receptors at low doses
- Theoretically blocks postsynaptic dopamine 2 receptors at higher doses
- * May be a partial agonist at dopamine 2 receptors, which would theoretically reduce dopamine output when dopamine concentrations are high and increase dopamine output when dopamine concentrations are low
- Blocks dopamine 3 receptors, which may contribute to its clinical actions
- * Unlike other atypical antipsychotics, amisulpride does not have potent actions at serotonin receptors

How Long Until It Works

- Psychotic symptoms can improve within 1 week, but it may take several weeks for full effect on behavior as well as on cognition and affective stabilization
- Classically recommended to wait at least 4–6 weeks to determine efficacy of drug, but in practice some patients require up to 16–20 weeks to show a good response, especially on cognitive symptoms

If It Works

- Most often reduces positive symptoms in schizophrenia but does not eliminate them
- Can improve negative symptoms, as well as aggressive, cognitive, and affective symptoms in schizophrenia

- Most schizophrenic patients do not have a total remission of symptoms but rather a reduction of symptoms by about a third
- Perhaps 5–15% of schizophrenic patients can experience an overall improvement of greater than 50–60%, especially when receiving stable treatment for more than a year
- Such patients are considered super-responders or "awakeners" since they may be well enough to be employed, live independently, and sustain long-term relationships
- Continue treatment until reaching a plateau of improvement
- After reaching a satisfactory plateau, continue treatment for at least a year after first episode of psychosis
- For second and subsequent episodes of psychosis, treatment may need to be indefinite
- Even for first episodes of psychosis, it may be preferable to continue treatment indefinitely to avoid subsequent episodes

If It Doesn't Work

- Try one of the other first-line atypical antipsychotics (risperidone, olanzapine, quetiapine, ziprasidone, aripiprazole)
- If two or more antipsychotic monotherapies do not work, consider clozapine
- If no atypical antipsychotic is effective, consider higher doses or augmentation with valproate or lamotrigine
- Some patients may require treatment with a conventional antipsychotic
- Consider noncompliance and switch to another antipsychotic with fewer side effects or to an antipsychotic that can be given by depot injection
- Consider initiating rehabilitation and psychotherapy
- Consider presence of concomitant drug abuse



Best Augmenting Combos for Partial Response or Treatment-Resistance

- Valproic acid (valproate, divalproex, divalproex ER)
- Augmentation of amisulpride has not been systematically studied

AMISULPRIDE (continued)

- Other mood stabilizing anticonvulsants (carbamazepine, oxcarbazepine, lamotrigine)
- Lithium
- Benzodiazepines

Tests

- * Although risk of diabetes and dyslipidemia with amisulpride has not been systematically studied, monitoring as for all other atypical antipsychotics is suggested

Before starting an atypical antipsychotic

- * Weigh all patients and track BMI during treatment
- Get baseline personal and family history of obesity, dyslipidemia, hypertension, and cardiovascular disease
- Get waistline circumference (at umbilicus), blood pressure, fasting plasma glucose, and fasting lipid profile
- Determine if patient is
 - overweight (BMI 25.0–29.9)
 - obese (BMI ≥30)
 - has pre-diabetes (fasting plasma glucose 100–125 mg/dl)
 - has diabetes (fasting plasma glucose >126 mg/dl)
 - has hypertension (BP >140/90 mm Hg)
 - has dyslipidemia (increased total cholesterol, LDL cholesterol, and triglycerides; decreased HDL cholesterol)
- Treat or refer such patients for treatment, including nutrition and weight management, physical activity counseling, smoking cessation, and medical management

Monitoring after starting an atypical antipsychotic

- * BMI monthly for 3 months, then quarterly
- Blood pressure, fasting plasma glucose, fasting lipids within 3 months and then annually, but earlier and more frequently for patients with diabetes or who have gained >5% initial weight
- Treat or refer for treatment and consider switching to another atypical antipsychotic for patients who become overweight, obese, pre-diabetic, diabetic, hypertensive, or dyslipidemic while receiving an atypical antipsychotic
- * Even in patients without known diabetes, be vigilant for the rare but life threatening onset of diabetic ketoacidosis, which always requires immediate treatment by

- monitoring for the rapid onset of polyuria, polydipsia, weight loss, nausea, vomiting, dehydration, rapid respiration, weakness and clouding of sensorium, even coma
- EKGs may be useful for selected patients (e.g., those with personal or family history of QTc prolongation; cardiac arrhythmia; recent myocardial infarction; uncompensated heart failure; or taking agents that prolong QTc interval such as pimozide, thioridazine, selected antiarrhythmics, moxifloxacin, sparfloxacin, etc.)
- Patients at risk for electrolyte disturbances (e.g., patients on diuretic therapy) should have baseline and periodic serum potassium and magnesium measurements

SIDE EFFECTS

How Drug Causes Side Effects

- By blocking dopamine 2 receptors in the striatum, it can cause motor side effects, especially at high doses
- By blocking dopamine 2 receptors in the pituitary, it can cause elevations in prolactin
- Mechanism of weight gain and possible increased incidence of diabetes and dyslipidemia with atypical antipsychotics is unknown

Notable Side Effects

- * Extrapyramidal symptoms
- * Galactorrhea, amenorrhea
- * Atypical antipsychotics may increase the risk for diabetes and dyslipidemia, although the specific risks associated with amisulpride are unknown
- Insomnia, sedation, agitation, anxiety
- Constipation, weight gain
- Rare tardive dyskinesia



Life Threatening or Dangerous Side Effects

- Rare neuroleptic malignant syndrome
- Rare seizures
- Dose-dependent QTc prolongation

(continued) **AMISULPRIDE****Weight Gain**

- Occurs in significant minority

Sedation

- Many experience and/or can be significant in amount, especially at high doses

What To Do About Side Effects

- Wait
- Wait
- Wait
- Lower the dose
- For motor symptoms, add an anticholinergic agent
- Take more of the dose at bedtime to help reduce daytime sedation
- Weight loss, exercise programs, and medical management for high BMIs, diabetes, dyslipidemia
- Switch to another atypical antipsychotic

Best Augmenting Agents for Side Effects

- Benztropine or trihexyphenidyl for motor side effects
- Many side effects cannot be improved with an augmenting agent

DOSING AND USE**Usual Dosage Range**

- Schizophrenia: 400–800 mg/day in 2 doses
- Negative symptoms only: 50–300 mg/day
- Dysthymia: 50 mg/day

Dosage Forms

- Different formulations may be available in different markets
- Tablet 50 mg, 100 mg, 200 mg, 400 mg
- Oral solution 100 mg/mL

How to Dose

- Initial 400–800 mg/day in 2 doses; daily doses above 400 mg should be divided in 2; maximum generally 1200 mg/day

**Dosing Tips**

- * Efficacy for negative symptoms in schizophrenia may be achieved at lower doses, while efficacy for positive symptoms may require higher doses
- Patients receiving low doses may only need to take the drug once daily
- * For dysthymia and depression, use only low doses
- * Dose-dependent QTc prolongation, so use with caution, especially at higher doses (>800 mg/day)
- * Amisulpride may accumulate in patients with renal insufficiency, requiring lower dosing or switching to another antipsychotic to avoid QTc prolongation in these patients

Overdose

- Sedation, coma, hypotension, extrapyramidal symptoms

Long-Term Use

- Amisulpride is used for both acute and chronic schizophrenia treatment

Habit Forming

- No

How to Stop

- Slow down-titration (over 6 to 8 weeks), especially when simultaneously beginning a new antipsychotic while switching (i.e., cross-titration)
- Rapid discontinuation may lead to rebound psychosis and worsening of symptoms

Pharmacokinetics

- Elimination half-life approximately 12 hours
- Excreted largely unchanged

**Drug Interactions**

- Can decrease the effects of levodopa, dopamine agonists
- Can increase the effects of antihypertensive drugs
- CNS effects may be increased if used with a CNS depressant
- May enhance QTc prolongation of other drugs capable of prolonging QTc interval

AMISULPRIDE (continued)

- Since amisulpride is only weakly metabolized, few drug interactions that could raise amisulpride plasma levels are expected.



Other Warnings/ Precautions

- Use cautiously in patients with alcohol withdrawal or convulsive disorders because of possible lowering of seizure threshold
- If signs of neuroleptic malignant syndrome develop, treatment should be immediately discontinued
- Because amisulpride may dose-dependently prolong QTc interval, use with caution in patients who have bradycardia or who are taking drugs that can induce bradycardia (e.g., beta blockers, calcium channel blockers, clonidine, digitalis)
- Because amisulpride may dose-dependently prolong QTc interval, use with caution in patients who have hypokalemia and/or hypomagnesemia or who are taking drugs that can induce hypokalemia and/or magnesemia (e.g., diuretics, stimulant laxatives, intravenous amphotericin B, glucocorticoids, tetracosactide)
- Use only with caution if at all in Parkinson's disease or Lewy Body dementia, especially at high doses

Do Not Use

- If patient has pheochromocytoma
- If patient has prolactin-dependent tumor
- If patient is pregnant or nursing
- If patient is taking agents capable of significantly prolonging QTc interval (e.g., pimozide; thioridazine; selected antiarrhythmics such as quinidine, disopyramide, amiodarone, and sotalol; selected antibiotics such as moxifloxacin and sparfloxacin)
- If there is a history of QTc prolongation or cardiac arrhythmia, recent acute myocardial infarction, uncompensated heart failure
- If patient is taking cisapride, intravenous erythromycin, or pentamidine
- In children
- If there is a proven allergy to amisulpride

SPECIAL POPULATIONS

Renal Impairment

- Use with caution; drug may accumulate
- Amisulpride is eliminated by the renal route; in cases of severe renal insufficiency, the dose should be decreased and intermittent treatment or switching to another antipsychotic should be considered

Hepatic Impairment

- Use with caution, but dose adjustment not generally necessary

Cardiac Impairment

- Amisulpride produces a dose-dependent prolongation of QTc interval, which may be enhanced by the existence of bradycardia, hypokalemia, congenital or acquired long QTc interval, which should be evaluated prior to administering amisulpride
- Use with caution if treating concomitantly with a medication likely to produce prolonged bradycardia, hypokalemia, slowing of intracardiac conduction, or prolongation of the QTc interval
- Avoid amisulpride in patients with a known history of QTc prolongation, recent acute myocardial infarction, and uncompensated heart failure

Elderly

- Some patients may be more susceptible to sedative and hypotensive effects



Children and Adolescents

- Efficacy and safety not established under age 18



Pregnancy

- Although animal studies have not shown teratogenic effect, amisulpride is not recommended for use during pregnancy
- Psychotic symptoms may worsen during pregnancy and some form of treatment may be necessary
- Amisulpride may be preferable to anticonvulsant mood stabilizers if treatment is required during pregnancy

Breast Feeding

- Unknown if amisulpride is secreted in human breast milk, but all psychotropics assumed to be secreted in breast milk
- * Recommended either to discontinue drug or bottle feed

THE ART OF PSYCHOPHARMACOLOGY**Potential Advantages**

- Not as clearly associated with weight gain as some other atypical antipsychotics
- For patients who are responsive to low dose activating effects that reduce negative symptoms and depression

Potential Disadvantages

- Patients who have difficulty being compliant with twice daily dosing
- Patients for whom elevated prolactin may not be desired (e.g., possibly pregnant patients; pubescent girls with amenorrhea; postmenopausal women with low estrogen who do not take estrogen replacement therapy)
- Patients with severe renal impairment

Primary Target Symptoms

- Positive symptoms of psychosis
- Negative symptoms of psychosis
- Depressive symptoms

**Pearls**

- * Efficacy has been particularly well demonstrated in patients with predominantly negative symptoms
- * The increase in prolactin caused by amisulpride may cause menstruation to stop
- Some treatment-resistant patients with inadequate responses to clozapine may benefit from amisulpride augmentation of clozapine
- Risks of diabetes and dyslipidemia not well studied, but does not seem to cause as much weight gain as some other atypical antipsychotics
- Has atypical antipsychotic properties (i.e., antipsychotic action without a high incidence of extrapyramidal symptoms), especially at low doses, but not a serotonin dopamine antagonist

- Mediates its atypical antipsychotic properties via novel actions on dopamine receptors, perhaps dopamine stabilizing partial agonist actions on dopamine 2 receptors
- May be more of a dopamine 2 antagonist than aripiprazole, but less of a dopamine 2 antagonist than other atypical or conventional antipsychotics
- Low dose activating actions may be beneficial for negative symptoms in schizophrenia
- Very low doses may be useful in dysthymia
- Compared to sulpiride, amisulpride has better oral bioavailability and more potency, thus allowing lower dosing, less weight gain, and fewer extrapyramidal symptoms
- Compared to other atypical antipsychotics with potent serotonin 2A antagonism, amisulpride may have more extrapyramidal symptoms and prolactin elevation, but may still be classified as an atypical antipsychotic, particularly at low doses
- Patients have very similar antipsychotic responses to any conventional antipsychotic, which is different from atypical antipsychotics where antipsychotic responses of individual patients can occasionally vary greatly from one atypical antipsychotic to another
- Patients with inadequate responses to atypical antipsychotics may benefit from a trial of augmentation with a conventional antipsychotic or switching to a conventional antipsychotic
- However, long-term polypharmacy with a combination of a conventional antipsychotic with an atypical antipsychotic may combine their side effects without clearly augmenting the efficacy of either
- Although a frequent practice by some prescribers, adding two conventional antipsychotics together has little rationale and may reduce tolerability without clearly enhancing efficacy

ANEXO 8

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Clinical Pharmacology, Clinical Efficacy, and Behavioral Toxicity of Alprazolam: A Review of the Literature

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Keywords: Alprazolam — Benzodiazepines — Anxiety — Depression — Panic — Sedation.

ABSTRACT

Alprazolam is a benzodiazepine derivative that is currently used in the treatment of generalized anxiety, panic attacks with or without agoraphobia, and depression. Alprazolam has a fast onset of symptom relief (within the first week); it is unlikely to produce dependency or abuse. No tolerance to its therapeutic effect has been reported. At discontinuation of alprazolam treatment, withdrawal and rebound symptoms are common. Hence, alprazolam discontinuation must be tapered.

An exhaustive review of the literature showed that alprazolam is significantly superior to placebo, and is at least equally effective in the relief of symptoms as tricyclic antidepressants (TCAs), such as imipramine. However, although alprazolam and imipramine are significantly more effective than placebo in the treatment of panic attacks, Selective Serotonin Reuptake Inhibitors (SSRIs) appear to be superior to either of the two drugs. Therefore, alprazolam is recommended as a second line treatment option, when SSRIs are not effective or well tolerated.

In addition to its therapeutic effects, alprazolam produces adverse effects, such as drowsiness and sedation. Since alprazolam is widely used, many clinical studies investigated its cognitive and psychomotor effects. It is evident from these studies that alprazolam may impair performance in a variety of skills in healthy volunteers as well as in patients. Since the majority of alprazolam users are outpatients, this behavioral impairment limits the safe use of alprazolam in patients routinely engaged in potentially dangerous daily activities, such as driving a car.

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INTRODUCTION

Alprazolam, 8-chloro-1-methyl-6-phenyl-4H-s-triazolo [4,3- α] (1,4) benzodiazepine, is a benzodiazepine derivative used in the treatment of a variety of disorders, including panic attacks, generalized anxiety, and depression. Since its introduction in the 1960s, alprazolam became one of the most used drugs in the USA and is currently the most often prescribed psychoactive drug.

The first section of this review article summarizes clinical pharmacology of alprazolam. In the second section its clinical use is discussed. The therapeutic efficacy of alprazolam is compared with the alternative pharmacological treatments that have been introduced over the years. The ideal drug should be clinically effective and should not produce adverse effects that interfere with daily activities, such as driving a car. Since the majority of alprazolam users are outpatients, many experimental studies have been conducted to investigate the behavioral effects of this drug. The results from these studies are summarized in the third section of this article. The fourth section discusses the future prospects for alprazolam.

CLINICAL PHARMACOLOGY

Alprazolam is a 1,4-triazolobenzodiazepine analog. The chemical structure of alprazolam differs from that of classical benzodiazepines such as diazepam by the presence of the triazole ring.

Greenblatt and colleagues studied pharmacokinetics of alprazolam during the 1980s and 1990s (108,109). At 1 to 2 h after administration (T_{max}) of a single 1 mg dose of alprazolam the peak plasma concentration (C_{max}) ranged between 12 and 22 $\mu\text{g/L}$, the volume of distribution (VD) from 0.8 to 1.3 L/kg, the elimination half-life ($t_{1/2}$) from 9 to 16 h and the clearance from 0.7 to 1.5 mL/min/kg. After oral administration, approximately 90% of alprazolam was absorbed and bound to plasma proteins (the fraction of unbound alprazolam is approximately 30%). The absorption rate was independent of the administered dose. However, plasma levels of alprazolam (and its metabolites) were linearly related to the administered dose (45). A steady state condition was reached after 2 to 3 days, independent of the dosage schedule.

Alprazolam is metabolized in the liver by hepatic microsomal oxidation. This process is mediated by cytochrome P4503A isoforms (CYP3A4). Up to now, 29 metabolites of alprazolam have been identified. Alprazolam's two principle metabolites, 4-hydroxy alprazolam and α -hydroxy alprazolam, are present at low plasma concentrations and show much less benzodiazepine receptor affinity than alprazolam. Hence, these metabolites are unlikely to contribute to the clinical effects of alprazolam. Approximately 80% of alprazolam is excreted by the kidney as unchanged drug.

Factors that Influence the Pharmacokinetics of Alprazolam

A comparison of pharmacokinetics of alprazolam (1 mg) after oral and sublingual routes in 12 healthy male volunteers (233) showed that peak plasma levels are reached significantly later after sublingual (2.8 h) than after oral administration (1.8 h). Other pharmacokinetic parameters do not differ significantly between these routes of adminis-

tration. Another study by the same author reported no significant differences (232). Pharmacokinetics after intravenous administration did not differ from that after oral administration, except for a faster T_{max} (0.48 h) after intravenous administration (250). The onset of alprazolam-induced sedation was more rapid after intravenous (10 to 20 min) than after oral administration (40 min).

The pharmacokinetics of alprazolam is not significantly influenced by gender (140, 150) or menstrual cycle (140,176). However, oral contraceptives have been reported to affect pharmacokinetics of alprazolam (151). In pregnant women alprazolam appears to increase the risk of congenital abnormalities when used during the first trimester (127). Alprazolam and 4-hydroxy-alprazolam have been found in breast milk (194). Pharmacokinetics of alprazolam in breast milk is similar to that in blood plasma of lactating women, suggesting that alprazolam readily passes the blood-milk barrier. Hence, alprazolam is classified as a pregnancy category D drug and lactating women are advised to stop breast-feeding if they are treated with alprazolam.

In elderly, peak plasma levels of alprazolam are higher than in young subjects. Furthermore, alprazolam clearance is reduced in older subjects resulting in a prolonged elimination half-life (104,106,182).

Alprazolam pharmacokinetics is similar in healthy volunteers and patients. In elderly depressed patients, pharmacokinetics of alprazolam did not change significantly from day 1 (starting dose of 0.5 mg) up to day 42 (mean daily dose of 1.6 mg). Metabolites of alprazolam do not accumulate after repeated dosing (63). In another study there was also no significant difference in the pharmacokinetics of alprazolam in healthy volunteers as compared to patients with panic disorder (134).

Since alprazolam is metabolized in the liver, patients with hepatic diseases should be carefully monitored when treated with the drug. The clearance of alprazolam is significantly reduced and elimination half-life significantly increased in patients with liver cirrhosis (131). Renal disease may also reduce alprazolam clearance leading to accumulation of alprazolam and its metabolites. Increased levels of free alprazolam have been reported in patients receiving either hemodialysis or Continuous Ambulatory Peritoneal Dialysis (CAPD) as compared to healthy controls (235). CAPD patients also showed a tendency to increased T_{max} , clearance and prolonged elimination half-life of alprazolam. Metabolism is significantly slowed by obesity (1), whereas smoking has been reported to accelerate alprazolam elimination (123,197,251). No significant changes in the pharmacokinetics of either drug have been observed during co-administration of alprazolam and buspirone to healthy male volunteers (35).

Drugs that inhibit cytochrome P450 enzymes are likely to alter the pharmacokinetics of alprazolam. Inhibitors of CYP3A4 may increase elimination half-life and elevate peak plasma concentrations of this drug, whereas other drugs may enhance alprazolam metabolism. Changes in the pharmacokinetics of alprazolam have been produced by drugs that alter CYP3A4 metabolism. They have been reported for erythromycin (285), ketoconazole (111), itraconazole (286), venlafaxine (3), nefazodone (112,154), fluoxetine (107, 160), fuvoxamine (91), carbamazepine (97), cimetidine (1,207), and propoxyphene (2). However, other substrates for CYP3A4 such as grapefruit juice (287) and sertindole (281) did not significantly alter alprazolam metabolism.

Because immediate release tablets are clinically effective for 3 to 6 h, alprazolam must be administered several times during the day. This can result in fluctuations in plasma levels, which may affect clinical efficacy. Sustained release (SR) formulations reduce

interdose variability in plasma levels of alprazolam and its adverse effects (5). Hence, SR formulations may improve treatment compliance. SR formulations have a considerably lower absorption rate, resulting in peak plasma concentrations that are half those observed with immediate-release formulation of alprazolam and a $T_{\max}$ of 8 to 12 h (38). Therefore, it has been suggested that sedative effects of alprazolam SR may be less pronounced than those with the immediate release tablets. A study on the pharmacokinetics of alprazolam SR at single doses of 2, 4, 8, and 10 mg reported a linear increment in peak plasma concentrations of alprazolam and its metabolites with increasing doses (283). Anxious patients, who were switched from immediate release tablets (first 2 weeks) to extended release alprazolam tablets (second 2 weeks), showed gradual clinical improvement during the study. However, patients reported a significant increase in sedation and anxiety symptoms during the first days after switching (271).

MECHANISM OF ACTION

Alprazolam easily crosses the blood brain barrier and enters central nervous system (CNS). Although the exact mechanism of action of benzodiazepines is unknown, alprazolam binds nonselectively to the gamma-amino butyric acid_A (GABA_A)-benzodiazepine receptor complex. Most GABA_A receptors are composed of three classes of subunits with several variants (α_{1-6} , β_{1-3} , γ_{1-3}). Benzodiazepine receptors can be differentiated based upon their subunit structure: type 1 receptors are composed of $\alpha_1\beta_{1-3}\gamma_2$ subunits whereas type 2 receptors have $\alpha_{2,3,5}\beta_{1-3}\gamma_2$ subunits. The functional significance of these subunits is still under investigation, but it has been established that sedation and anterograde amnesia are mediated by α_1 subunits, whereas α_2 subunits mediate anxiolytic effects (59,65,226, 231). Benzodiazepine α_1 subunits are found in high density in the cerebellum and in low density in hippocampus. Benzodiazepine α_2 subunits are abundantly present in hippocampus, striatum, and spinal cord.

At the receptor complex, alprazolam facilitates the binding of GABA and increases the influx of chloride ions. The presence of GABA, in turn, inhibits the action of several connected brain structures. It is, however, difficult to limit the action to "therapeutic meaningful" sites of action: GABA binds nonselectively to more than 30% of all brain synapses. The inhibition exerted by GABA results in a general slowing of brain activity, known as sedation. Further, the GABA system interacts with other neurotransmitter systems, including noradrenergic, serotonergic, cholinergic, and opioidergic systems. Especially alprazolam's interactions with the serotonergic and noradrenergic pathways to the limbic system and brain stem structures (e.g., locus coeruleus) contribute to its clinical effectiveness in the treatment of anxiety and depression.

CLINICAL EFFICACY OF ALPRAZOLAM

Generalized Anxiety Disorder (GAD)

During the 1980s and 1990s a number of studies investigated the efficiency of alprazolam in the treatment of GAD. The efficacy of alprazolam during 4 to 8 weeks-long periods of therapy was determined by means of anxiety symptom scales such as the Hamilton

Rating Scale for Anxiety (HAM-A). The average alprazolam dosage used in GAD ranged from 0.5 to 3.0 mg. Relative to placebo, most studies reported significant clinical improvement during alprazolam treatment (4,49,61,62,75,83,170,171,177,181,213). However, some studies failed to demonstrate any significant difference between alprazolam and placebo (40,95). This was probably caused by a significant clinical improvement during placebo treatment. As in panic disorder studies, global improvement rates of 50% and higher after placebo treatment has been reported in GAD patients.

Alprazolam has been shown to be at least equally effective in the treatment of GAD as other benzodiazepines, such as oxazepam (218,272), diazepam (4,49,61,62,71,83,95,171,213), lorazepam (50,51,119,269), clobazam (40,118), etizolam (19,30,200), bromazepam (19), and the partial benzodiazepine agonist abecarnil (170). Alprazolam has also been shown to be at least equally effective as TCAs, such as imipramine (121,178). Alprazolam was more effective than imipramine in the relief of somatic symptoms, whereas imipramine had a positive effect on psychic symptoms such as dysphoria and negative anticipatory thinking.

In a 6-week double-blind placebo-controlled study in 94 outpatients with GAD (75) alprazolam (1.9 mg daily) was equally effective as buspirone (18.7 mg daily). However, the onset of clinical improvement was much faster with alprazolam (within the first week) than with buspirone (gradual improvement over weeks). Another study reported that alprazolam was at least equally as effective as buspirone in the treatment of anxious outpatients (51). Buspirone produced significantly fewer adverse effects when compared to alprazolam. However, dropout rate during buspirone treatment was rather high, since the therapeutic effect was apparent only at 2 to 4 weeks after the start of therapy.

Alprazolam was found to be as effective as diazepam in the treatment of moderate to severe anxiety during alcohol withdrawal in alcoholics (145). Also, 98% of GAD patients with comorbid irritable bowel syndrome showed a significant reduction in anxiety and 89% reported a reduction of gastrointestinal complaints after 4 weeks of alprazolam therapy. These benefits were maintained during a 4-week tapering period and were also present at 4 weeks after discontinuation of therapy (268). In the treatment of anxiety associated with cancer alprazolam has been reported to produce significant improvement that was evident within one week of treatment and was maintained thereafter. However, in similar studies clinical improvement was also achieved with muscle relaxants (122) and even with placebo (276).

Panic Disorder (PD)

During the eighties two large-scale international multi-center studies, the first and second Cross-National Collaborative Panic Study (CNCPS I and II), were initiated to examine the efficacy of alprazolam in the treatment of PD. The first CNCPS was a multi-center study performed in the USA, Canada, and Australia (11,142,165,190,203). Patients with PD or agoraphobia with panic attacks (Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, DSM III, criteria) were included. Patients received either placebo or alprazolam for 8 weeks. Daily dosages were increased to reach 6 mg alprazolam after 3 weeks of treatment. The mean ($\pm$ SD) daily alprazolam dose was 4.2 ± 1.1 mg after 3 weeks and 5.7 ± 2.3 mg after 8 weeks of treatment. Results showed that after 4 weeks alprazolam was significantly more effective than placebo. However, after 8 weeks the dif-

ference between alprazolam and placebo was only moderate and all therapeutic gains disappeared after discontinuation of alprazolam.

Clinical improvement was maximal when alprazolam plasma levels ranged from 20 to 40 ng/mL (110). However, plasma levels and corresponding treatment responses showed great individual differences and there was no linear relationship between plasma levels and clinical improvement (280). Therefore, individual dose adjustments are necessary to gain optimal treatment benefits with alprazolam (166).

With few exceptions (99,270), other placebo-controlled studies also reported clinical superiority of alprazolam as compared to placebo (48,68,162,169,186,238,266,267). However, there were high rates of noncompliance in the placebo groups. This was also a problematic issue in the first CNCPS trial when approximately 50% of patients that received placebo dropped out of the study between weeks 4 and 8. It is likely that a large number of noncompleters affected the study outcome. Hence, the results from the first CNCPS have been a matter of debate (172). Several relatively small studies compared the efficacy of alprazolam with that of other benzodiazepines. For example, double-blind studies showed that alprazolam was at least as effective in the treatment of PD as diazepam (68,192,278), etizolam (179), clonazepam (266,267), adinazolam (208), or lorazepam (43,237). Alprazolam was also superior to non-benzodiazepine drugs such as propranolol (186,209). Unlike alprazolam, trazodone (42) and ibuprofen (243) were ineffective.

The traditional treatment in PD is imipramine, a TCA. The largest study comparing the effects of imipramine and alprazolam versus placebo was the second CNCPS (55). A total of 1168 PD patients were randomly assigned to one of the treatment groups. During an 8-week, double blind treatment period, clinical changes were recorded. The onset of clinical improvement was faster in the alprazolam group (week 1) as compared to the imipramine group (week 4). After 8 weeks, both drugs were equally effective, and both were superior to placebo. The average daily dose was 5.7 mg of alprazolam and 155 mg of imipramine. The number of patients that did not complete the study was considerably higher in the placebo (43%) and imipramine groups (30.2%) than in the alprazolam group (17.4%).

Several smaller double-blind studies compared the efficacy of alprazolam (at doses ranging from 2 to 6 mg) with that of imipramine (at doses ranging from 100 to 150 mg); the treatment periods in these trials ranged from 8 to 12 weeks (42,48,58,162,163,219,245,265,270). In line with the CNCPS, these studies consistently showed that in the treatment of PD alprazolam was at least as effective as imipramine. Again, the onset of the therapeutic effect of alprazolam (week 1) was significantly faster than that of imipramine (weeks 2 to 4). Symptoms of the disease often exacerbated during the first weeks of therapy with imipramine. Due to its anticholinergic side effects, imipramine was not well tolerated by a substantial number of patients. As a result, dropout rates in patients receiving imipramine were higher than with alprazolam.

Long-term (8 months) treatment of PD patients with alprazolam showed that clinical improvement was maintained without significant dose changes. However, the number of completers in patients receiving the active drug was twice that in the placebo group. At 1.5 years after participation in a 6-week clinical trial (267) 78% of PD patients continued using alprazolam without dosage increments and most of these patients continued to benefit from long-term therapy with alprazolam (205). Long-term efficacy of alprazolam was also reported in other studies (135,136,216,239,246). Nevertheless, many patients still had occasional panic attacks or agoraphobia. Hence, long-term therapy with alprazolam does not cure panic patients, but only reduces the symptoms of PD.

Klosko and colleagues (143) treated patients with PD over a period of 15 weeks with alprazolam (mean dose, 4.6 mg), behavioral therapy, or placebo; one group of patients received no treatment at all. These authors reported that behavioral therapy was significantly more effective than placebo or no treatment. Although in both groups of patients clinical improvement was observed, there was no significant difference in the improvement of alprazolam- and placebo-treated patients. In 1992, a meta-analysis comparing the effectiveness of treatments in PD with agoraphobia revealed that alprazolam was effective for the treatment of panic and anxiety symptoms, whereas behavioral treatment (exposure therapy) was significantly more effective for phobia (53).

The London/Toronto study in 154 PD patients with agoraphobia compared the effects of combined treatment with alprazolam (5 mg/day) and exposure therapy, alprazolam and relaxation therapy (a psychological placebo), placebo and exposure therapy, and placebo and relaxation therapy (173). Patients received 8 weeks of treatment, followed by a tapering period of 8 to 16 weeks and follow-up visits until week 43. Unlike in the CNCS studies, placebo dropout percentage in the London/Toronto study was low. Clinical improvement was shown in all four groups, but the onset was faster with alprazolam than with exposure therapy. In contrast to the results from the 1992 meta-analysis (53), exposure therapy was significantly more effective than alprazolam and placebo. Improvement after alprazolam did not differ significantly from that after placebo. Thus, regarding clinical efficacy, the advantage of alprazolam or exposure therapy in the treatment of PD is not yet obvious.

The improvement with the combination of alprazolam and exposure therapy was not significantly greater than after exposure therapy combined with placebo. Furthermore, in contrast to those patients receiving exposure therapy, sedation was reported up to week 8 during alprazolam treatment. Taking into account these adverse effects, exposure therapy may be regarded more favorably than treatment with alprazolam.

An 8-week double-blind study in 92 PD patients showed that alprazolam was significantly superior to buspirone or placebo. Moreover, buspirone was not superior to placebo in the treatment of PD (244).

Selective Serotonin Reuptake Inhibitors (SSRIs) were introduced more recently as an alternative treatment option for PD. Unfortunately, up to now, no direct comparison of the therapeutic efficacy of alprazolam and any of the SSRIs has been made. A meta-analysis of 27 clinical studies in PD patients showed that SSRIs (including paroxetine, fluvoxamine, zimelidine and clomipramine) were significantly superior ($p < 0.0004$) to alprazolam (mean dosage of 4 mg/day), imipramine (mean dosage of 150 mg/day) or placebo (29). Although there was a significant relationship between dose and the magnitude of the effect for either alprazolam or imipramine, SSRIs were also superior to higher dosages of alprazolam and imipramine. There was no significant difference between imipramine and alprazolam ($p < 0.11$). Nevertheless, both treatments were significantly superior to placebo in alleviating panic symptoms. Taken together, these results suggest that SSRIs should be viewed as the first-line treatment of panic disorder. Alprazolam may be used as a second-line treatment, if SSRIs prove to be ineffective or are not tolerated well.

Depression

Although alprazolam use has been associated with suicide ideation, a review of the literature (149) and a meta-analysis of 22 studies in depressed patients (130) showed that al-

prazolam use is not associated with an increased risk of suicide. Moreover, in contrast to other benzodiazepines, alprazolam is known for its antidepressant properties.

First-line treatments in depression are tricyclic antidepressants (TCAs). Clinical studies in depressed patients showed that alprazolam is at least equally effective as TCAs, such as amitriptyline (13,66,76,126,157,249), imipramine (84,86,87,180,212,277), dosulepine (41), desipramine (85,210), or doxepin (9). However, other studies reported that, although alprazolam provided symptom relief, amitriptyline (124,159,227) and imipramine (103,161) were significantly more effective. Analyses of early dropouts revealed that whether alprazolam or TCAs are the most favorable treatment choice depends on the diagnosis of depression (mild versus severe) (199). Early dropouts were most frequently observed during imipramine treatment (in patients with severe depression). Imipramine, however, seems to be more efficacious in the long run in patients that remain in treatment (patients with mild depression). Patients with severe depression seem to benefit most from alprazolam treatment. Meta-analyses confirmed that alprazolam is significantly superior to placebo and is as effective as TCAs (204,256).

Premenstrual Dysphoric Disorder (PDD)

Most women experience mild to moderate premenstrual symptoms during their lifetime, but 3 to 5% of women experience a severe syndrome, referred to in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) as Premenstrual Dysphoric Disorder or PDD (146). Double blind placebo-controlled studies in women with PDD showed that alprazolam caused improvement during the luteal phase, but had no postmenstrual effects (64,115,253). Other studies reported, however, no significant improvement with alprazolam as compared to placebo in any phase of the menstrual cycle (234). Mixed results, depending on the presence of mild anxiety or depression, were reported for alprazolam, 0.75 mg/day. (18). Conflicting reports are not surprising, since in the treatment of PDD the placebo effect is rather high, providing at least 50% symptom relief in 20 to 60% of patients (93), independent of the time spent with the physician (94).

Studies with SSRIs have shown that drugs such as fluoxetine (201,257,258) or sertraline (288,289) are highly effective in the treatment of PDD, without producing adverse CNS effects of alprazolam. Hence, SSRIs are recommended as the first-line treatment strategy in PDD. Alprazolam may be considered in case SSRIs do not provide sufficient relief.

Other Therapeutic Areas

A placebo-controlled study investigated the effects of alprazolam (0.5 to 1.5 mg) on tinnitus over a 12-week period (128). In thirteen out of 17 patients suffering from tinnitus alprazolam, 1.5 mg/day reduced the intensity of tinnitus, while at 0.5 mg alprazolam was effective in only 4 patients. It appears that more crossover studies are needed to confirm the effectiveness of alprazolam (125,248).

Alprazolam has also been examined in the treatment of Post Traumatic Stress Disorder (PTSD), but found to be ineffective. Although improvement in anxiety related symptoms was evident, specific PTSD symptoms remained unimproved (32).

In flight phobia, a single dose of alprazolam, 1 mg, (but not placebo) significantly reduced anxiety but increased heart rate and respiratory rate during flight. Patients treated

with alprazolam during one flight expected to be less anxious on future flights. This expectation did not, however, materialize. If the patients were not medicated, rebound anxiety, panic attacks and physiological responses were observed during the second flight (279).

In a more recent study six patients suffering from seasonal affective disorder were treated successfully with alprazolam, 1.2 to 2.4 mg/day for 2 weeks (284). Since the number of patients in this study was limited, the results will have to be confirmed by future placebo-controlled and active treatment-controlled (light therapy) studies. When co-administered with haloperidol in the treatment of schizophrenia, alprazolam reduced symptoms of excitement and uncooperativeness in 28 psychotic patients (15). Alprazolam has also been reported to be effective in the treatment of primary fibrositis/fibromyalgia syndrome when co-administered with ibuprofen (229). Its effectiveness has been described in essential tremor (113), social phobia (99), and in patients with disruptive behavioral episodes (44).

Adverse Events

A post-marketing study in the UK, involving almost 10,000 patients treated with alprazolam, reported no serious adverse events. The most commonly reported adverse events were drowsiness and sedation (70). A literature review including 3574 patients that participated in the active drug-controlled studies reported similar results (129).

In the London/Toronto study significantly more adverse effects were reported after alprazolam than after placebo (195). Generally, adverse effects diminished during treatment, but were still present at the end of the study. Analysis of the number of adverse events reported in the second CNCPS showed marked differences between the treatments (39). Patients using alprazolam reported an increment in typical adverse effects of benzodiazepines, such as sedation, fatigue, weakness, memory problems, ataxia and slurred speech, whereas patients using imipramine reported an increment in anticholinergic adverse effects such as blurred vision, tachycardia, palpitations, insomnia, nervousness, malaise, dizziness, headache, nausea, vomiting, and decreased appetite. In patients that received placebo anxiety symptoms were the most reported adverse events. In total, alprazolam produced less adverse effects than either imipramine or placebo. The number of adverse events decreased over time with either of the treatments. Clinical improvement was not correlated with either number or severity of adverse effects.

Withdrawal and Rebound Effects after Discontinuation of Therapy

Discontinuation of daily alprazolam use is preferably tapered to reduce rebound symptoms. During tapering over a period of several weeks or months the daily dosage is gradually decreased to zero.

In the London/Toronto study, the therapeutic benefits from alprazolam were lost during tapering and follow-up, whereas the gains from exposure therapy continued. The results from this study enhanced the debate on alprazolam's efficacy in the treatment of PD (173,174,175,254). With few exceptions (164), other studies also showed that treatment of PD with benzodiazepines is followed by withdrawal and rebound symptoms (anxiety scores higher than baseline), especially if the drugs are discontinued abruptly. High rates (50% or more) of symptomatic withdrawal and rebound effects have been reported

after 4 to 8 weeks of successful treatment with alprazolam, followed by a rather short (several weeks) tapering period (69,202,215,239). However, also after much longer tapering periods rebound effects were still present in a substantial number of patients. Rebound anxiety has been often reported with the dose reduction from 1 to 0 mg. Hence, a slower tapering period from 1 to 0 mg (in gradually lower dosages over several weeks) seems necessary to avoid the occurrence of rebound symptoms. In this context, it is important to note that an effective tapering period could last longer than the duration of the actual alprazolam treatment.

In contrast, discontinuation effects are not observed after buspirone (214), imipramine (215), SSRIs, or cognitive-behavioral therapy (173). Withdrawal and rebound effects during alprazolam tapering are not reduced by concurrent therapy with a non-benzodiazepine, such as the 5-HT₃ receptor antagonist ondansetron (224). Combining alprazolam tapering with cognitive behavioral therapy reduces withdrawal symptoms and thus facilitates alprazolam discontinuation (198,255).

Frequency of panic attacks or of episodes of phobia before the start of therapy did not predict recurrent pathology after long-term (4 years) treatment with alprazolam (136). However, other studies showed that baseline levels of anxiety can predict treatment outcome. Age over 40, low levels of baseline anxiety (282), or reduced anxiety and panic symptoms during treatment (33) correlated with less rebound and withdrawal effects. The severity of rebound phenomenon differs among benzodiazepines and seems to be related to the half-life of the drug. For example, rebound and withdrawal effects after alprazolam are more severe than after diazepam, which has a much longer half-life (191).

Dependence, Addiction, and Abuse Liability

In contrast to general beliefs, and taking into account a huge number of prescriptions for alprazolam, the incidence of abuse and dependence in the patient population is very low (221,223,241). However, like other benzodiazepines, alprazolam is abused for non-medical reasons, often in combination with alcohol, drugs of abuse and/or other prescription medications. Alprazolam is used by drug addicts and has become a popular party drug during the last decade.

Mood changes associated with abuse liability in subjects with a history of drug abuse have been assessed with the Addiction Research Center Inventory (ARCI). The following factors are assessed by the ARCI questionnaire: euphoria, dysphoria, sedation, intellectual efficacy and energy, and activation. Studies have consistently shown that the use of alprazolam is accompanied by a dose-dependent sedation (37,77,78,183,184,185,228,291) and euphoria (46,47,185,291). Especially the euphoric effects of alprazolam may reinforce usage of the drug by subjects with a history of drug abuse. In contrast, in healthy volunteers and patients the sedative effects of alprazolam are more pronounced. Decreased intellectual efficacy and energy (185,291) and increased dysphoria (37) have been reported. It has been shown that patients' tendency to consume drugs more frequently predicts dependence to a much greater extent than the preference for alprazolam (drug liking) (196). Thus, the simple fact of increased "drug liking" or euphoria does not correlate well with the risk for alprazolam abuse.

BEHAVIORAL EFFECTS

Brain Activity

Cerebral blood flow (CBF) and brain activation were found to be decreased after alprazolam. By intravenous administration alprazolam caused an acute decrease in whole-brain CBF of 25 to 30% (225). More specifically, cerebral blood flow was significantly decreased (>15%) in the left lateral superior and right medial inferior frontal areas as well as in the cingulate cortex (259).

The decrease in brain activity after administration of alprazolam has also been demonstrated by studies recording event-related potentials. For example, significantly reduced N1 and P1 amplitudes have been found after alprazolam administration (6,25,242), reflecting the inhibitory activity of alprazolam. Significantly decreased P300 amplitude and a prolonged P300 latency have also been shown (187,242). The reduction of P300 amplitude reflects a worsening of cognitive functioning, reduction in information processing, and the quality of processing. The prolonged P300 latency implicates a prolonged stimulus evaluation time. In line with these changes in P300 activity, performance on a reaction speed test was significantly impaired. In this context, it has been shown that the P300 correlates highly to alprazolam's effects on a choice reaction time (117).

Acute Effects of Alprazolam in Healthy Volunteers

Psychomotor performance

The majority of behavioral studies were performed in healthy volunteers after a single dose of alprazolam. Table 1 provides an overview of the results from these studies.

The doses of alprazolam in these studies ranged from 0.25 to 2.0 mg, reflecting those used in clinical practice. Studies in Table 1 are double blind and placebo-controlled. They were designed as crossover or comparison group trials. Acute administration of alprazolam led to a general slowing of performance expressed in increased reaction speed on the tests. The performance impairing effects are significantly different from placebo at doses of 0.5 mg and higher. Performance impairment is not limited to specific skills or abilities. The effects of alprazolam are evident in all tests.

Some remarks must be made regarding the clinical relevance of the findings summarized in Table 1. That is, several of the studies were performed with a limited number of subjects. Hence, the results have low statistical power and their reliability is questionable. Furthermore, most tests do not represent daily activities. For example, the Digit Symbol

TABLE 1. Psychomotor performance after acute administration of alprazolam

Alprazolam dosage (mg)	0.25	0.50	0.75	1.0	1.5	2.0
% of tests showing impairment	13.0	28.6	66.7	84.2	92.9	75.0
Total number of tests	23	28	15	38	14	12

Data are from references 10,17,22-25,54,60,72-74,78,89,92,105,114,116,120,133,137,147,155,160,167,187-189,206,211,228,230,233,240,250,261,263,273-274,283. Tests included reaction time tests, tapping, cancellation, tracking, DSST, symbol copying, CFFT, card sorting, vigilance, stroop, and balance tests. A detailed summary of study results can be obtained upon request from the authors.

Substitution Test (DSST), Cancellation Test (CT), and Symbol Copying Test (SCT) are very popular in human psychopharmacology. Their popularity is probably due to the fact that they are cheap, easy to apply, and of short duration (1 to 3 min). Their results are readily obtained by counting the number of correct digit-symbol pairs (DSST), cancelled items (CT), or copied symbols (SCT). Unfortunately, these tests have no clear theoretical background. That is, it is unclear what is actually measured by these tests, and as a result interpretation of the data differs greatly among investigators. Tests such as the DSST measure several skills and abilities simultaneously resulting in a global measure of response (98), which in turn cannot be related easily to distinct brain regions or specific neurotransmitter systems.

Performance on these tests is also very sensitive to learning effects. Despite exhaustive training sessions, significant performance improvement on subsequent test sessions can be observed. Unfortunately, learning effects are not always taken into account and other studies do not report whether subjects were trained to a baseline performance level before the start of the study.

Whereas it is likely that performance decrement in short cognitive and performance tests (usually 5 to 10 min) can be compensated by increased effort and alertness during test performance, this is generally not true in tests of longer duration (>30 min). In this context, performance of daily activities, such as highway driving, requires sustained attention over a long period of time (vigilance). Benzodiazepines are known to affect vigilance performance by producing gradual performance decrement (144). At 0.5 mg alprazolam impaired performance in a 60 minutes vigilance test (147,148) and at 0.8 mg it reduced speed and accuracy in a 20 min visual vigilance test at 3.5 h after its administration (263).

As a consequence of the limitations observed in the majority of studies summarized in Table 1, one should be cautioned about making judgments regarding real-life performance based upon results from psychomotor test batteries. This is particularly true since daily activities often represent overlearned skills or abilities, whereas laboratory tests are more often of an artificial nature and are not practiced on a daily basis. In addition, daily activities such as driving a car are examples of complex behavior. Individually tested skills and abilities related to driving may be unimpaired, whereas results on a driving test may show significant impairment. Also, the severity of impairment found in laboratory tests does not predict to what extent daily activities will be impaired. For example, in a study which showed both significant impairment in laboratory tests and in actual driving performance after acute alprazolam treatment, a comparative analysis showed that results of laboratory tests measuring skills and abilities related to driving do not accurately predict actual driving performance in normal traffic (274).

Driving ability

Driving is an example of complex, skilled behavior practiced on a daily basis. Epidemiological evidence showed that benzodiazepine users have an increased risk of traffic accidents (16). A review showed that benzodiazepine hypnotics significantly impaired driving ability during normal traffic the day following bedtime administration (275). Although the effect of benzodiazepines on driving performance depends on various factors, such as the dose of the drug, time after drug intake, or gender, driving impairment due to benzodiazepines is generally higher than observed with blood alcohol concentrations of 0.05% (the legal limit for driving in The Netherlands).

Recently, Verster and colleagues examined the acute effects of alprazolam (1 mg) on driving ability (274). Twenty healthy volunteers performed an on-the-road driving test during normal traffic, one hour after treatment administration. Subjects were instructed to drive with a steady lateral position and a constant speed (60 mph) over a 100 km highway circuit. The Standard Deviation of Lateral Position (SDLP, cm) and speed variability (SD Speed, km/h) were the primary and secondary parameters of the test, expressing the amount of vehicle control of the subjects. Driving performance was severely impaired after alprazolam. That is, performance on the test was characterized by significantly increased side-to-side weaving of the car, repeated excursions out-of-lane into both the adjacent traffic lane and the road shoulder and significantly increased speed variability. Hence, vehicle control was seriously affected. SDLP increment after alprazolam (1 mg) was equal to that observed after blood alcohol levels of 0.15%, clearly above the legal limit of driving, and corresponding to a significantly increased traffic accident risk. Moreover, after alprazolam six out of twenty subjects had to stop their driving test before completion, because they fell asleep behind the steering wheel. In conclusion, driving is unsafe after acute administration of alprazolam.

Effect on memory

Brain structures involved in memory processes (cerebellum, hippocampus, amygdala and cerebral cortex) have a high affinity for alprazolam, due to the abundant presence of GABA_A benzodiazepine receptor complexes. Several reviews concerning the effects of benzodiazepines on memory have pointed to the dose-related impairment of acquisition (56,100) and retrieval (262) of newly learned information, whereas previous learned information (i.e., before drug administration) remains intact. The results from studies with alprazolam are summarized in Table 2. The effects of alprazolam were similar to those of other benzodiazepines.

Immediate and delayed recall, and recognition are impaired by alprazolam at doses of 0.5 mg and higher. In contrast, working memory is not significantly affected after administration of this drug. Although studies using the Sternberg Memory Scanning Test, Digit Span and Mental Arithmetic showed significant impairment, this impairment involves reaction speed parameters, and not the accuracy of test performance. Hence, impairments in these tests do not indicate an effect on memory processes, but suggest only slower responsiveness (reaction speed) after alprazolam.

TABLE 2. Memory functioning after acute administration of alprazolam

Alprazolam dosage (mg)	0.25	0.50	0.75	1.0	1.5	2.0
% of tests showing impairment	11.8	36.4	53.3	75.0	85.7	100.0
Total number of tests	17	22	15	24	7	2

Data from references 14,22,23,52,60,92,105,114,116,120,137,155,167,187,206,228,230,233,260, 263,273-274. Tests included immediate and delayed recall, recognition, digit span, arithmetics, Sternberg memory scanning, and story recall. A detailed summary of study results can be obtained upon request from the authors.

Dose dependency of the effects

It is evident from Tables 1 and 2 that with increased alprazolam blood levels impairment in memory and psychomotor tests are reported more often. Also, the impairment is more severe with higher doses of the drug. Behavioral and adverse effects of alprazolam are less pronounced after small and more frequent dosing (252).

Interestingly, Bourin and colleagues (28) reported that alprazolam at a low dose (0.125 mg b.i.d. for 14 days) improved performance in DSST, picture recall, choice reaction time test and the Critical Flicker Fusion Test (CFFT). The authors reported that the test performance was trained to baseline before the start of the study. The placebo group showed less improvement in the performance than the alprazolam group. However, the clinical relevance of the effects of alprazolam at such a low dose is questionable.

Routes of administration

By either oral or sublingual routes of administration alprazolam produced similar impairment in the DSST, a reaction time test, and in immediate and delayed recall tests (233). DSST and performance on a perceptual speed test, measured at 1.25 to 12.5 h after drug administration, were equally affected by alprazolam, by either oral or intravenous routes (250). These data suggest that alprazolam impairs performance independent of its route of administration. However, due to the differences in the pharmacokinetic parameters the time of maximal effects of the drug may vary substantially with the route of administration.

Interactions with alcohol

Interactions between alprazolam and alcohol are important to address since many patients with anxiety or panic disorder self-medicate with alcohol. Bond and colleagues (24) examined the acute effects of alprazolam (1 mg) and alcohol (0.5 g/kg) alone and in combination in a variety of psychological tests. Alprazolam caused significant impairment in all tests. The impairment after alcohol was less pronounced. The effects of both drugs administered simultaneously were not greater than predicted from the sum of the single dose effects, suggesting no interaction (25). With higher doses of alcohol, impairment in combination with alprazolam was greater than with either drug alone (167,228). Alprazolam and alcohol combination increased behavioral aggression and irritability in moderately drinking healthy volunteers and in patients with panic disorder more than would have been predicted from the sum of single effects (26,27).

Interactions with CYP3A4 inhibitors

Erythromycin did not modify the acute effects of alprazolam (0.8 mg) in DSST performance (285). DSST performance worsened significantly when alprazolam was co-administered with ketoconazole, but immediate and delayed word recall test after alprazolam was not affected by ketoconazole (111). Inhibition of alprazolam metabolism by itraconazole significantly impaired performance in the DSST (286). DSST and symbol copying were not affected by alprazolam and venlafaxine combined (3). Test performance (DSST and RT tasks) after alprazolam are worsened by drugs that alter CYP3A4 metabolism, including nefazodone (112,154), fluoxetine (107,160), fluvoxamine (91), carbamazepine (97), cimetidine (1,207), and propoxyphene (2).

Acute tolerance

It has been shown that after a single dose of benzodiazepines, including alprazolam, psychomotor impairment in simple tests such as the DSST, symbol copying and tracking is greater during the absorption phase than at a later time point after acute administration, even if blood levels of a drug are similar (72,73,74,105,152,155,252,283). This effect, called proteresis, suggests the development of acute tolerance and many authors support this conclusion. However, because these tests are known to be very sensitive to learning and practice effects the "tolerance" may also reflect a practice effect.

Repeated Administration to Healthy Volunteers

The effects of alprazolam by chronic administration were initially studied in healthy volunteers. Tables 3 and 4 summarize the results of these studies.

It is evident from Tables 3 and 4 that after 1 to 3 weeks of daily administration of alprazolam, the drug produced no significant impairment in most of the tests. In contrast, Tables 1 and 2 show that in the same tests a single dose of alprazolam produced significant impairment of performance. Hence, many authors concluded that tolerance to the performance impairing effects of alprazolam was evident. However, as discussed earlier, learning effects can also cause the absence of impairing effects in these tests.

Fleishaker and colleagues (90) reported that sedation scores after three days of therapy with alprazolam were independent of the dose of the drug (1, 3, or 6 mg daily, sustained release) and were lower than after a single dose of alprazolam. Alprazolam's blood levels after 3 days of therapy were, however, 1.5 times higher than after a single dose of the drug.

This finding may be explained by the results of a study that measured changes in GABA_A benzodiazepine receptor density with single photon emission computed tomography. During 24 days of daily alprazolam administration (2 mg) to healthy volunteers receptor density decreased (96). At the baseline, and at 3, 10, 17, and 24 days after the start of therapy, sedation was measured and the Hopkins verbal learning test was per-

TABLE 3. Psychomotor performance after repeated administration of alprazolam

Alprazolam dosage (mg)	0.25	0.50	0.75	1.0	1.5	2.0
% of tests showing impairment	0.0	0.0	14.3	0.0	0.0	66.7
Total number of tests	3	3	7	7	4	3

Data from references 10,28,116,132,156,160,206,240,252,261. Tests included reaction time tests, DSST, symbol copying, card sorting, tracking, vigilance, and the CFFT. A detailed summary of study results can be obtained upon request from the authors.

TABLE 4. Memory functioning after repeated administration of alprazolam

Alprazolam dosage (mg)	0.25	0.50	0.75	1.0	1.5	2.0
% of tests showing impairment	0.0	0.0	0.0	0.0	50.0	0.0
Total number of tests	2	2	2	2	2	2

Data from references 28, 96,116,156,206. Tests included immediate and delayed recall, and recognition. A detailed summary of study results can be obtained upon request from the authors.

formed. Results were compared with GABA_A receptor density. Sedation was associated with 16% receptor occupancy, but tolerance developed on day 17. This rather low occupancy rate had significant performance effects. After 3 and 10 days of therapy delayed recall was significantly worse as compared to baseline. After 24 days, delayed recall performance returned to the baseline performance level, suggesting the development of tolerance. Immediate recall was unaffected at all measurements. Correlation between tolerance effects in sedation and memory tests and receptor density was poor, suggesting the involvement of other mechanisms.

In this context, it is important to note that tolerance lasts only as long as drug therapy is continued on a daily basis. Patients requiring chronic drug treatment comply poorly with treatment instructions (21) and often use the drug only if needed, use other medication concurrently, or change dosages depending on their health complaints (222). In such instances, tolerance to the impairing effects of alprazolam will not develop easily, and patients are still at risk that behavioral impairments will compromise their daily activities.

Gender

None of the behavioral studies summarized in Tables 1 to 4 reported significant gender differences in the test performance. Also, menstrual cycle phases did not affect performance during alprazolam therapy. Progesterone did not affect performance in alprazolam-treated patients. DSST and card sorting test were equally impaired during the luteal and follicular menstrual cycle phases (176). However, women using oral contraceptives were more sensitive to alprazolam-induced impairment in the DSST, an aiming test, and a reaction time test. The increased impairment did not, however, correlate well with the pharmacokinetic parameters (151).

Age

Elderly

Old age is associated with an increased sensitivity to pharmacodynamic and behavioral effects of benzodiazepines. That is, at a given alprazolam plasma concentration performance will be more impaired in older than in young subjects. However, the relationship between pharmacokinetic differences and behavioral effects does not always correlate well, and some studies failed to find pharmacokinetic and pharmacodynamic differences between young and old subjects (133). In a study comparing young and elderly healthy volunteers, the elderly showed a longer duration of performance impairment, as measured by DSST and tracking tests (188). The difference between young and elderly subjects was more pronounced with increased task difficulty. By intravenous administration to young and elderly healthy volunteers alprazolam had a significant sedative effect. Elderly subjects were more impaired in the card sorting test and DSST, but there was no significant difference in the performance of elderly and younger subjects in the cancellation as well as in the immediate and delayed recall tests (20).

Children

Alprazolam is not frequently prescribed to children and behavioral studies in the pediatric population are scarce. In an open trial with alprazolam (88) twelve children with overanxious disorders received placebo (week 1), alprazolam (4 weeks), and post-drug

placebo treatment (2 weeks). Children were subjected to a continuous performance task, a paired associate learning test, a semantic word processing task, and a test to assess visuo-motor performance. No significant performance decrement was reported during alprazolam treatment. On the contrary, performance improved in most tests, probably due to practice effect.

In another study the effects of alprazolam (mean dose 1.57 mg/day) were studied in children and adolescents with overanxious and avoidance disorders (mean age 12.6 years). After 1 week of placebo, followed by 4 weeks of alprazolam, there was no significant change in performance in the continuous performance test and visual matching task that assess long-term memory. However, in the Sternberg memory scanning test, children in the placebo group performed significantly worse than those on alprazolam (247). Based upon these limited results, it seems that the behavioral effects of alprazolam in children and adults are similar.

Current or Past Drug Abusers

Mumford and colleagues (184) investigated the single-dose effects of alprazolam (0.5, 1.0, and 2.0 mg) in 14 male subjects who met the DSM-III-R diagnosis for psychoactive substance dependence. Urine drug tests revealed that subjects were drug-free on test days. Tests included DSST, balance, a choice reaction time test, and immediate and delayed picture recall and recognition. After 2 mg of alprazolam the subjects showed significantly greater impairment than with placebo in all tests. At lower doses alprazolam produced no significant impairment of performance, except in the choice reaction time test. In similar studies with polydrug users the same authors reported significant impairment in digit span, balance, DSST and choice reaction tests after a single dose of alprazolam (37,77, 183,185).

Light, moderate and heavy drinkers are at increased risk to abuse benzodiazepines. Women are more vulnerable to this effect than men. Moderately drinking women reported equally increased alprazolam and buspirone liking when compared to light drinking women. Although alprazolam produced a dose-dependent impairment in performance tests, including DSST, a word learning test, divided attention, and tracking, drug liking was independent of the dose of alprazolam (82). Females with a paternal history of alcoholism performed significantly worse in the DSST and immediate word recall test than control subjects (81).

Behavioral Effects in the Patient Population

The presence of pathology is known to affect human behavior. For example, it has been shown that anxiety and depression itself significantly impair cognitive and psychomotor performance (31,36,67,101,139,141,220,264). However, the number of studies that investigated possible impairment caused by the illness itself is limited and the results are often conflicting.

For example, in a direct comparison (67), 14 panic patients in a non-panic state (background anxiety) performed worse than healthy controls in a variety of psychological tests, including immediate and delayed recall, digit span, DSST, symbol copying, digit cancellation test, and a reaction time test. Panic patients in the non-panic state are physiologically more aroused and reported significantly higher levels of anxiety than the healthy

controls. However, performance differences in the tests reached significance only for immediate and delayed recall tests. In contrast, in another study with 69 patients with mild to moderate panic disorder, there was no significant impairment in similar tests (101).

In patients with unipolar and bipolar depression, significant impairment was found in laboratory tests assessing motor speed (reaction time and tapping tests) and visual tracking (trial making and dot replacement tests) (264). There was no significant difference in the impairment of patients with unipolar as compared to bipolar depression. In contrast, another study reported that patients with unipolar or bipolar depression had no significant impairment in a laboratory test battery, including tests of executive functioning, verbal and visual memory, attention, intelligence, psychomotor speed, and sensory-perceptual skills, than matched control subjects (220). However, memory functioning in patients scoring high on depression was significantly worse than those having mild depression.

A study examining memory functioning in 3999 Vietnam veterans (141) reported that those experiencing depressive symptoms (without anxiety) had significantly impaired immediate recall on the California Verbal Learning Test. The veterans experiencing anxiety (without depression) had no significant impairments. However, veterans suffering from both, anxiety and depression, had immediate recall impairment and were significantly worse on the retrieval of newly learned information than control subjects.

These studies illustrate that it is important to take comorbidity into account when discussing possible impairments accompanying panic disorder, anxiety, or depression. Taking into account the mixed and conflicting results described above, more studies are needed to get a better understanding of the impact of the pathology itself on human performance.

The complex interaction between therapeutic effects (presumably improving performance) and adverse effects (presumably worsening performance) of alprazolam cannot easily be predicted from studies with healthy volunteers. However, these studies will examine the adverse drug effects of alprazolam, without interference of changes in the pathology itself. Since alprazolam's activity may be modified by the disease, it is important to perform these studies in a patient population as well.

A limited number of studies investigated the effects of alprazolam on cognitive and psychomotor performance in patients. For example, patients receiving hemodialysis or continuous peritoneal dialysis performed significantly worse on the DSST, choice reaction and short-term memory tests suggesting that patients with renal dysfunction are more sensitive to some of the psychomotor and memory effects of alprazolam (236).

Behavioral effects after acute administration of alprazolam (0.25, 0.5, 0.75 mg) and placebo have been investigated during the premenstrual and postmenstrual phases in women with premenstrual syndrome (79,80). Performance tests were performed at 0.5 to 4 h after treatment; they included DSST, balance, divided attention, repeated acquisition, and word learning tests. Relative to placebo, alprazolam produced significant dose-related decreases in performance on all tasks regardless of menstrual cycle phase. However, significant practice effects were found in the DSST and repeated acquisition test.

A study comparing the performance impairing effects of a single dose of alprazolam (1 mg) in healthy volunteers and PD patients showed that alprazolam significantly impaired DSST performance in both groups. The difference between patients and healthy volunteers was not significant (134).

A controlled longitudinal study (57) examined memory functions in PD patients from the London Toronto Study. The patients received alprazolam, placebo, and/or psychologi-

cal therapy (exposure or relaxation). Patients were treated for 8 weeks with alprazolam (mean daily dose of 5.25 mg in week 8) or placebo and tested at baseline (week 0), after chronic treatment (week 8), and during follow-up period (24 weeks after the start of the study). As compared to placebo, alprazolam significantly impaired free word recall after 8 weeks. After 24 weeks, performance in alprazolam-treated patients returned to the baseline, whereas performance in the placebo-treated patients improved. There was no significant difference in the performance of alprazolam- and placebo-treated patients in digit span, news recall and psychomotor tests (cancellation and tapping speed). Psychological therapy had no significant effects on test performance. A 3.5-year follow-up of these patients showed that the test performance of PD patients that belonged to the alprazolam treated group was similar to that in the placebo-treated group (138). Performance in both groups was equal to the pre-treatment level. The authors concluded that their post-treatment findings after 24 weeks should be explained as practice effects rather than drug-induced effects.

Gladso and colleagues (102) investigated neuropsychological functioning of PD patients treated for six weeks with alprazolam (4 mg/day, extended release) or placebo in combination with cognitive-behavioral therapy. Relative to baseline, both groups showed significant improvement on tests measuring attention, executive functioning (Card Sorting Test), psychomotor speed (Trial Making Test, Finger Tapping Test), and visual memory tests. No significant improvement was found on tests measuring learning, verbal memory and reaction time. However, these effects were caused by a practice effect on the tests, making the results of this study hard to interpret.

Like alprazolam (274), driving ability after diazepam can be severely impaired (193). Driving ability after chronic alprazolam treatment has not been investigated in patients. A 4-week study (158) examined the effects on actual driving performance in anxious outpatients treated with buspirone (15 mg daily) or diazepam (15 mg daily). Chronic treatment with buspirone did not significantly impair driving performance. In contrast, diazepam significantly impaired driving performance during the first three weeks of treatment. In addition, speed control after diazepam was significantly impaired during the first treatment week. From these results it could be expected that driving will be significantly impaired after chronic administration of alprazolam as well. It is, therefore, important to investigate whether alprazolam would affect performance of PD patients in the-road driving test and whether tolerance develops to driving impairment that is evident after acute alprazolam administration (274).

A study that examined the effects of long-term (8 years) benzodiazepine therapy on DDST performance and a Symbol copying test before, during, and after discontinuation of the therapy showed that performance of PD patients, who were successfully tapered off from alprazolam therapy, significantly improved as compared to patients continued on alprazolam (217). An earlier study (168) compared cognitive and psychomotor performance in anxious outpatients who were treated with benzodiazepines (including alprazolam) and those who received no pharmacological treatment. Performance in benzodiazepine-treated patients was significantly worse in the Critical Flicker Fusion Test (CFFT), but no significant differences were observed on the DSST, symbol copying, letter cancellation and immediate and delayed recall tests. However, after acute administration of benzodiazepines to these patients, performance in the DSST significantly improved, whereas delayed recall was significantly impaired. Treated patients were also tested at 4 to 8 days after discontinuation of therapy (during benzodiazepine withdrawal). In comparison with their base-

line performance, there were no significant differences in the test results, except for an increase in CFF threshold. Furthermore, patients reported significantly increased sedation and less feelings of tranquilization.

DISCUSSION

This exhaustive literature review shows that alprazolam is effective in the treatment of panic disorder, generalized anxiety and depression. The major conclusion from the studies discussed is that alprazolam is as effective as TCAs, such as imipramine, in the relief of symptoms. However, in panic disorder SSRIs appear to be significantly superior to alprazolam, as well as TCAs, and should, therefore, be viewed as the first line therapy of this disorder.

In panic attacks alprazolam produces rapid clinical improvement during the first week of therapy, reducing anxiety, depressive symptoms and frequency of the attacks. TCAs and SSRIs, in contrast, may worsen the symptoms initially and may become effective only after 2 to 4 weeks of continuous administration. Patients receiving psychological therapy reported no worsening at the onset, but it took several weeks before clinical improvement was established. The aggravation of symptoms or slow onset of action has serious implications for compliance and may result in premature discontinuation of therapy. Hence, alprazolam treatment is favored, if rapid relief of symptoms is required.

During treatment, alprazolam is safe and well tolerated. No dose increments are necessary and the risks of dependence and abuse are small in the patient population. However, like other benzodiazepines, when treatment is discontinued, alprazolam causes withdrawal and rebound effects; the symptoms that were reduced or absent during treatment may reappear. The rebound symptoms may be more severe than prior to therapy. Furthermore, it has been reported that all advantages of alprazolam therapy are lost after discontinuation of the drug. In contrast, improvement remains stable long after discontinuation of psychological therapy. However, psychological treatment is effortful, time-consuming and costly.

Alprazolam produces a variety of unwanted effects of which drowsiness and sedation are the most prominent. As reviewed above, alprazolam at clinically effective doses produces significant behavioral toxicity. The results summarized in Tables 1 and 2 show that a single dose of alprazolam can impair memory and psychomotor performance. As summarized in Tables 3 and 4, after repeated administration of the drug tolerance develops to the performance impairing effects of alprazolam. However, it must be taken into account that the tests used in these studies (e.g., the DSST) are known for persistent learning and practice effects. Hence, it cannot be ruled out that improvement in the performance after repeated administration of the drug is caused by practice effects. Moreover, a large number of patients use alprazolam not on a daily basis, but only as needed. In such cases, tolerance to alprazolam's impairing effects does not develop easily. This becomes evident from the few behavioral studies conducted in patients. Although some of these studies reported learning and practice effects, others reported significant performance impairment after long-term therapy with alprazolam. It can be questioned whether at higher doses of alprazolam the therapeutic effect of alprazolam outweighs its adverse effects.

In conclusion, the impairing effects on daily activities such as driving a car limit the safe use of alprazolam in outpatients. Taking into account alprazolam's effects after treatment discontinuation (withdrawal and rebound effects) and its adverse effect profile

(drowsiness and sedation), it is not surprising that alprazolam is not recommended as first-line therapy in panic disorder (12), generalized anxiety (7,12) or depression (8). However, alprazolam may be considered as a second-line treatment if SSRIs are not effective or tolerated well.

Prescribers may prefer a combination therapy of SSRIs and alprazolam. It is known that SSRIs have a slow onset of action (several weeks) and may produce initial symptom worsening. The co-administration of alprazolam may provide adequate symptom relief during the first 6 to 8 weeks after the start of the therapy. Alprazolam can be gradually withdrawn when SSRIs become clinically effective (290). The combination therapy with alprazolam will presumably enhance treatment compliance.

A recent study in 443 panic patients reported that despite efforts to promote the use of SSRIs in the treatment of panic disorder only a modest increase in their use took place (34). Despite all recommendations to use SSRIs instead of alprazolam in the therapy of panic disorder, the use of alprazolam remained stable over the past decade. The prescribers are apparently conservative in changing their treatment regimen, despite clear evidence that SSRIs have a greater clinical efficacy combined with a favorable adverse effect profile when compared to alprazolam.

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EDITORIAL

Dear colleagues,

As we have already announced earlier, Bosnian scientists working abroad prepared three reviews for this issue of our Journal. Editor of all the contributions published in this and following issues is Associate Professor Ernes Karić, MD, MSc, PhD, FASC from the USA. Under the auspices of UNDP (TOKTEN PROJECT in B&H) he is invited to deliver a series of lectures as a visiting professor as follows: March 29 - Cantonal Hospital Zenica, March 30 - State Hospital Sarajevo, April 1 - University Clinica Center in Tuzla.

As you are aware, as a consequence of events which occurred between April 1992 and Dayton Agreement Ratification in 1995, Bosnia and Herzegovina will be a land of disabled persons for a long time. It is therefore of paramount importance that the information on modern achievements in orthopedics and bordering areas of science be available to our scientists and experts.

We plan to publish articles from 10 areas that are defined by the respected professor. He has contacted renown authors, provided adequate peer reviews and performed all the necessary editorial duties.

As always, our primary intention is to serve as a tool of communicating knowledge in basic medical sciences as well as bordering areas of science.

Sarajevo, May, 2005

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UDRUŽENJE RAZLIČNIH MEDICINSKIH ZNANOSTI

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PERIPHERAL NERVE BLOCKS
FOR PERIOPERATIVE
MANAGEMENT OF PATIENTS
HAVING ORTHOPEDIC
SURGERY OR TRAUMA OF
THE LOWER EXTREMITY

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ABSTRACT

Over the past decade several developments have led to an increased interest in lower extremity PNBs including transient neurologic symptoms associated with spinal anesthesia, increased risk of epidural hematoma with the introduction of new antithrombotic prophylaxis regimens, and evidence of improved rehabilitation outcome with continuous lower extremity PNBs. Simultaneously, the field of lower extremity blockade has been revolutionized through our better understanding of functional regional anesthesia anatomy, introduction of new drugs, better and more sophisticated equipment and wider teaching of lower extremity nerve block techniques. This review focuses on techniques and applications of lower extremity nerve blocks in patients having orthopedic surgery or trauma of the lower extremity, as well as potential complications and means to avoid them.

KEY WORDS: regional anesthesia, nerve block, trauma, neurologic complications

UDRUŽENJE RAZLIČNIH MEDICINSKIH ZNANOSTI

5

LOWER EXTREMITY NERVE BLOCK TECHNIQUES

PSOAS COMPARTMENT BLOCK (LUMBAR PLEXUS BLOCK)

The psoas compartment block (PCB) is a deep block of the lumbar plexus approached posteriorly, and can be performed either as a single injection or as a continuous technique with catheter placement for prolonged analgesia. The PCB block provides anesthesia for thigh surgery and hip fracture repair in combination with a paravertebral nerve block.¹ The PCB is successfully used for analgesia following total hip arthroplasty (THA),^{2,3} total knee arthroplasty (TKA),⁴ and in the treatment of chronic hip pain.⁵ At the level of L4-L5 the following anatomic structures would be encountered from posterior to anterior: posterior lumbar fascia, paraspinous muscles, anterior lumbar fascia, quadratus lumborum, and the psoas muscle. The common iliac artery and vein lie anterior to the psoas muscle, which is located inside a fascial sheath, the psoas compartment. The most consistent approach to block the entire lumbar plexus with a single injection is via the posterior approach, since the lumbar plexus travels through the body of the psoas muscle. The PCB provides consistent anesthesia in the distributions of the femoral, lateral cutaneous nerve of the thigh, and the obturator nerves. Several descriptions of the needle entry site for the PCB have been described.^{1,6-12} (Figure 1).

was significant ($p<0.05$). There was no statistically significant difference in the expression of HER-2 protein in cases with and without signs of vascular invasion. In cases with no metastases in axillary lymph nodes, expression of the HER-2 protein was significantly ($p<0.05$) higher in comparison to cases with no metastases. Most descriptions rely on bony contact with the transverse process as a guide to depth of needle placement. The distance from the skin to the lumbar plexus ranges from 6.1–10.1 cm in men and 5.7–9.3 cm in women and this distance correlates with gender and body mass index (BMI). The distance from the transverse process to the lumbar plexus is typically less than 2 cm, and this is independent of BMI or gender. Contact with the transverse process provides a consistent landmark to avoid excessive needle penetration during PCB.¹ The depth of needle insertion is emphasized because of the complications associated with deep needle penetration including renal hematoma, pneumothorax, total spinal anesthesia, and unintended intrabdominal and intervertebral disk catheter placement.¹³⁻¹⁵ Epidural spread of local anesthetic is a common side effect of PCB occurring in 9–16% of adult patients.¹⁶⁻¹⁸ In children, Dalens reported a greater



FIGURE 1. Lumbar Plexus Block: A 10-cm long needle is inserted at L3-L4 level and 3–4 cm lateral to the midline. (Reproduced with permission from Hadzic A. *3-in-1 Popliteal Nerve Blocks: Principles and Practice*. McGraw-Hill, New York, NY, 2003.)

than 50% incidence of epidural spread when using the original landmarks of Chayen compared to no epidural spread when using the landmarks as modified by Winnie.¹⁹ The epidural spread is attributed to diffusion of the local anesthetic into the epidural space when large volumes of local anesthetic (greater than 50 mL) are used. In most cases of epidural spread, residual lumbar plexus blockade is apparent after resolution of the contralateral block. However, there are case reports of total spinal anesthesia occurring during PCB and vigilance must be maintained during the management of this block.^{19,20}

CONTINUOUS PSOAS COMPARTMENT BLOCKS
Continuous PCB techniques have been described to provide analgesia following a variety of operations including THA, TKA, open reduction and internal fixation (ORIF) of acetabular fractures, ORIF of femur fractures and anterior cruciate ligament (ACL) reconstruction.²¹⁻²⁹ Interest in this block developed as practitioners sought alternatives to central neuraxial techniques that could provide consistent analgesia following hip, femur and knee surgery. One advantage of PCB over other continuous approaches to the lumbar plexus is the decreased likelihood of catheter dislodgement because of the large muscle mass that must be traversed to reach the lumbar plexus. Continuous infusion of local anesthetic should be started after an initial bolus is given through the catheter. Care must be taken to watch for local anesthetic toxicity, intravascular injection, and the unilateral sympathetic blockade that accompanies a PCB.

FEMORAL NERVE BLOCK

Indications for single injection femoral nerve block (FNB) include anesthesia for knee arthroscopy in com-

bination with intraarticular local anesthesia and analgesia for femoral shaft fractures, ACL reconstruction, and TKA in multimodal regimens.³⁰⁻³² The femoral nerve divides into the posterior and anterior divisions shortly after it emerges from under the inguinal ligament and undergoes extensive arborization. Commonly the anterior branch of the femoral nerve, which innervates the sartorius muscle, will be identified first. Vluka and Hadzic reported this to be a common first motor response.³³ Stimulation of this branch leads to contraction of the sartorius muscle on the medial aspect of the thigh and should not be accepted, as the articular and muscular branches arise from the posterior part of the femoral nerve. The needle should be redirected slightly laterally and deeper to encounter the posterior branch of the femoral nerve. Stimulation of this branch is identified by patellar ascension as the quadriceps contract (Figure 2).

A 3-IN-1 BLOCK

During FNB, it has been advocated to use a higher volume of local anesthetic and apply firm pressure just distal to the needle during and a few minutes after injection to block the femoral, lateral femoral cutaneous, and obturator nerves, the so named 3-in-1 block.³⁴ However, despite many efforts to consistently produce a 3-in-1 block, the efficiency of these maneuvers has not been demonstrated. In most reports, the femoral nerve is the only nerve consistently blocked with this approach.³⁵⁻³⁷ Occasional blockade of the lateral femoral cutaneous (LFC) nerve occurs through lateral diffusion of local anesthetic and not through proximal spread to the lumbar plexus.³⁸ The obturator nerve is less frequently anesthetized during 3-in-1 block than the LFC nerve, which is not surprising given the number of fascial barriers between these structures at the level of the inguinal ligament.

CONTINUOUS FEMORAL NERVE BLOCK

Continuous FNB has been shown to improve surgical outcome following major knee and vascular surgery of the lower extremity compared to intravenous narcotic therapy or continuous intraarticular infusions of analgesics.³⁹⁻⁴² Two prospective randomized studies examined three different modes of analgesia: continuous FNB, epidural analgesia, and intravenous narcotic therapy following TKA.⁴³⁻⁴⁴ These studies demonstrated improvement in postoperative rehabilitation scores and a decreased duration of stay in a rehabilitation center for patients receiving the regional anesthesia techniques. Continuous FNB was shown to have equivalent analgesia with fewer side effects than epidural analgesia in both of these studies.^{43,44} However, not all investigators



FIGURE 2. Femoral Nerve Block: A 5 cm needle is inserted at the femoral crease and immediately laterally to the pulse of the femoral artery. The nerve is typically stimulated at a depth of 1–3 cm. (Reproduced with permission from Hadzic A. *3-in-1 Popliteal Nerve Blocks: Principles and Practice*. McGraw-Hill, New York, NY, 2003.)

have been able to demonstrate these improvements in outcome with continuous FNBs. Lang found no difference between patients receiving a single injection FNB and patients receiving a continuous FNB after TKA.⁴⁵ The accuracy of catheter placement may play a role in these conflicting findings. In a prospective study, Capdevila showed that continuous FNB using a standard approach led to unpredictable catheter placement. In the Capdevila study, a vastus intermedius muscle response was elicited at 0.5 mA, the sheath was distended with 5 mL of saline, a catheter was inserted 16–20 cm, then a bolus of local anesthetic was given through the catheter.⁴⁶ Most of the catheters tended to course medially in the direction of the psoas muscle or laterally in the direction of the iliacus muscle. The accuracy of final catheter placement correlated well with the quality of analgesia following proximal lower limb surgery, although VAS values were generally low in all groups.⁴⁷

LATERAL FEMORAL CUTANEOUS NERVE BLOCK

The LFC nerve of the thigh is a sensory nerve that supplies the cutaneous sensation to a large but variable area from the inguinal ligament to the knee on the lateral aspect of the thigh.⁴⁸ LFC nerve block is most commonly used as the sole anesthetic during diagnostic muscle

biopsy and harvesting of split thickness skin grafts.^{39,40} It has also been used to provide analgesia in elderly patients undergoing hip fracture repair.⁴¹ However, in a study comparing LFC nerve block, FNB, and patients receiving no block following femoral neck repair, LFC nerve block was not as effective at controlling postoperative pain as FNB.⁴² Typically this block is done as a blind, "fan" technique with a variable success rate. This may be due to variability in the innervation of the nerve or imprecise localization of the nerve. Shannon compared the traditional fan technique for LFC nerve block to the use of a nerve stimulator technique seeking tingling in the distribution of the nerve.⁴³ A 40% success rate with the "fanning" technique was reported compared to 100% with the nerve stimulating technique. There was no difference in the extent of the blockade in successful blocks. FNB has been reported following LFC block,⁴⁴ which is not surprising given the fact that most FNB literature reports that FNB results in anesthetic spread to the LFC nerve.

SAPHENOUS NERVE BLOCK

The saphenous nerve follows the saphenous vein to the medial malleolus and supplies the cutaneous area of the medial aspect of the calf and foot to the level of the midfoot. The saphenous nerve block is often combined with a sciatic block to provide anesthesia and analgesia for surgery involving the medial aspect of the lower leg and foot. The saphenous nerve is a sensory nerve and does not contribute to the bony innervation of the foot. Approaches to the saphenous nerve along its entire course, from the adductor canal to the ankle, have been described. Success rates vary widely between techniques. For example, successful block is reported in 33–65% of cases with a field infiltration performed medially at the level of the tibial plateau,^{45,46} 70–80% of cases with the transarticular approach,^{45,47} 93–100% of cases with femoral paracutaneous approach⁴⁷ and nearly 100% of cases with the paravenous approach.⁴⁶ The saphenous nerve has been reported to be selectively blocked, sparing of the quadriceps musculature, in the adductor canal.⁴⁸ However, this finding has not been confirmed in a large series of patients receiving this approach for a saphenous nerve block.

PSOAS COMPARTMENT BLOCK VERSUS FEMORAL NERVE BLOCK

Parkinson compared the extent of blockade following FNB and the posterior approach to the lumbar plexus.¹³ They compared the extent of blockade of the lumbar plexus with four different methods: posterior approach at L3 (posterior approach of Dekrey)

and L4–5 (Chayen's approach) with a nerve stimulator using noninsulated needles, and anterior FNB (approach of Winnie) with a paresthesia technique and nerve stimulation technique.¹³ The FNB had a 100% success rate, while the LFC nerve block success rate was 85–95%. The obturator nerve, as assessed by thigh adduction, was blocked 100% of the time with the posterior approaches and never with the anterior approaches. Limitations of this report include lack of details regarding the type of nerve stimulation, the small sample size, and exclusion of patients in whom FNB failed to develop. A more recent comparison has been made between PCB and FNB.⁴⁹ In this study, patients receiving a PCB developed a sensory block of the femoral, LFC, and obturator nerves in 100, 97, and 77% of patients versus 93, 63 and 47% of the patients receiving a FNB.

CONTINUOUS PSOAS COMPARTMENT BLOCKS VERSUS EPIDURAL ANALGESIA

Advantages of continuous PCB compared to epidural block include unilateral analgesia and motor block, lack of impairment of bladder function, and improved risk/benefit ratio in patients receiving anticoagulation medications following surgery. These advantages must be weighed against the disadvantages of incomplete blockade for anesthesia, and the need for supplementation in a balanced analgesic regimen for effective analgesia. Türker recently compared continuous PCB with epidural block for analgesia following THA under combined general/regional technique and demonstrated that continuous PCB provided excellent intra- and postoperative analgesia with a low incidence of complications.⁴ Epidural block took longer to perform and had a significantly higher incidence of hypotension, while the analgesia and patient satisfaction provided by the two blocks were similar. Epidural block also provided more motor blockade, longer time to ambulation, and significantly more complications.

CONTINUOUS PSOAS COMPARTMENT BLOCKS VERSUS CONTINUOUS FEMORAL BLOCKS

Following TKA, continuous FNB and continuous PCB reduce narcotic consumption and pain scores compared to intravenous morphine usage alone.⁵⁰ However, no differences in outcome were observed between the two peripheral nerve block groups despite a more "consistent" presence of obturator nerve block in the psoas compartment group. Both the continuous FNB and continuous PCB can be effective for pain control after a TKA and the choice of which continuous nerve block to choose should be based on patient profile and

preference, comfort of the anesthesiologist in placing the block and catheter, and preference of the surgeon.

PARASACRAL BLOCK

The parasacral nerve block (PSNB) described by Mansour in 1993 has been described as more than an isolated sciatic nerve block.^{50,51} It has been used to provide analgesia following major foot and ankle reconstruction. PSNB will consistently block both components of the sciatic nerve and the posterior cutaneous nerve of the thigh. Spread of local anesthetic may also anesthetize other branches of the sacral plexus including the superior and inferior gluteal and pudendal nerves. The pelvic splanchnic nerves, the terminal portion of the sympathetic trunk, the inferior hypogastric plexus, and the obturator nerve all lie in close proximity to the elements of the sacral plexus and may all be anesthetized with this approach. For procedures around the knee this may be advantageous over more distal approaches to the sciatic nerve.^{52,53} For procedures below the knee, the adductor weakness from the obturator and superior gluteal nerve block may actually be disadvantageous for mobilization of the patient following blockade. The sympathetic nerve supply to the bladder is in close proximity to the sacral plexus, but problems with voiding and the need for bladder catheterization after PSNB have not been reported.⁵² A notable difference from other approaches to the sciatic nerve is the type of muscle response deemed acceptable as an endpoint for injection. Mansour described contraction of the hamstring muscles (biceps femoris semitendinosus) above the knee as the endpoint for PSNB with consistent success.⁵⁰

CONTINUOUS PARASACRAL BLOCKS

Continuous PSNBs have been used in combination with PCBs to provide lower extremity anesthesia for TKA, above the knee amputation, ACL repair and a variety of other lower extremity procedures.⁵⁴ Gertner reported successful catheter placement, as confirmed by radiographic contrast dye, in 86 out of 87 consecutive patients undergoing lower extremity surgery. All patients developed analgesia in the distribution of the tibial, peroneal and cutaneous nerve of thigh.

SCIATIC NERVE BLOCK AT THE LEVEL OF THE GLUTEUS MAXIMUS

The sciatic nerve, the largest nerve derived from the sacral plexus, innervates the posterior thigh and almost the entire leg below the knee. The most common indications for sciatic nerve block (SNB) are anesthesia and analgesia for foot and ankle surgery. Gaston



FIGURE 3. Sciatic Nerve Block. Anatomical relationships of importance to the technique.

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Hodges J., White J. *Regional Anesthesia: Principles and Practice*.
McGraw-Hill, New York, NY, 2003.

Labat first described the approach to the SNB, now referred to as the Classic Approach of Labat, at the beginning of the 20th century. This approach is based on the bony relationship of the posterior superior iliac spine (PSIS) and the greater trochanter with the patient positioned in a modified Sims position (Figure 3). Winnie was the first to modify the original description by adding another landmark, the sacral hiatus, to more precisely account for varying body habitus.⁵⁵ The reported success rate of this approach is ranges from 33% to 95%.⁵⁶ More recently, Franco described a simplified approach to the SNB in the prone position. The needle entry site is perpendicular to the floor 10 cm lateral from the middle of the inguinal sulcus regardless of the patient's gender or body mass index.⁵⁷ The sciatic nerve was found by trainers in three passes or less in 83% of the cases reported. Whether the success of this simple approach will be replicated in a larger sample size remains to be seen.

SUBGLUTEAL APPROACHES TO THE SCIATIC NERVE

Raj described a supine approach to the sciatic nerve in the flexed hip position initiating the block at the midpoint between the greater trochanter of the femur and the ischial tuberosity.⁵⁸ The positioning of the patient was thought to be advantageous compared to the classic approach of Labat by "thinning the gluteus maximus muscles, making the sciatic nerve more superficial". However identifying these bony landmarks in very obese patients is sometimes difficult and maintaining this position requires additional personnel to assist the patient. A lateral subgluteal approach to the sciatic nerve using the greater trochanter of the femur as a landmark was first described by Ichijiyangji in 1959. Other investi-

gators have described a high success rate using this high lateral approach with a slightly more caudal entry point.¹⁷ When using this approach, the success rate of the blockade of the posterior cutaneous nerve of the thigh was 93%. Although theoretically this nerve should reliably be blocked in most proximal approaches to the sciatic nerve, the success rate of blockade of the posterior cutaneous nerve of the thigh is not usually reported. The anterior approach to the sciatic nerve has the appeal of supine positioning and one skin prep when performing a combined femoral and sciatic nerve block. However, its clinical utility has been limited by its complexity, patient discomfort, and low success rate.^{20,21} Numerous variations of the original Beck's technique description have been described.^{4,22} In separate studies, Vlika and Hadzic²³ and Moore²⁴ described the importance of internal rotation of the leg if the path to the sciatic nerve is obstructed by the lesser trochanter.²⁵ An MRI study of the anatomy of this area found that in 63% of patients the sciatic nerve is inaccessible from the anterior approach at the level of the lesser trochanter.²⁶ These authors suggested needle placement 4 centimeters lower, where obstruction to the sciatic nerve occurred in only 28 of the patients. Dales²⁷ compared the success rate of the posterior, lateral and anterior approaches to the sciatic nerve in children.^{6,28} Although a success rate of 90% with all approaches was reported, fewer manipulations were required to perform either a lateral or posterior approach compared to the anterior approach. Recently, a medial approach to the sciatic nerve at the level of the lesser trochanter was reported in series of 10 children.²⁹ Advantages of this approach are the lack of obstruction from the femur and no muscle mass to transverse. The authors reported a 70% rate of blockade of the posterior cutaneous nerve of the thigh with this medial approach. di Benedetto recently described results from 135 consecutive patients using a posterior subgluteal approach to the sciatic nerve.^{6,8} The time to perform the block was 41 ± 25 s (mean $\pm$ SD), with an average of a needle redirections. The degree of discomfort reported was very low and only 16 patients (12%) reported severe pain during placement of the block. In contrast to this, Faull³⁰ reported patient discomfort in 50% of patients receiving a classic Labat approach to the sciatic nerve.³¹

SCIATIC NERVE BLOCK AT THE LEVEL OF THE POPLITEAL FOSSA

Popliteal block is typically used for foot and ankle surgery.³² This technique offers advantages over spinal anesthesia in patients having short saphenous vein

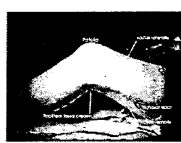


FIGURE 4. Popliteal Sciatic Block through the Lateral Approach, Surface Landmarks.
(Reproduced with permission from: Hadzic A, Vlika J. *Popliteal Nerve Block: Principles and Practice*. 1st Edition. New York, NY, 2003.)

stripping.³³ The block has also been successfully utilized in the pediatric population.³⁴ As opposed to an ankle block, popliteal block anesthetizes the musculature of the lower leg which likely improves tolerance for a calf tourniquet and provides an immobile foot for surgery. Performance of popliteal fossa block in patients with previous TKA or vascular bypass (femoral-popliteal) should be done with care even though there are no reported cases of joint infections or graft disruption relating to needle placement in these patients. The components of the sciatic nerve may be blocked at the level of the popliteal fossa via a posterior or lateral approach. Access to the sciatic nerve may also occur with the patient in the lithotomy position.³⁵ Continuous techniques have been described using both the posterior^{36,37} and lateral³⁸ approaches. The posterior approach to the popliteal fossa is accomplished with a patient in the prone position.³⁹ In order to block the sciatic nerve before it divides, injection of local anesthetic is given 7–10 cm above the popliteal crease (Figure 4).^{40–42,43,44} With a single injection technique, inversion may be best predictor of complete neural block of the foot.⁴⁵ A lateral approach to blockade of the sciatic nerve in the popliteal fossa has recently been described.^{46,47} Success rate with all approaches to the popliteal sciatic nerve block is typically 90–95% with approximately 28 of patients requiring supplemental general anesthesia. It is believed that incomplete block is the result of poor diffusion (due to the size of the sciatic nerve), separate fascial coverings of the tibial and peroneal nerves, or blockade of only a single component of the sciatic nerve. This has led some practitioners to endorse the practice of dual stimulation to improve success rate.⁴⁸

CONTINUOUS SCIATIC NERVE BLOCKS

Continuous PNBs can theoretically be achieved at any place along the course of the sciatic nerve. Continuous SNBs have been used for analgesia following major foot and ankle reconstruction, ankle fracture fixation, and below the knee amputation.^{49–51,52,53} Three studies have been published on the use of continuous popliteal blocks for analgesia after extensive foot and ankle surgery.^{54–56} All three studies reported excellent analgesia with few side effects. Compared to intravenous analgesia or placebo infusion, a continuous infusion of local anesthetic via a popliteal catheter reduces pain scores, opioid consumption, and sleep disturbances.^{54,55} Successful catheter placement has been reported with both the lateral and posterior approach. The only consistent problem reported with popliteal catheters is a high incidence, 15–25%, of kinking or dislodgement.^{54,55} di Benedetto compared the subgluteal approach to the posterior popliteal approach for continuous infusions in a prospective study.⁶ In the 24-hour observation period following surgery, 13.3% of the catheters in the popliteal group were either occluded or dislodged compared to 6.6% of the catheters in the subgluteal group. This difference did not reach statistical significance.

ANKLE AND FOOT BLOCK

The main indication for blockade of the lumbosacral plexus distally, at the ankle and midtarsal levels, is anesthesia for surgery to the foot.^{57,58} A diagnostic ankle block for sympathetically mediated ankle pain has also been described.⁵⁹ The peripheral nerves blocked at the ankle are the terminal branches of both the sciatic (posterior tibial, superficial peroneal, deep peroneal, and sural) and femoral (saphenous) nerves. The five peripheral nerves that supply the foot are relatively easy to block at the ankle. There are no important variants in the innervation of the distal musculature. However, there is considerable variation in the branching and distribution of the sensory nerves of the foot. For this reason, blockade of all five nerves has been advocated.⁶⁰ Neural blockade of the posterior tibial nerve has been described at the supramalleolar,^{61,62} midmalleolar,⁶³ subcalcaneal,^{64,65} and midtarsal⁶⁶ levels with no evidence of superiority of one technique. The three superficial nerves are consistently blocked with simple field infiltration. Few studies evaluating perioperative outcomes with ankle block exist⁶⁷ although the technique has been in clinical practice for decades. Rather, most publications describe variations to improve success rate. Peak blood levels of local anesthetic occur around 90 minutes after blockade and are very low even after bilateral ankle block.⁶⁸

PHARMACOLOGIC CONSIDERATIONS

Complete unilateral lower extremity blockade involves multiple nerve blocks and a large volume of local anesthetic. Attention must be given to total local anesthetic dose; the concentration should be adjusted to accommodate the volume required for initial blockade as well as the ongoing local anesthetic administered as part of a continuous postoperative infusion. The concentration must also take into account the degree of sensory and/or motor block desired. Residual blockade may impede the ability of the patient to ambulate or actively participate in physical therapy. Thus, it is critical for the anesthesiologist to be aware of the postoperative rehabilitation program. Finally, the efficacy of adjuvants such as clonidine, opioids and ketorolac in improving the quality or duration of blockade has not been consistently demonstrated. Relatively few randomized studies have compared local anesthetics for lower extremity block. Casati evaluated the onset and duration of combined femoral sciatic block performed with 0.5%, 0.75%, or 1% ropivacaine versus 2% mepivacaine, 1.00, 0.75%, or 1% ropivacaine had an onset similar to that of mepivacaine, but with a duration of analgesia longer than mepivacaine. These results are similar to those involving upper extremity blocks, which suggest that 0.75% ropivacaine and 0.5% bupivacaine produce similar block quality and duration. In a single comparative study of SNB, levobupivacaine has block characteristics similar to ropivacaine. Although 0.5% ropivacaine took a longer time to onset than 2% mepivacaine, the duration of analgesia with 0.5% ropivacaine was much longer (mean of 12 hours versus 5 hours).

EPINEPHRINE

Epinephrine prolongs the duration and quality of most local anesthetics used for lower extremity peripheral nerve blocks. The effects are the result of vasoconstriction of the perineural vessels, which decreases uptake and increases the neural exposure time to the local anesthetic. The difference in effect is only somewhat dose-dependent. For example, the addition of epinephrine 500 µg/mL (1:200,000 dilution) significantly increases the duration of lidocaine from 186 minutes to 264 minutes. Epinephrine 2.2 µg/mL (1:400,000 dilution) prolongs the block to nearly the same extent (240 minutes) without any effect on nerve blood flow.^{69,70} The addition of epinephrine to local anesthetics with vasoconstrictive properties, such as ropivacaine, may not significantly increase block duration, but may facilitate detection of intravascular injection.⁷¹ The decision to add epineph-

rine and the dose of epinephrine added is based on the concerns related to cardiac or neural ischemia versus the ability to discern an intravascular injection. In general, since seizures related to intravascular injection are highest in patients undergoing PNBs,¹⁰ the benefits of adding epinephrine may outweigh the risks. However, the nearly equivalent effects on block quality and duration reported with epinephrine 2.5 µg/mL when compared to 5 µg/mL suggest that the lower concentration is sufficient. It is a personal bias of this author that epinephrine should not be used for sciatic blocks, in particular the anterior SNB, due to the long duration of sciatic blockade and risks of nerve ischemia compounded by vasoconstriction, the possibility of arterial puncture, pressure on the anesthetized sciatic nerve, and application of the tourniquet over the upper thigh.

BICARBONATE

The addition of bicarbonate has been recommended to increase the speed of onset of peripheral and plexus blockade. However, most studies that have demonstrated a statistically significant difference utilized commercially prepared epinephrine-containing solutions of local anesthetics (which have a much lower pH due to the addition of anticoagulants) compared to plain local anesthetic solutions. A recent review of the literature involving brachial plexus block concluded that there was little reason to add sodium bicarbonate with plain local anesthetics or those with freshly added epinephrine.¹¹ These results were substantiated in a study by Candia,¹² which reported no difference in the onset or duration of combined lumbar plexus-sciatic block in patients that received 0.5% bupivacaine with bicarbonate compared to those who received a non-alkalinized solution.

COMPLICATIONS OF LOWER EXTREMITY PERIPHERAL NERVE BLOCKS

Auroy prospectively evaluated serious complications following 21,378 PNBs in a 5-month period in France.¹³ Using a 95% confidence interval, the potential for serious complications was estimated per 10,000 PNBs to be 0 to 2.6 deaths, 0.3 to 4.1 cardiac arrests, 0.5 to 4.8 neurologic injuries, and 5.0 to 11.2 seizures. There is a paucity of reports of complications specifically associated with lower extremity PNBs as compared to upper extremity PNBs. This is most likely related to decreased use rather than inherent safety of the techniques. Complications can occur with lower extremity PNBs and it is up to the anesthesiologist performing the block to be aware of these complications and be vigilant in preventing them.

LOCAL ANESTHETIC SYSTEMIC TOXICITY

The potential for systemic local anesthetic toxicity would seem to be very high for lower extremity PNBs. Relatively large doses of local anesthetic are employed for combined femoral and sciatic nerve blocks to anesthetize the entire lower extremity, but there are only a few case reports of local anesthetic toxicity associated with lower extremity PNBs. For instance, Fanelli reported a series of 2175 patients undergoing femoral-sciatic combined blocks in which there were no systemic adverse local anesthetic reactions reported (86). The apparent margin of safety seems to vary with individual block techniques. For instance, there are no case reports of toxicity after popliteal sciatic blockade, whereas there are several case reports of severe toxicity following lumbar plexus and sciatic nerve blocks.¹⁴⁻¹⁷ Differences in anatomy, primarily in the vasculature and presence of deep muscle beds in the area blocked, are the most likely explanation for this discrepancy. Interestingly, severe toxic reactions typically occur during or immediately after the injection. This suggests that the mechanism of these events is most commonly an unintentional intravascular injection of local anesthetic into the circulation, rather than absorption.^{18,19,20} A forceful rapid injection of local anesthetic carries a much higher risk of local anesthetic toxicity than a slow, gentle injection.^{14,21} This is because the mean dose of local anesthetic that elicits the signs of CNS toxicity is much less during rapid intravascular injection as compared to that associated with slower absorption after appropriate deposition of the local anesthetic. After a lower extremity PNB, local anesthetic levels peak at approximately 60 minutes following injection. Perhaps this slow time-to-peak level in the bloodstream offers an explanation for the low incidence of toxic complications associated with absorption of local anesthetics after PNB. Important measures to decrease the risk of severe toxicity include the use of epinephrine as an intravascular marker, slow and methodical injection while avoiding high-injection pressures, frequent aspiration and constant assessment of the patient and vital signs, and prudent selection of local anesthetic concentration and volume.

PROXIMAL SPREAD (NEURAXIAL BLOCK)

Another potential needle misadventure is intrafascicular spread of the local anesthetic proximally toward the spinal cord, resulting in central neuraxial blockade (10,11,17). This is of particular concern with block techniques that involve needle placement at the level of the nerve roots or spinal nerves, such as paravertebral and PCBs. Forceful, fast injections under high pressures

into dural cuffs or perineurium can result in unintentional spinal or epidural anesthesia (15,18,19). In a large series of severe complications associated with regional anesthesia, Auroy found that the posterior approach to the lumbar plexus has the highest incidence of complications of the lower extremity PNBs (120). Of the 294 posterior lumbar plexus blocks reported, there were 5 serious complications reported. Three of these complications, 1 cardiac arrest and 2 respiratory arrests, were directly attributed to cephalad diffusion of the local anesthetic to the epidural or intrathecal space. Managing this block with the same degree of vigilance as for a neuraxial block is essential to detect and treat these complications early if they should occur.

HEMORRHAGIC COMPLICATIONS

Several approaches for PNBs of the lower extremity involve deep needle insertion, including the PCB, the obturator nerve block, and the parasacral and classical approaches to the sciatic nerve. Despite the proximity to vascular and hollow viscous structures of these deep nerves, there are relatively few reports of needle misadventures in published reports. Retroperitoneal hematoma formation following PCB has been reported(4). In order to reach the lumbar plexus, the needle must transverse multiple muscle and other tissue layers. The combination of deep location and inability to apply direct pressure after an inadvertent puncture of deep blood vessels makes this block less suitable in the setting of anticoagulation as compared to other, more superficial lower extremity PNBs.

INFECTIOUS COMPLICATIONS

There are no case reports of infection following single injection, lower extremity PNBs. However, Cavillon reported incidences of bacterial complications associated with the use of continuous PNBs.²² In a cohort of 208 patients, 57% had positive bacterial colonization of the catheter at 48 hours postoperatively. Three patients had transitory symptoms of bacteremia that resolved after removal of the catheter. There were no long-term sequelae related to these positive catheter cultures. Two case reports of *psa* abscesses requiring drainage and intravenous antibiotic therapy has been reported in patients who received a continuous PNB. This stresses the importance of keeping a sterile field when performing a continuous catheter placement.

NEUROLOGIC COMPLICATIONS

Neurologic injury is an infrequent but often feared complication of PNBs and may be present in 0.4% to 1.8% of

all nerve block procedures.^{23,24,25} Most reports of neurologic complications after PNBs are related to upper extremity nerve blocks, a fact that more likely reflects the higher use of upper extremity blocks rather than an unique sensitivity to nerve injury in the upper extremity. The symptoms of nerve injury after PNB usually manifest shortly after block resolution. The perception and presentation of symptoms are typically influenced by the origin of the nerve lesion and other confounding factors, such as postoperative pain, immobility, effects of surgery, operative position, and the application of casts, dressings, and bandages. Intensity and duration of symptoms also vary with the severity of the injury. From light, intermittent tingling and numbness lasting a few weeks, to persistent and painful paresthesias, neuropathic pain, and sensory and/or motor deficits that can last for several months or years.²⁶⁻²⁹ Some nerve injuries result in a permanent neurologic deficit or evolve into severe reflex sympathetic dystrophies.³⁰ Unfortunately, current understanding of the factors that lead to neurologic complications after PNBs is limited. This is partly the result of our inability to conduct meaningful retrospective studies owing to a lack of standard and objective documentation procedures for PNBs. In the absence of objective data, published discussions of the factors that lead to nerve injury, as well as medico-legal reviews, are often speculative at best. Intraneural injection is a well-known mechanism of neurologic injury associated with PNBs.³¹⁻³⁴ However, in current clinical practice, there is no consensus on the techniques or methods that can reduce the risk of intraneural injection. Much of the debate has focused on methods of nerve localization (i.e., paresthesia versus nerve stimulation). However, there is still no evidence that one method is safer than the other, and neurologic injury can occur even with experienced practitioners using either technique.³⁵ Recent reports have also suggested that nerve stimulator assisted nerve localization may not eliminate the risk of intraneural needle placement. These reports document that paresthesia (presumably caused by needle-nerve contact or intraneural placement of the needle) may be present without a motor response to nerve stimulation even with current intensity as high as 1.5 mA.^{36,37} In addition, there are currently no manufacturing standards for nerve stimulators, and older models may be inaccurate.³⁸ Use of short-bevel needles and a nerve stimulator is generally believed to result in a lower risk of nerve trauma.³⁹ Still, the advantages of short-bevel over sharper, long-bevel needles remain controversial.⁴⁰ Educational material in regional anesthesia suggests

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that lancing pain and high injection pressure may portend intraneural injection of local anesthetic, thus increasing the potential for nerve injury.^{14,15} However, a number of case reports suggest that pain is not a reliable warning sign of impending nerve injury.¹⁶⁻¹⁸ Because greater force required to perform an injection may be associated with intraneural injection, anesthesiologists often rely on a subjective evaluation of injection pressure during PNB to detect abnormal resistance to injection and prevent intraneural injection.^{19-21,22-24,25} Indeed, an earlier study performed in rabbits suggested that intraneural injections result in higher injection pressures than perineural (Tourniquet) injections.²⁶ More recently, studies in large animal models documented that high injection pressures (>20 psi) with intraneural needle placement lead to nerve injury.²⁷ Unfortunately, clinical perception of an abnormally high resistance and pressure required to inject is impossible to verify because clinicians vary widely in what they perceive as appropriate force and rate of injection during PNB.²⁸ Since current clinical practice does not allow for meaningful assessment, monitoring, and documentation of the injection technique, objective monitoring of injection pressures may prove beneficial to avoid pressures known to be capable of injuring the nerve fascicles.²⁹ Neuronal ischemia can occur from a variety of sources including disruption of the neuronal microvasculature, high endoneurial pressures, addition of vasoconstricting agents, and exogenous compression from tourniquets,³⁰ although a combination of these events is most likely required for development of nerve injury. The perineurium is a tough and resistant tissue layer. An injection into this compartment or into a fascicle can cause a prolonged increase in endoneurial pressure, exceeding the capillary perfusion pressure, which can result in endoneurial ischemia.³¹ Addition of vasoconstricting agents theoretically can enhance ischemia because of the resultant reduction in blood flow. Addition of epinephrine has been shown in vitro to decrease blood supply to intact nerves, however this has not been shown to be a risk factor for development of post block nerve dysfunction in patients having lower extremity PNBs.³²⁻³⁴ An additional source of ischemia may be the application of a tourniquet, particularly over

the site of a nerve block. Post-tourniquet palsy (Tourniquet paralysis) has been reported to occur in 1 in 3,000 operations.³⁵ However, the paucity of reported severe complications, despite the widespread use of tourniquets, suggests that peripheral nerves are relatively resistant to ischemia of limited duration and magnitude.³⁶ The potential for neurotoxicity with a local anesthetic is a function of its potency, concentration, and the length of exposure of the neuronal tissue to the agent. Local anesthetics are used in concentrations that, under normal clinical conditions, do not cause irreversible nerve damage. Under normal circumstances, the concentration of local anesthetic at the injection site decreases quickly because of dilution by interstitial fluids and absorption in the blood. For this reason, local anesthetic solutions used in vivo are of a higher concentration than what is needed for nerve block in vitro. Nonetheless, exposure of the endoneurium (as may occur during intraneural injection) to a very high concentration of local anesthetic, particularly when coupled with intraneural pressure and ischemia, may contribute to the increased vulnerability of the nerve to neurologic injury.³⁷⁻⁴⁰ While neurologic complications of PNBs are uncommon, they can and do occur even in the hands of experienced practitioners⁴¹ and may result in significant patient suffering, distress to the practitioner, and medico-legal consequences. In summary, recent developments in the field of regional anesthesia have led to an increased interest in lower extremity PNBs. These include reports of transient neurologic symptoms associated with spinal anesthesia, increased risk of epidural hematoma with the introduction of new anti-thrombotic prophylaxis regimens, and evidence of improved rehabilitation outcome with continuous lower extremity PNBs. Research and focus on functional regional anesthesia anatomy have significantly contributed to the ease and success rate of lower extremity nerve blocks. More widespread acceptance of nerve blocks in clinical practice will depend on our ability to complete the transformation of this subspecialty field of anesthesiology to a more objective, standardized and reproducible practice with more clearly defined indications (Table 1) both to improve their clinical utility and to reduce the risk of complications.

LOWER EXTREMITY SURGERY

Knee arthroscopy Patella tendon and ACL repair Saphenous vein stripping Open reduction internal fixation of patella fracture Pain management after hip and knee surgery	Lumbar plexus block Femoral nerve block	Genic, portion of the hip and knee anterolateral and medial thigh, and medial shin below knee are anesthetized Femoral nerve block combined with a peritendinous nerve block is an effective choice for saphenous vein stripping For complete anesthesia of the leg, femoral or lumbar plexus blocks must be combined with static block When choosing femoral or lumbar plexus block - a lumbar plexus block should be chosen when anesthesia of the lumbar plexus (lateral femoral cutaneous nerve, obturator nerve) is sought Femoral nerve block with large dose of local anesthetic will not result in lumbar plexus blockade regardless of volume used
Above and below knee amputations Any surgery on the tibia and/or fibula Achilles tendon repair Foot surgery Pain management after surgery on the lower extremity	Sciatic nerve block	Combined with a lumbar plexus or femoral block results in complete anesthesia of the lower extremity Can be combined with a saphenous block or "low-volume" femoral block to achieve complete anesthesia of the leg below the knee Femoral nerve block is not an adequate supplement for above the knee surgery because the obturator nerve and lateral femoral cutaneous nerve of the thigh are not anesthetized and may be important for complete anesthesia a lumbar plexus block is a better choice
Ankle surgery Foot Achilles tendon repair Shunt saphenous vein stripping	Popliteal nerve block	When surgery involves the medial aspect of the lower leg a saphenous nerve block is needed Success rate is volume dependent Popliteal block is chosen over ankle block for more proximal, more extensive surgery and surgery requiring the use of tourniquet

TABLE 1. Choice of peripheral nerve block technique for common surgical procedures of the lower extremity Reproduced with Permission from: New York School of Regional Anesthesia (NYSORA). <http://www.nysora.com> (December 29, 2004)

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ABSTRACT

KEY WORDS: thoracolumbar spine fractures, load-sharing classification, short segment fusion

Fracture anatomy should never be used as a single criterion to determine fracture treatment because if there is no neurological deficit, any fracture can be treated either operatively or nonoperatively. The majority of the spine fractures, especially in the thoracic spine can be successfully treated nonsurgically with bracing or adequate length of the bed rest. Patient selection is fundamental for nonoperative management. Besides anatomical characteristics of a fracture other factors critical for fracture management include patient's age, general health, expectations, and compliance.¹⁶ An overactive, debilitated, demented or noncompliant patient who is unwilling or unable to follow treatment instructions is particularly at risk

BED REST Treatment with bed rest is not a preferable choice any more. Prolonged bed rest even for a few days increases a risk of deep venous thrombosis (DVT), pulmonary complications and pressure decubiti formation. If for any reason patients with spine fractures should be on a prolonged bed rest a chemical and/or mechanical DVT prophylaxis with aggressive pulmonary toilet should be initiated. Patients with spinal cord injuries treated with or without surgical stabilization are especially prone to these complications. Careful daily skin inspection especially around bony prominences and skin care with washing, massage, powdering and application of lotions are essential. Because of the above mentioned complications related to prolonged recumbency, bed rest is no longer considered as an accepted initial treatment method. If a fracture can not be maintained stable enough in a brace and patient

It is accepted that compression fractures with up to 50% loss of kyphosis can be successfully treated with braces. Spine fracture types such as a flexion-distraction fractures with bony involvement (Bony distal belt fractures, bony Chance fractures) can be successfully treated with bracing. Flexion-distraction injuries of the thoracolumbar spine with complete disruption of the ligamentous structures and, or no minimal bony involvement (Soft seat belt injuries, soft tissue Chance fractures) are not suitable for bracing and should be stabilized surgically. A minority of authors has made the attempts to popularize bracing for certain types of burst fractures but that has not been widely accepted. For the majority of the thoracolumbar fractures use of a high profile thoracolumbar orthosis (TLSO) is recommended. High profile TLSOs extend from the sternum

CASTING

Body casting with either plaster of Paris or fiberglass is not used any more due to a patient's inability to effectively perform skin care and hygiene. Casts are heavier and their fit and effectiveness depends on the skill of the person applying them. Soft limboskeletal casts are used in treatment of osteoporotic fractures in elderly patients whose diminished physical strength reduces their ability to carry a somewhat heavy TLSO. They are usually used in conjunction with a walker in order to decrease axial load of the upper body on the broken vertebra.

skin of the spinal canal can cause long-standing radicular symptoms and/or spinal cord myelopathy much later from the time of injury. Possible consequences of chronic neurological compromise include arachnoiditis, syringomyelia, and late-onset vascular compromise.

LENGTH OF FUSION: SHORT VERSUS LONG SEGMENT FUSION

In preoperative planning of a surgical treatment of a spine fracture, the surgeon must determine the length of fusion and the surgical approach that will achieve stable fixation but preserve maximal spinal motion postoperatively. The fracture location (thoracic, thoracolumbar, or lumbar spine) influences the choice of length of fixation. Longer fixation in the upper or middle thoracic spine does not reduce the patient's mobility much, because this part of the spine is already naturally limited in motion from its connection with the ribs.

SHORT SEGMENT FUSION

Short-segment fixation (instrumentation one level above and one level below the damaged vertebra) and fusion limits the levels of fusion. The introduction of the pedicle screw-based implants, anterior spinal implants, and better understanding of fracture biomechanics have led to the ability to perform short-segment instrumentation and fusion from either anterior or posterior approach to preserve motion segments.

LONG SEGMENT FUSION

Long-segment fixation and fusion involves instrumentation two or more levels above and below the fracture. In our practice, only patients with grotesque, severely displaced, and comminuted fracture dislocations are treated with long-segment instrumentation and/or combined anterior and posterior procedures, and for patients who are to be noncompliant in brace-wear, based on their premorbid personality, their injuries, or both.

PEDICLE SCREW FIXATION

Pedicle screw fixation has improved the stability of the spinal fusion constructs, produced better correction of the fracture, allowed an application of short segment instrumentation and fusion, produced better retention of intraoperative correction of spinal deformity, and has nearly eliminated nonunion as a complication.^{3,9,17} Use of pedicle screws in fixation of the thoracic and lumbar spine is widespread and there use is popularized even in the cervical spine.⁷ Although complications associated with placement of pedicle screws are multiple (nerve root, spinal cord, and vessel

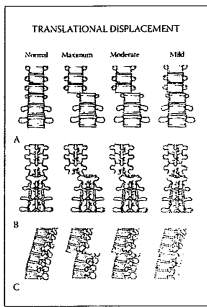


FIGURE 3. Anteroposterior (A), posterolateral (B), and lateral (C) views of translational displacement in a thoracolumbar spine. The drawings on the left represent an intact spine, followed by the drawings of different degrees of translation from gross to subtle. Published with permission.¹⁰

injuries, etc.), their incidence is very rare with the use of a proper technique.^{18,19} We recommend using a technique of direct visualization of the pedicle during placement, the so called "funnel technique". The accurate placement of the pedicle screws is confirmed using intraoperative fluoroscopy. The "funnel technique" was proved to be very accurate based on anatomical and clinical data. Placement of pedicle screw based solely on a tactile feedback, landmarks, or fluoroscopy is dangerous and can produce grave consequences to a patient.

SURGICAL APPROACH: POSTERIOR VS. ANTERIOR

The fundamental principle of load sharing between the implant system and injured spinal column provides good bony apposition at any internally fixed fracture site to permit healing and prevent implant failure.^{10,15} A preoperative analysis of bone fracture anatomy, as well as individual characteristics of the patient, guide the selection of successful candidates for either anterior and/or posterior instrumentation and fusion, and for long- or short-segment instrumentation.⁸

POSTERIOR APPROACH

Posterior approach, especially short-segment fusion can be successfully used only if the support through the anterior and middle columns is adequate to transfer residual load that extends the capacity of the posterior instrumentation.¹⁷ Only when pedicle screws are applied posterior can short segment instrumentation be utilized.¹⁴ Strict analysis of the fracture character using the principles of Load Sharing Classification will allow one to apply posterior short-segment instrumentation and fusion.¹² Utilization of hook and wire constructs require long- or longer segment fusion due to their inherent instability in short-segment constructs.

ANTERIOR APPROACH

In highly comminuted vertebral body injury at the thoracolumbar junction (a total point of 6 or higher on the Load Sharing Classification scheme), anterior strut

grafting and instrumentation is superior to fixation with a posterior instrumentation with pedicle screws. Anterior spinal fusion surgery is a safe procedure which can be used with confidence when the patient's disorder dictates its use. The total complication rate for all complications that were directly attributed to the anterior approach is 11.55%. Serious complications, such as death (0.6%), paraplegia (0.2%), and deep wound infection (0.6%) are rare.¹ Any anterior device (screw-rod and a plate) must be applied laterally on the vertebral bodies, with bicortical purchase assuring no instrumentation penetrating into the spinal canal or contralateral foramen, and performed under intraoperative radiographic control. The success of any anterior instrumentation depends on adequate vertebral body reconstruction. Autologous bone struts are preferable (tricortical iliac crest or fibula), although allografts and titanium or carbon fiber cages, can be used successfully.^{4,10,18}

CONCLUSIONS

Posterior and anterior spinal instrumentation systems are very effective for the reconstruction of severely damaged vertebral bodies of the thoracic and lumbar spine. They can provide immediate adequate reconstruction and retention of the fragments as a solid supporting construct of the spinal columns until the body fusion takes over the load. Most of the complication that occur during or after the surgery can be avoided by careful and appropriate patient selection and excellent operative technique. Operative versus nonoperative management of thoracolumbar fractures should NEVER be made based solely on one factor. Only after a thorough physical, neurological, and spinal examination, and an assessment of patient's prior activity, social and educational background and future plans, we then review the patient's radiographs and CT scans to determine the risks and benefits of operative versus nonoperative care. For those patients in whom operative care is recommended we then determine whether the patient is reliable enough to allow for short-segment instrumentation and fusion.

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EXTRACORPOREAL SHOCK WAVE THERAPY IN ORTHOPAEDIC DISEASES

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ABSTRACT

The application of extracorporeal shock wave therapy (ESWT) as a treatment for different orthopedic conditions has experienced a rapid increase over the last several years. However the mechanism of action and the therapeutic effect is not clear. The aim of this study was to review the literature about the efficacy of ESWT in the treatment of plantar fasciitis, lateral epicondylitis, shoulder painful disorders and non-union fractures. Only randomized controlled studies published in the last 5 years were retrieved from electronic database and manual search. Results on efficacy of ESWT are controversial. Studies that have claimed therapeutic benefit did not fulfill scientific criteria and controlled, randomized trials were not able to confirm significant improvement after treatment with ESWT.

KEY WORDS: ESWT, plantar fasciitis, lateral epicondylitis, shoulder disorders, non-union fracture.

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INTRODUCTION

Originally used for the treatment of kidney stones ESWT has found its place in the treatment of variety of chronic orthopaedic conditions including plantar fasciitis, lateral epicondylitis (tennis elbow), calcific tendinitis of the shoulder, delayed union or non union of fractures, osteonecrosis of the femoral head and other tendinopathies. Declared as a non invasive and safe treatment it has been applied as an alternative to surgical procedures especially in European clinics. Naturally generated shock waves can be observed in thunder and lightning, explosion and supersonic aircrafts. They can produce a dramatic increase in pressure amplitudes. Sometimes this pressure can break the windows and damage ear membranes. The energy released is usually expressed in joules (J) or millijoules (mJ) and pressure in kilopascals (kPa) and megapascals (MPa). Animal experiments performed 20 years ago revealed that shock waves had the potential to activate osteoblast cells and stimulate bone formation. Delayed and non-union fractures healed faster after being exposed to the shock waves (1). This and other research papers published in the eighties and early nineties introduced the new concept of orthopaedic "Extracorporeal Shock Wave Therapy (ESWT)". Before that shock waves were used mainly for kidney stone lithotripsy.

SHOCK WAVES:

PHYSICAL PRINCIPLES

A shock wave can be defined as a sonic pulse with the following characteristics:

1. High Peak Pressure (50 MPa)
2. A short life cycle (10 ns)
3. Fast pressure rise (less than 10 ns)
4. A broad frequency spectrum

In practice there are three methods applied in shock wave generation:

1. Electrohydraulic principle
2. Electromagnetic principle
3. Piezoelectric principle

All these techniques convert electrical to mechanical energy. They are called focused shock waves and their main characteristics are a very high peak pressure (10 – 100 MPa) and a very short rise time (less than 1 micro second).

Recently another principle for generating pressure waves was implemented, so called a ballistic, radial or unfocused pressure wave (Figure 1).

Ballistic pressure pulses are significantly lower in peak pressure (0.1-1 MPa), feature a longer rise time (more than 1000 micro seconds) and pulse duration. The effect of ballistic pressure pulses is strongest at the surface point of impact and that is why their therapeutic effect is limited to superficial targets. Depth of penetration is 3 to 3.5 cm. This type of ESWT has been used mainly in physiotherapy. The shock waves propagate through a homogeneous soft tissue with a speed of 1500 m/sec with negligible distortion. However, facing the tissue inhomogeneities the shock wave will be modified by deviation from straight propagation, reflection, absorption etc. As a result a localized release of shock wave energy occurs. The direct effect of the energy released at interfaces of different tissue as well as the indirect effects of colliding cavitation bubbles is assumed to initiate the healing effects of shock wave therapy (2).

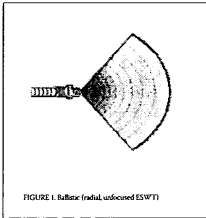
MOLECULAR BIOLOGICAL MECHANISMS OF ESWT

Although ESWT has been used in orthopaedic practice for many years, the molecular mechanism of its action is poorly understood. It has been postulated that mechanical energy converted from high frequency electricity, triggers the release of free radicals, vasodilatation and successive angiogenesis. Among the free radicals the role of nitrogen oxide (NO) was particularly investigated.

Properties of NO include:

- Vasodilatation by relaxation of smooth vascular musculature through cGMP stimulation.
- Macrophage activation to increase immune defense and cytotoxicity.
- Neurotransmitter

There are three isoenzymes of NO synthase (NOS) which using L-arginine as a substrate catalyses the synthesis of NO and citrulline. These are: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Experimental work on rats confirmed that calcium independent NOS activity was significantly increased after treatment with ESWT. Increase was observed 1. day and persisted for 28 days after ESWT. Calcium dependent NOS activity was evidently elevated 14 days after treatment (3). Immunohistological observation demonstrated that mesenchymal cells at fibrous tissue and chondral cells at hypertrophic cartilage displayed intensive iNOS expression. Chondral cells and osteoblasts at newly formed woven bone



junction appeared evident eNOS expression. Mature osteoblasts and osteoclasts at vascularized woven bone area showed strong nNOS immuno-reactivity. Based on these results one can conclude that NOS acts an important mediator to translate physical ESW treatment into biological reaction for mesenchymal cell proliferation and differentiation in the area of bone defect after exposure to ESW treatment. The eNOS is involved in new cartilage and bone formation. The iNOS is probably to mediate bone remodeling and angiogenesis (3). The role of mitogen activated protein kinase (MAPK), including extracellular signal-regulated kinase (ERK) and p38 kinase (p38) on bone formation was also investigated. Animal model was used for experiment. Results suggest that ERK is active in the process of bone regeneration of segmental defect. The p38 is likely act important role in signaling cartilage formation in callus. ERK and p38 act as an important mediator to translocate biological signal into nuclei of cells to initiate mitogenic and osteogenic transcription of osteochondrogenic cells (4).

ANALGESIC EFFECT OF ESWT

Experimental studies in vitro confirmed three major effects of ESWT. These are:

- Physical effects (mechanical pressure, cavitation)
- Chemical effects (diffusible radicals)
- Biologic effects (increase of membrane permeability) (5)(6)(7).

The relationship between these effects and pain relief is not clear. So far the mechanism of analgesia produced by ESWT is uncertain. Induction of neovasculariza-

tion associated with mechanical and cavitation properties of shock waves are the most common proposed model (8). However gate control mechanism is also suggested (9). Decreased level of calcitonin gene-related peptide (CGRP) in dorsal root ganglia in rats was found after treatment with ESW. This could be related to pain relief (10). The possible role of spinal endogenous opioids (met-enkephalin and dynorphin) was also investigated. The level of these substances remains the same at 4 and 72 hours after exposure to ESWT. According to these results there is no proof that endogenous opioids are responsible for analgesic effect of ESWT (11).

CLINICAL STUDIES ON ESWT

Throughout the last 10 years there have been many publications about ESWT in orthopaedic treatment. Most of these publications were based more on clinical than on scientific background. While ESWT has been proved effective particularly for treatment of plantar fasciitis and tennis elbow, there have been very few randomized, prospective placebo controlled, double blinded studies performed. After reviewing the literature published in the last 5 years we selected only randomized control study articles. In a prospective randomized placebo-controlled double blind study an experimental group received ESWT of 1000 impulses of 0.08 mJ/mm for treatment of plantar fasciitis. 88% were pain free or had good results. None of placebo group was pain free, only 33.3% had good results. The treatment group showed significantly better outcome for morning and resting pain, pressure stamp-tolerance and walking ability. ESWT has been proved effective in treatment of plantar fasciitis (12). In another single blind randomized study on 60 patients with calcaneal enthesophytosis (calcaneal spur) it was found that the group treated with ESWT showed significant decrease of pain on the visual analog scale (VAS) and reduction of enthesophytosis larger than 1 mm on x-ray. Such improvement was not registered in placebo group (13). The effect of ESWT in patients with chronically painful proximal plantar fasciitis was compared with conventional conservative treatment including kinetophoresis with Doleferac and oral nonsteroidal anti-inflammatory drugs. Two years after treatment both groups showed pain decrease during activities of daily living. There was no statistically significant difference between groups (14). A randomized placebo-controlled, multiply blinded, crossover study conducted on patients with chronic plantar fasciitis concluded that the application of electrohydraulic high-energy shock waves to the heel is a safe and effective noninvasive method to treat chronic

ic plantar fasciitis, lasting up to and beyond one year (15). Contrary to previous results, randomized, blinded multicentre trial study comprised 272 patients with chronic plantar fasciitis did not confirm better result with ESWT than with placebo. Authors concluded that ESWT is ineffective in treatment of chronic plantar fasciitis (14). After reviewing clinical trials on ESWT and plantar fasciitis Boddeker and co - authors found that many studies are incomplete and methodologically inadequate. After analysis of 21 relevant articles authors concluded that efficacy of ESWT can be neither confirmed nor excluded (17). Like plantar fasciitis results of treatment of lateral epicondylitis (tennis elbow) are also controversial. Perlick and co-authors evaluated the outcome of the ESWT and the operative treatment. They concluded that taking into account duration of symptoms, large scale of the primary treatment and operative hazards ESWT could be considered beneficial in the treatment of patients suffering from lateral epicondylitis (18). In a multicentre, blinded, randomized, placebo-controlled study 228 patients with chronic lateral epicondylitis were randomized to either active ESW treatment or to placebo group. Treated group received 1500 shocks directed at the affected site at a power of 18 kV. Authors concluded that ESWT was more effective than placebo in the treatment of chronic lateral epicondylitis, although both treatments might need to be performed to enhance the level of successful outcome (19). However, recently published papers were not able to confirm efficacy of ESWT in treatment of tennis elbow. Study performed on 40 patients randomly allocated to receive 1 session per week for 3 weeks of either sham or active ESWT revealed significant difference neither in pain reduction nor in quality of life between two groups (20). Review of the literature about ESWT and lateral epicondylitis was published in two articles. In the first one, a total of 20 studies were identified, 2 of which had been carried out as prospective, randomized and controlled studies. Regarding to clinical success, 40-80% of the patients achieved a good or very good result at follow up examinations after 3-12 months. However, in the single placebo-controlled and blinded study with the independent observer there was no difference between the ESWT group and the group treated with local anesthetic (21). In another study, 20 relevant articles were analyzed and it was concluded that at this point the efficacy of ESWT can be neither confirmed, nor excluded (22). The next common conditions treated with ESWT were degenerative painful disorders particularly calcifying tendinitis of the rotator cuff. In prospective study 115 patients diagnosed as tendinitis calcarea of the rota-

tor cuff were treated with high energy ESWT once or twice. Subjectively 78% of group A (once treated) and 87% of group B (twice treated) judged the treatment to be successful. A radiographic examination 4 years after treatment revealed complete or partial resorption of the calcium deposit in 93% in both groups (23). In another study after comparing ESWT with radiation therapy authors concluded that ESWT appeared to be equivalent but not superior to radiotherapy (24) (25). A controlled, randomized study on the effects of low-energy shock wave therapy on the function and pain in tendinitis of the supraspinatus without calcification was performed. The patients receiving either ESWT or sham ESWT were examined 6 and 12 weeks after treatment by an independent observer. The statistical analysis showed no difference in results between the groups (26). The therapeutic effect of ESWT in shoulders with chronic calcific tendinitis was compared with transcutaneous electric nerve stimulation (TENS). Although the pain and calcium deposits were reduced significantly in both groups ESWT appeared to be more effective than TENS (27). Another double-blind, randomized, placebo-controlled trial to compare effect of high-energy, low energy (ESWT) or placebo on patients with chronic calcifying tendinitis of the rotator cuff was conducted. The result showed that both high energy and lower energy ESWT were significantly more efficient than placebo. Furthermore, high-energy ESWT appeared to be superior to low energy ESWT (28). Treatment of fracture non-union was another interesting field for ESWT. Birnbaum and co authors reviewed 52 papers referring to ESWT of the locomotor system. Conclusions regarding to possible applications in therapy were taken only from high quality publications of types A and B according to internationally accepted system of the American Association that treat the spinal problems. Most of the investigations showed a consolidation of the non union during a period of 3 months following ESWT. However long term results are not available (29). ESWT is usually considered as a safe therapeutic procedure with mild and temporary side effects. However particular studies on the side effects of ESWT are very rare. One of them compared side effects of ESWT with placebo in patients with lateral epicondylitis. A total of 272 patients from 15 centers were allocated at random to active ESWT or placebo ESWT. More side effects were recorded in the ESWT group than in placebo group. Most frequently were reddening of the skin (21.4%), pain (6.8%) and small haematomas (5.4%). Migraine was registered in 4 and syncope in 3 patients after receiving ESWT (30).

CONCLUSION

For the past 10 years ESWT has been intensively used in orthopaedic practice. At the moment three machines have been FDA approved for the use on humans, two for plantar fasciitis and one for lateral epicondylitis (tennis elbow). Almost all study emphasized high safety of this therapy with minimal side effects. However, results on efficacy of ESWT are still controversial. Many studies that claimed ESWT was efficient in treatment of musculoskeletal conditions did not fulfill scientific criteria. On the other side, most of the well established controlled studies which included double blind or single blind trials were not able to produce such results. There are multitude reasons to consider ESWT experimental and investigational rather than well documented therapeutic modality. The mechanism of action is speculative, difficult to quantify and prove. Treatment parameters have not yet been standardized and optimal dosages and frequency has not yet been established. There is still no consensus on when to differentiate between low and high energy applications. There are no studies to compare the effect of focused versus ballistic (radial, not focused) techniques. One of the biggest advantages of ESWT in comparison to surgery was lower cost and faster healing of the treated tissue. However the surgery for plantar fasciitis, lateral epicondylitis and supraspinatus tendinitis with and without calcification are not very often and there are no enough studies to compare efficacy of ESWT and the surgical treatment on pain relief and functional outcomes. An opinion prevails that ESWT should be used only if other ordinary conservative treatments failed. Taking into account that in many patients, spontaneous resolution of pain and calcification is possible, the abuse of ESWT in the clinical practice should be considered as a serious problem. More studies need to be done for better understanding of the mechanism of action, to define optimal parameters and to create a specific clinical protocol for different musculoskeletal conditions.

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SORE SHOULDER IN PHYSICAL PRACTICE

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ABSTRACT

Sore shoulder or shoulder region is frequently encountered in general population. According to reference data prevalence ranges between 15 and 20% in 40-45 age group. A weak bone support enables extensive excursions of this joint and simultaneously reduces its stability. We analyzed successfulness of sore shoulder treatment in a sample of 1114 patients treated in the Center for physical medicine and rehabilitation between 1996 and 2000. All the patients reported to the Center either in acute phase or in the phase of chronic state exacerbation, with limited function that was graded on the scale 0 to 5. Type and kind of sore shoulder cause was determined by clinical examination and, where needed, by X-ray. Of the total number of cases, 33 patients did not report for follow-up examination, 166 patients were forwarded to other physical therapy centers for treatment so the final analysis included 916 patients (82%) whose treatment success was evaluated on the scale 0 to 5. In 659 (59%) patients the inflammation of musculus biceps long tendon was identified as a cause of sore shoulder. The least frequent cause was the blow syndrome (impingement) – 20 (2%). In 466 patients (51%) the problem was resolved by local instillation of depo corticosteroids (betamethasone 7 mg) so the physical treatment was not required.

KEY WORDS sore shoulder, sore shoulder treatment

INTRODUCTION

Sore shoulder or shoulder region is frequently encountered in general population. According to reference data prevalence ranges between 15 and 20% in 40-45 age group. A weak bone support enables extensive excursions of this joint and simultaneously reduces its stability. Considering that shoulder movement is supported by numerous joints (gleno-humeral, sterno-clavicular, acromio-clavicular, scapulo-humeral) as well as soft tissues, anatomical and biomechanical complexity of shoulder region may hinder evaluation and treatment of shoulder dysfunction. Therefore, detailed evaluation of each of the mentioned structures is of utmost importance. Evaluation of spine function in jugular area is of particular importance considering that its dysfunction frequently causes referral shoulder pain.

MATERIAL AND METHODS

We analyzed the results of the treatment of patients suffering from sore shoulder in polyclinic Praxis, in the period 1996-2004. The treatment outcome was evaluated by successfulness estimation. The treatment success is given as an assessment of clinical condition after the treatment, objectively evaluated according to the following scale:

1. grade «0»: zero condition unchanged (no results);
2. grade «1»: minimal improvement;
3. grade «2»: satisfactory functional improvement with sequelae (sensory or motorical);
4. grade «3»: good improvement and satisfactory functional restitution with minimal sequelae;
5. grade «4»: good restitution without consequences of injury or illness.

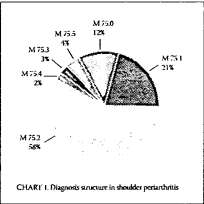
By retrospective analysis we thus registered and sorted all the patients with sore shoulder who were treated with local instillation and physical therapy procedures in physical medicine polyclinic "Praxis". Accompanying clinical findings and physiological measurements such as functional tests, muscle tonus, pain intensity were used in treatment success evaluation for each individual patient according to the given scale. The data are statistically analyzed and presented in Tables and Charts.

RESULTS AND DISCUSSION

Between 1996 and 2004 total of 1115 patients reported for examination in the Center for physical medicine and rehabilitation "Praxis". Table 1 and Chart

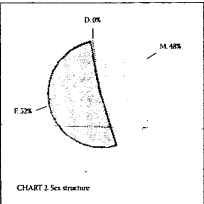
Reported for treatment	48 patients with dg. M75.1
Reported for treatment	26 patients with dg. M75.4
Reported for treatment	30 patients with dg. M75.3
Reported for treatment	659 patients with dg. M75.2
Reported for treatment	229 patients with dg. M75.1
Reported for treatment	129 patients with dg. M75.0
TOTAL M75	1115 patients

TABLE 1. Diagnosis structure in shoulder periarthritis



MALE	538
FEMALE	575
CHILD 7 YEARS AND YOUNGER	2
CHILD 14 YEARS AND YOUNGER	0

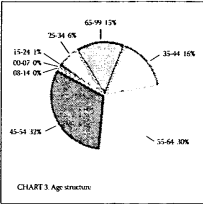
TABLE 2. Sex structure



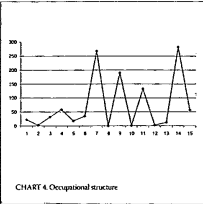
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0-40	05-14	15-24	25-34	35-44	45-54	55-64	65-99
2	2	15	75	177	353	329	182

TABLE 3. Age structure



illustrate numerical data on structure and type of sore shoulder causes. Inflammation of long tendon of musculus biceps was identified as sore shoulder cause in 65% (58%) patients. The least frequent cause was the blow syndrome (impingement) - 20 (1%). Table 2 and Chart 2 represent sex structure of the patients that reported for checkup. The structure is quite balanced with slight prevalence of female patients.



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
22	3	32	59	18	15	268	0	190	1	133	3	12	286	27

TABLE 4. Occupational structure

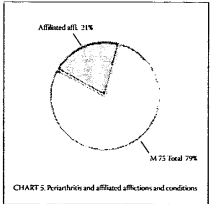
Total of 1115 patients with dg. M75 reported for examination and treatment

Subjected to physical treatment in "Praxis" polyclinic: 250 patients (22.4%)
Forwarded to other centers for physical treatment: 166 patients (14.8%)
Subjected to physical treatment: 416 patients (37.3%)
Did not report for follow up examination after the intervention: 33 patients (2.9%)
Completed the treatment and evaluated: 916 patients (82.1%)
Relapse: 53 patients (4.7%)

Type and kind of sore shoulder cause was determined by clinical examination and, where needed, by X-ray. Of the total number of cases, 33 patients did not report for follow up examination, 166 patients were forwarded to other physical therapy centers for treatment so the final analysis included 916 patients (82%) whose treatment success was evaluated on the scale 0 to 5. Evaluation of treatment success is presented in Table 5 and Chart 5. It is evident that 827 (90%) patients were successfully treated with grade 4 and 5

0	2	3	4	5
2	2	85	230	297

TABLE 5. Treatment results - "Praxis"

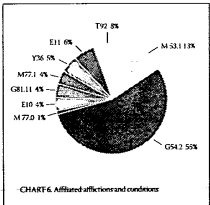


1. LOCAL INJECTION FLUOSTERON-A (BETAMETHASON 2 MG)	989 (27%)
2. MANUAL THERAPY	138 (11%)
3. ACUPUNCTURE	150 (13%)
4. PHYSICAL THERAPY	150 (13%)
TOTAL PROCEDURES	1602 64 procedures /patient
AVERAGE TREATMENT	16 days
PASSIVE EXERCISES	1943
ACTIVELY SUPPORTED EXERCISES	2305
ACTIVE EXERCISES WITH WORKLOAD	68
EXERCISES IN ROTATING CIRCLE	983
SPECIAL EXERCISES FOR SPINE CONNECTION	133
GALVANIC CURRENT THERAPY	40
GALVANIC CURRENT UNDERWATER THERAPY - LOCAL	123
DIADYNAMIC CURRENT THERAPY	1722
ELECTROSTIMULATION- INDIVIDUAL NERVE- BIPOLAR	9
ELECTROSTIMULATION- INDIVIDUAL NERVE- MONOPOLAR	2
INFRARED RAYS THERAPY	10
INTERFERENT CURRENTS THERAPY	135
ULTRASOUND THERAPY	1589
MANUAL MASSAGE OF INDIVIDUAL REGION	1958
MANUAL MASSAGE - GENERAL	23
VACUUM MASSAGE	130
HYDROCOLATOR THERAPY- REGIONAL	2901
PARAFIN THERAPY - REGIONAL	332
TENS	1688

TREATMENT "PRAXIS"

2 to 7	8 to 15	16 to 21	22 to 30	31 to 60	61 to 90
310	294	84	93	97	30

TABLE 6. Treatment length



CONCLUSIONS

Between 1996 and 2004 total of 1115 cases of sore shoulder were registered in the Polyclinic for physical medicine and rehabilitation "Praxis". 916 patients were treated and analyzed. 33 patients did not report for follow up examination while 166 were forwarded to other centers for physical treatment. Inflammation of long tendons of musculus biceps (Mys-a) was identified as sore shoulder cause in 639 (51%) patients. The least frequent cause was the blow syndrome (impingement) - 30 (2%). Most of the patients (639 - 87%) were treated by local instillation of depo-corticosteroids (Betamethason 7 mg). Thus, only 416 (32%) patients required physical treatment (350 patients in Praxis polyclinic and 166 in other centers). In 666 patients (71%) the problem was resolved without physical treatment. Affiliated afflictions are frequent problem with sore shoulder (330 cases or 32.2%). Amongst those, the most frequent affliction is cervical syndrome (16.5%) which requires more complex treatment that involves manual treatment, acupuncture and physical treatment. Evaluation of treatment success is presented in Table 5 and Chart 5. It is evident that 827 (60%) patients were successfully treated with grade 4 and 5. Excellent result (clinical findings evaluation 5 and 4) were achieved in 90% patients in the analyzed sample of 916 cases. Minimal improvement or no result was assigned in 4 patients or 0.4 %.

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MODERN APPROACH
IN TREATMENT OF
DIABETES INSIPIDUS

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ABSTRACT

In this paper we have reviewed the position of desmopressin in the treatment of diabetes insipidus. Desmopressin is a synthetic analog of vasopressin, with more pronounced antidiuretic effect. It is treatment of choice in substitution therapy of diabetes insipidus. Its application before sleeping time can reduce nocturnal enuresis, so it has a place in the treatment of *enuresis nocturna*. Antidiuretic effect of desmopressin is the result of agonistic effect on V₂ receptors in the renal tubules. The efficacy and safety of desmopressin in mentioned indications was confirmed in clinical studies.

KEY WORDS: desmopressin, diabetes insipidus

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INTRODUCTION

Desmopressin is a synthetic vasopressin analog, with enhanced effect and longer duration of action than vasopressin or lysine-vasopressin. Desmopressin differs from naturally-occurring vasopressin by deaminated hemicysteine at position 1 and substituted D-arginine for L-arginine at position 8. This has been shown to diminish vasopressor activity, enhance antidiuretic-to-pressor potency, and prolong the duration of action of the compound. Vasopressin, hormone of neurohypophysis, is released when the plasma osmolality is increased and/or blood pressure reduced. Diabetes insipidus, as a consequence of vasopressin deficit, is characterized with reduced reabsorption of water from collecting tubules of the kidney and patients suffer from polydipsia and polyuria (1).

PHARMACODYNAMIC
CHARACTERISTICS

Desmopressin was synthesized in 1967, and from 1974 is widely used in treatment of diabetes insipidus. Neurohypophysis hormones, vasopressin and oxytocine, are released from endings of neurosecretory neurons which somas are located in paraventricular and supraoptic nuclei of hypothalamus. Vasopressin is released when the plasma osmolality is increasing and/or blood pressure dropping. The actions of vasopressin are mediated through interaction with two types of receptors, V₁ and V₂. Vasopressin acts on V₁ receptors, enhances generation of cyclic AMP in collecting tubules of the kidney and increases reabsorption of water according to the osmotic gradient. That is the essence of its antidiuretic effect. Diabetes insipidus, as a consequence of vasopressin deficit, is characterized with reduced reabsorption of water from collecting tubules of the kidney and patients suffer from polydipsia and polyuria. Desmopressin is a drug of choice in the treatment of diabetes insipidus. Desmopressin is used in management of nocturnal enuresis. Desmopressin given at bedtime to patients with nocturnal polyuria was associated with reduced nocturnal diuresis. With naturally occurring vasopressin (arginine vasopressin) the antidiuretic-to-pressor ratio is 1 (approximately 400 units/mg). Antidiuretic-to-pressor potency of desmopressin is 2000 to 4000x and the duration of action ranges from 6 to 24 hours. This is considerably longer than the duration of action for arginine vasopressin (1 to 6 hours). The prolonged duration of action of desmopressin as compared to arginine vasopressin is most likely due to resistance to enzymatic

cleavage, delayed absorption from nasal mucosa, and enhanced generation of cyclic AMP in the renal medulla (1). In addition, desmopressin may be useful in the treatment of some coagulopathies, hemophilia A and von Willebrand's disease (2). Desmopressin significantly shortens bleeding time in healthy volunteers treated with aspirin in the duration of up to 4 hours. Evaluation of dose and effect ratio in patients with diabetes insipidus has shown that oral doses of desmopressin ranging from 0.025 milligrams to 0.4 milligrams induce significant antidiuretic effects. Desmopressin in dosage range from 0.1 to 0.2 milligrams per day has the optimal antidiuretic effect, in the most patients with duration of 8 hours. In doses of 0.4 milligrams antidiuretic effect lasts for 12 hours. With increasing oral dosages the concentration of desmopressin in plasma is increasing in dose-dependent manner (3). In a study of patients with nocturnal enuresis, patients who were classified as responders to desmopressin therapy experienced increased fractional excretion of sodium at night compared to those classified as non-responders and a group of normal control subjects (4). A lack of circadian variation in the plasma osmolality was observed in both responder group and non-responder group. Normals and responders experienced a steady increase in plasma potassium during the night. Plasma sodium was stable without circadian rhythm in all three groups. Desmopressin responders did not experience significant fluctuation in diurnal variation of plasma vasopressin values but the other two groups did experience significant increases peaking at 05:00 hours (p < 0.05). Only normal control patients experienced significant circadian variations in plasma atrial natriuretic peptide levels (p < 0.001). Patients with complete form of diabetes insipidus experienced significant decrease in plasma triglyceride levels following desmopressin therapy (p = 0.007). However, similarly treated patients with partial diabetes insipidus did not experience significant change in triglyceride levels. Favorable effects of desmopressin on lipid and lipoprotein metabolism in complete diabetes insipidus patients may be due to either direct effects or through modifications of factors that contribute to lipid metabolism (5).

MECHANISM OF ACTION

Desmopressin increases reabsorption of water in collecting tubules of the kidney. Desmopressin, also, stimulates factor VIII and plasminogen activated release. Antidiuretic effect is the result of agonistic activity on V₂ receptors placed in tubules of the kidney. This effect is the result of enhanced reabsorption of water, probably

based on opening of pores and channels which play role in water reabsorption according to the osmotic gradient. Molecular mechanisms of action include stimulation of adenylate cyclase and enhanced synthesis of cAMP. Vasopressin-2 receptors are also placed extrarenally, and play role in factor VIII release. Desmopressin has minimal effect on V1 receptors that are located in smooth muscles of blood vessels walls and numerous different organs (1)

PHARMACOKINETIC CHARACTERISTICS

Desmopressin is absorbed in nasal mucosa. Bioavailability of desmopressin after intranasal administration is 3.3% to 4.1% compared to intravenous or subcutaneous administration (1). After oral administration, large part of desmopressin dose is degraded in gastro intestinal tract, but after high doses there absorption is sufficient for the therapeutic effect (6). After oral administration bioavailability is .5% compared to intranasal administration, and 0.16% compared to intravenous administration. Maximal concentration in plasma and AUC do not increase proportionally to administered doses. Regardless of administered doses, plasma half-life of desmopressin is 1.5 to 2.5 hours (1). Terminal plasma half-life of desmopressin is 4 to 5 hours (7). Distribution half-life of intranasal desmopressin is 7.8 minutes. Desmopressin does not penetrate hemato-encephalic barriers. Volume of desmopressin distribution is 0.2 to 0.3 L/kg. *In vivo* studies using human liver microsomes showed that desmopressin was not metabolized by liver. Desmopressin is almost completely excreted in urine. Elimination half-life after intranasal administration is 0.2 to 0.6 hours.

INDICATIONES AND DOSAGE

Desmopressin is used in the treatment of neurohypophyseal diabetes insipidus, enuresis nocturna primaria, nocturia associated with nocturnal polyuria in adults and in testing of renal function capacity. Also, it is used in management of mild-to-moderate hemophilia A or mild-to-moderate type I von Willebrand's disease. Formulated as nasal spray desmopressin is used in crise prevention of sickle cell anemia by inducing hyponatremia. In patients with uremia, desmopressin reduces bleeding time by rising concentration of factor VIII. Desmopressin dosage should be individualized. Doses are titrated according to the patient's responses and pharmaceutical formulation of desmopressin. The food can reduce intensity and duration of desmopressin small doses antidiuretic effect.

PRECAUTIONS

Drugs which are releasing antidiuretic hormone (tricyclic antidepressives, chlorpromazine, and carbamazepine) can cause additional antidiuretic effect with increased risk of fluid retention. Nonsteroidal anti-inflammatory drugs can cause fluid retention and hyponatremia. Loop-eramide can increase concentration of desmopressin in plasma as well as the risk of fluid retention and hyponatremia. Concomitant desmopressin and glyburide therapy has resulted in inhibition of desmopressin's effects and decreased antidiuretic effect (13). Concomitant desmopressin and dexametson therapy can reduce desmopressin absorption. The food can reduce intensity and duration of desmopressin small doses antidiuretic effect. Precaution is needed in patients with coronary artery disease and/or hypertensive vascular disease, in patients with fluid and electrolyte disbalance, in patients with allergic rhinitis, nasal congestion, and upper respiratory infections who may experience decreased effectiveness (1). In the treatment of primary nocturnal enuresis or nocturia no fluid should be taken 1 hour before and 8 hours after desmopressin administration. Precaution is needed in pediatric patients. Testing of renal concentration capacity in children less than 1 year of age should be done only in hospital and with continuous surveillance. According to U.S. Food and Drug Administration desmopressin is classified in the pregnancy risk category B (3). In diuretic doses desmopressin does not have uterostonic effect. Physician should determine risk to benefit ratio for every individual case. Precaution is needed during lactation, considering that many of drugs are excreted in human milk (3).

CLINICAL STUDIES

Three children with primary nocturnal enuresis had resolution of this condition within 3 days after initiation of desmopressin in dose of 20 micrograms intranasally. One child had primary nocturnal enuresis only, while the other 2 had primary nocturnal enuresis and congenital nephrogenic diabetes insipidus. In patients with congenital nephrogenic diabetes insipidus, mutations in vasopressin-2 receptor (V2R) or aquaporin-2 (AQP2) water-channel genes inactivate arginine-vasopressin proteins. Patients with this mutation are unable to concentrate their urine in response to secretion of arginine-vasopressin. Serum osmolality is therefore high and urinary osmolality low, despite high plasma arginine-vasopressin concentrations. After the 3 children were placed on desmopressin therapy, the child

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with primary nocturnal enuresis slept through the night and did not wet the bed. The 2 youngsters with nephrogenic diabetes insipidus woke up during the night and went to the toilet to empty their bladder. All returned to their previous bed-wetting habits when desmopressin was temporarily halted. With restoration of desmopressin, all 3 ceased bed-wetting. Desmopressin may act outside of the renal area and may act in the central nervous system to resolve nocturnal enuresis (14). Results of a randomized, double-blind, placebo-controlled cross-over study demonstrated that oral desmopressin given to elderly patients with nocturnal polyuria at bedtime (n=17) was associated with reduced nocturnal diuresis and nocturnal voiding and increased uninterrupted sleep. Patients with nocturnal urinary output ≥ 150 ml/min and ≥ 3 nocturnal voids were eligible to participate in this study. Each patient had their dose titrated one week at a time beginning with 0.1 mg followed by 0.2 mg then 0.4 mg. Patients with nocturnal diuresis ≥ 0.5 ml/min were allowed to progress to the next dose level. The optimal dose of oral desmopressin in this population was identified as 0.1 mg administered at bedtime with no dose-response relationship observed (15). Significant mean reductions in nocturnal diuresis and nocturnal voids were experienced by desmopressin patients compared to placebo after 2 weeks of therapy (p<0.0001). No change in 24-hour diuresis was observed. In patients with higher nocturnal contribution to the baseline 24-hour diuresis experienced greatest decreases in nocturnal diuresis with desmopressin therapy. The mean total number of hours slept per night was not changed by desmopressin treatment; however, the longest period of uninterrupted sleep was extended by 1.4 hours. No patient experienced serious adverse event during the study period. Likewise, the amount of sodium excretion, the temporal excretion pattern of sodium, and the difference in mean serum osmolality were not different between the placebo period and the desmopressin treatment period. No dose-response relationship was observed for desmopressin (16). Results of a randomized, double-blind, crossover, and placebo controlled study indicate that children with desmopressin resistant enuresis have nocturnal bladder instability yet some of these patients (2/33) respond to high-dose desmopressin therapy. Children 6 to 16 years of age, who had primary monosymptomatic enuresis at least 6 wet nights during baseline and less than 30% decrease in the number of wet nights while receiving desmopressin dose of 0.4 mg, were enrolled in the study. During the study, patients were treated with desmopressin 0.4 mg, desmopressin 0.8 mg, and placebo for 5

consecutive nights with a washout period of at least 48 hours between treatment periods. During double-blind therapy, the probability of enuresis and nocturia was highest with placebo compared to desmopressin 0.4 mg and desmopressin 0.8 mg (p<0.0001). However, no difference between the two desmopressin groups was observed. The mean number of hours between bedtime and first bladder voiding during treatment periods was significantly smaller for placebo compared to desmopressin (p<0.0001) but again no differences between the desmopressin groups were observed. Nine children were dry during all 5 nights of at least 1 of the 3 treatment periods and were treated with open-label high-dose desmopressin 0.8 mg. Complete dryness was achieved in 5 of these patients. Of 38 patients who received open-label desmopressin 0.4 mg at bedtime combined with oxybutynin 5 mg twice a day, 13 were complete responders and 7 were intermediate responders (number of wet nights decreased by more than 50% compared to baseline but still with enuretic accidents) (17). Intranasal desmopressin therapy administered at bedtime to a cohort of 7 children with ectrophy-epispadias complex and nocturnal incontinence was associated with decreased urinary output at night and six of the seven achieved nocturnal dryness. All patients aged 8 to 12 years had daytime continence yet experienced bed wetting 7 nights per week. Desmopressin therapy was titrated from 10 to 30 µg until patients were dry all night. Nighttime urinary output was decreased from a mean of 230 ml at baseline to 134 ml while on desmopressin. After 2 months of successful therapy, dose was down titrated to the minimum effective dose. Nocturnal dryness was achieved in 6 patients at 20 µg desmopressin and 1 required 30 µg. After initial 1 month, long-term therapy was successful at 5 to 10 µg per night in 4 patients, 20 µg in 2 patients and 30 µg in 1 patient. At 14 months follow-up, all patients remained on desmopressin therapy. Six patients are dry at night while 1 still has nocturia. The only side effect reported was nosebleed in one patient. Body weight, arterial blood pressure, and serum electrolytes were not significantly modified on desmopressin therapy in this patients group (18). One trial (n=155, average age: 8 years) employed intranasal desmopressin 30 µg titrated to a maximum of 30 µg if not dry within 48 hours, then continued for 4 to 6 weeks maintenance therapy. A gradual dosage reduction of 10 µg per month was undertaken after 4 weeks of complete dryness if relapse occurred during tapering or after cessation of therapy; previous dosage was resumed. After an average 28-week treatment duration and 18-month median follow-up, the response

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rate of 85% included 71% who remained completely dry without relapse, 2% who achieved complete dryness with relapse, and 7% who showed some improvement (less than 3 enuretic episodes per week) (19). In a study of 25 patients (average age 14 years) with severe enuresis (5 nights/week at baseline), oral desmopresin 200 to 400 µg applied for 6 months resulted in complete dryness in 8 of 23 evaluated patients (35%). An additional 30% and 17% achieved cure within 2 and 6 years after the study, respectively. After 7 years, in a follow-up, 9% still experienced enuresis and 9% required continuous desmopresin to maintain dryness (20).

CONCLUSION

Desmopresin is a drug of choice for substitution treatment of diabetes insipidus. Antidiuretic effect of desmopresin is a result of agonistic activity on V2 receptors placed in tubules of the kidney. Molecular mechanism of action includes stimulation of adenylate cyclase and enhanced synthesis of cAMP. Except enhancing reabsorption of water in collecting tubules of kidney, desmopresin plays role in factor VIII release and it is plasminic activator. In addition, it is used in the treatment of coagulopathies, hemophilia A and von Willebrand's disease. Desmopresin is, mostly, well tolerated. The most common adverse effects are fluid retention, hyponatremia, seizures, abdominal cramps, headache, nausea, vomiting and epistaxis.

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OUR EXPERIENCES
IN KIDNEY
TRANSPLANTATION AND
MONITORING OF KIDNEY
GRAFT OUTCOMES

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ABSTRACT

Aim of kidney transplantation is to keep the functions of graft as long as possible, with an improvement of survival and quality of patients' lives. Aim of this article was to show the outcome of kidney transplantation in patients who were treated and monitored on Institute of Nephrology, CCU of Sarajevo in period between 1990 and 2004, and to identify the factors which can interfere with graft surviving. Retrospective analysis of data from the register of kidney transplanted patients was done. In the mentioned period 29 kidney transplants were performed, and at the same time 15 previously transplanted patients (total of 44) were monitored. Patients were followed until death or graft insufficiency. Most often cause of chronic renal failure before the transplantation were glomerular diseases (54.5%), and chronic pyelonephritis in 29.5% cases. Living-donor related kidney transplantation was performed in 56.8% of patients, living donor unrelated in 27.3% of patients and cadaveric in 15.6% of patients. Post-transplant complications occurred in 29.5% of patients. Analysis of graft surviving on 12 months, 2 years and 10 years monitoring showed functional grafts in 87.3%, 80% and 75.4% of patients. Cumulative survival of patients on one year monitoring is 100%, on 2 year 100%, and on 10 year 93.8%. Primary causes of graft function loss were recurrent kidney diseases. Three patients (6.8%) died due to concomitant diseases, irrespective of the transplantation. Kidney transplantation is a successful treatment of a chronic renal failure with a high percentage of patients survival and long-term graft survival, but also with serious post-transplant complications.

KEY WORDS: kidney transplantation, survival, complications

($p < 0.05$ was considered as being significant). Multivariate analysis was determination method of relation between the individual variables and graft surviving.

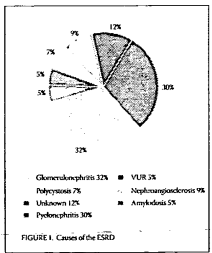
Causes of the ESRD before the kidney transplantation are shown on figure 1. The most often causes were glomerular diseases (32%) and chronic pyelonephritis (30%). Living-donor related kidney transplantation (Tx) was performed in 56.8% of patients, living-donor unrelated in 27.3% of patients and cadaveric in 15.9% of patients (Table 1).

Demographic characteristics of patients are shown on Table 2, referring to the average age of donors and recipients of the organ at the time of transplantation and their gender structure. Analysis of recipient distribution according to the gender shows that the percentage between the genders is almost equal in all observed groups (3:1

PATIENTS AND METHODS

LIVING-DONOR RELATED TX	56.8%
LIVING-DONOR UNRELATED TX	27.3%
CADAVERIC TX	15.9%

TABLE 1. Kidney transplantation types



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TABLE 2. Demographic characteristics of patients

TABLE 3. Duration of the dialysis treatmentTABLE 4. Frequency of occurring the DGF

TABLE 4. Frequency of occurring the DGF

TABLE 5. Frequency of occurring the acute graft rejection

TABLE 6. Other posttransplantation complications

Frequency of occurring other complications in the early period after transplantation is shown in the table 6. The acute renal insufficiency (ARI) occurred in 6.8% of patients, and the reasons for this clinical syndrome were urosepsa (*Escherichia coli*), CMV infection, sepsis due to fleigmon of foot (*Staphylococcus aureus*).

Within the observed population 11.3 % of patients had CMV infection. The recidive thromboembolism and the malaria infection occurred in the group of living unrelated transplantation. Mentioned complications are shown in comparison with the number of patients within a certain forms of transplantation.

Survival analysis for 12 months, 5 years and 10 years show that proper functional graft during the first post-transplant year is present in 87.3% of cases, in patients on 5 year monitoring in 80% of cases, and in patients on 10 years of monitoring in 75% of cases (figure 2). Survival of patients on one year monitoring is 100%, 5 years of monitoring 100% and 10 years of monitoring 93.8%. Pre-

The acute rejection (AR) manifested in 15.0% of patients and in almost same ratio between the groups of living related and living unrelated transplantation (table 5).

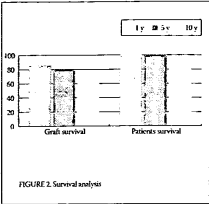


FIGURE 3. Survival analysis

NUMBER OF PATIENTS	AGE AT THE MOMENT OF GRAFT LOST (Y)	DURATION OF THE GRAFT SURVIVAL (Y)	DURATION OF POST TX SURVIVAL OF PATIENTS (Y)	CAUSES OF GRAFT LOST
8 (18.2%)	37.0±10.1 (21-69)	8.6±7.2 (3-22)	11.5±6.3 (3-22)	transmitted diseases 37.5% +not taking medications 72.5% CAN 13.5% death with function graft 37.5%

TABLE 9. Characteristics of patients with graft lost

TOTAL OF PATIENTS	DECEASED	CAUSES OF DEATH	NUMBER (%)
44 (100%)	3 (6.8%)	Cerebrovascular Alzheimer CVD	1 (2.3%) 1 (2.3%) 1 (2.3%)

TABLE 10. Causes of death

survival (table 3) showed that the risks of graft insufficiency within the first year after the transplantation refer to the appearance of the acute rejection ($p=0.003$). Primary cause of the graft function lost in patients on 5 years of monitoring are recurrent kidney diseases (15% of patients, table 8), while chronic allograft nephropathy + CAN (13.5% of patients) is main cause of losing graft function in patients on 10 years of monitoring. In overall period in 18.2% of patients graft lost occurred. Recurrent kidney diseases and death with functional graft were the most common cause of late graft lost. Causes and duration of graft survival and patients in the group with lost grafts is shown in table 9. In observed population of patients, 3 patients (6.8%) died due to comorbidity, all with functional renal graft. Causes of mortality are shown on table 10. Median of patients survival is 16.7 years. Malignancy of the skin (basocellular epithelioma) appeared in one patient, 10 years after the transplantation and post-transplant diabetes in 4 patients (9.0%).

	ODDS RATIO	P-VALUES
ACUTE REJECTIONS	0.09	0.003
DDG	0.007	0.002
DONOR > 50 YEARS	0.0535	<0.001
LOW HLA MACH	0.0000022	<0.001
MMF	0.233	0.004

TABLE 7. Multivariate analysis of graft survival

MEMBRANOPROLIFERATIVE GN TYPE II	5%
MEMBRANOUS GN	5%
AMYLOIDOSIS OF KIDNEYS	5%

TABLE 8. Diseases recurrence on graft – graft loss

DISCUSSION

In post-war period transplant program is limited on living related kidney transplantation and is very modest in quantity due to the lack of living donors. This work is an attempt to show the experiences in the field of kidney transplantations in our Center, and mostly covers patients who were transplanted outside of the country, and afterwards monitored in our hospital. Living unrelated transplantation in India (34.5%) was performed in almost 1/3 of the cases, with various complications after transplantation. Leading basic causes of ESRD before transplantation are chronic glomerulonephritis and chronic pyelonephritis, as well as in patients on dialysis treatment in our country (1). More than a half of performed transplantations are living related transplantations. The age of donors and recipients are in opposite relation between living related and living unrelated transplantation, where in the case of living related transplantation the average

age of donors is below 50 years, while recipients are younger (30.92±7.6 years), and in the case of living unrelated transplantation donors are very young (26.03±3.39) and recipients are in a middle age (40.44±8.6). Cadaveric kidney accepted the oldest patients in the observed population of patients (46.2±12.32). More kidney transplantation was performed in men in comparison with women. However, no difference was observed in distribution of donors according to the gender in all forms of transplantation (3:1 in favor of men). Early complications during the first year after transplantation were cumulative present in 25.2% of patients. AR in 15.0% of patients, DGF in 11.3%, CMV infection in 11.3% of patients, ARI in 6.8% of patients. The delayed graft function is more present in cadaveric and living unrelated transplantation (possible causes longer duration of dialysis before the transplantation and many hepatitis positive patients, older age, HLA-mismatching), while AR is almost equal in living related and living unrelated transplantation (possible causes HLA-mismatching and early not usage of MMF). In estimation of graft survival, serum creatinin <170 μmol/L, proteinuria less than 0.5 g/d and stable clinical condition were taken as parameters of satisfying graft function (2). In observed population of patients proper functional graft at the end of the first year was present in 87.5% of patients. In the study of Mc Laren with associates one year graft survival is 93.0% (3). Based on UNOS (United Network for Organ Sharing) data register 1996-2001, Gjertson says that one year graft survival in living related transplantation is 94.0% and that smaller centers (c or = 100 grafts) have range of one year survival 87-100% (4). Hariharan with his associates confirmed that one year graft survival from living donors increased from 88.8% in 1988 to 93.5% in 1998, and cadaveric grafts from 75.7 to 89.5% (5). One year outcome of grafts in our patients is much better in the group of living related transplantation (91.66%) than in the group of all observed patients (87.5%). Multiple logistic regressive analysis confirmed that 5 factors influence the appearance of graft insufficiency within the first year, and those are AR ($p=0.003$), DGF ($p=0.002$), low HLA matching ($p=0.001$), donors older than 50 years ($p=0.0001$) and no usage of MMF in immunosuppression ($p=0.014$). Significant connection

of AR with had graft outcome was proven in the study of Toki and his associates (6). Strongly connection of graft survival with DGF or episodes of AR was found also by Gjertson in his study about living kidney transplantation (4). The most of the authors recognize donors age as an independent risk factor of graft survival (6,7). Spanish authors found significantly higher values of creatinin and proteinuria on 3rd and 12th post-transplant month where donors were older than 60 (8). They also confirm that the donors age had significant effect on graft survival and patients survival, due to more often appearance of DGF. Similar to our results, Pascual and his associates confirmed that the donor older than 50 years is connected to worse graft outcome (6). Same authors highlight positive effect of MMF on one year survival, as well as the 3 year survival, especially in combination with tacrolimus (9,10). Five years of graft survival in our population of patients is 80.0%, and 10 years survival 75.0%. Recurrent kidney diseases are the main cause of graft loss in 5 years of monitoring and CAN in 10 years of monitoring. In 5 years European multicentric study, which compared the effects of tacrolimus with original formulation of cyclosporin, 5 years graft survival was 67.0%, thus 65.0% (10). Serum creatinin > 120 μmol/L in 6th and 12th month after transplantation and changes in serum creatinin > 42.75 μmol/L between 6th and 12th month, were related with the declining of 5 years graft survival. Mortality of our monitored patients is 6.8% and refer to comorbidity conditions (cerebrovascular insult, cirrhosis hepatitis, Alzheimer), with median of survival of 16.7 years. Group of Spanish authors registered mortality of 8.0% after the first transplantation year and the most often cause of death were cardiovascular diseases, malignancy and infectious diseases (11). Similar data were previously published by other authors (12, 13). Post-transplant atrogenic factors are traditional risk factors (14,15). Post-transplant diabetes mellitus (PTDM), which is considered to be the consequence of reduced secretion of insulin and/or increase of insulin resistance (16), increases the risk of graft loss and is related with the increase of cardiovascular morbidity and mortality (17). The incidence of PTDM is between 2-50% (18), so we can say that the percentage-presence of PTDM in population of our patients (9%) is satisfying.

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INTRODUCTION

Cerebro-vascular (brain) insult is defined as sudden focal neurological deficit, caused by a cerebro-vascular disease lasting more then 24 hours. Cerebro-vascular insult in general is divided as ischemic and hemorrhagic (bleeding brain insult). Ischemic insult is more often, while around 10% of all brain insults are manifested as brain bleeding (1). Together with cardiac and malignant diseases, cerebro-vascular diseases are the most common mortality and invalidity cause of modern humans. Each year about 5.5 millions of people all around the Globe suffer from brain insult. Because of that fact, this disease is the leading cause of disability (5, 9, 16). Complications are occurred among 25-40% of the cases; the most common are pneumonia, hearth decompensation, and pulmonary embolia (10). Brain insult occurs because of brain blood circulation problems, which results in insufficiency in oxygen supply for some parts of the brain. That leads to partial brain damages resulted in functional disability for the function performed by that part of the brain (2, 3, 4). It is estimated worldwide that as much as 46% of brain insult cases is among people between 45 and 59 years of life, while from 30 to 45 years the incidence is about 3%. Major cause in etiology of brain insult is blood vessels damaging due to atherosclerotic process by mechanical (blood pressure) and/or chemical (hypercholesterolemia) factors; inflammation process of blood vessels, or within some immune diseases (lupus eritematosides) (1, 6, 10). In literature major risk factors for developing of brain insult are: arterial hypertension, cardiac diseases, diabetes mellitus, adiposity, smoking stress, high cholesterol, and rare hyper-uric diathesis (2, 8). Together with treatment, preventing the risk factor (except for the unchangeable) is the best way to avoid insult.

OBJECTIVE

The objective of this paper was to analyse the frequency of cerebrovascular insult in West Herzegovina Canton from 1998 to 2002 as well as research on hospitalized cases according to the sex and age distribution, by demographic and epidemiological characteristics.

PATIENTS AND METHODS

Retrospective epidemiological study of hospitalized brain insult patients from West Herzegovina Canton was conducted in Clinical hospital Mostar. This hospital is the only hospital for 88,257 inhabitants of West Herzegovina Canton. Municipalities in this Canton

are: Ljubuski, Grude, Siroki Brijuni and Poustuje. This population lives there for years - there were no recent migrations. As a source of data we used medicinal documentation of Neurology department. For each of the cases we needed: age, sex, place of living and clinically confirmed cerebro-vascular insult diagnosis. From 1 January 1998 until 31 December 2002 a total of 393 insult cases were hospitalized. Among them, 189 were male and 204 were female patients. The cases were divided in three age groups: 30-49, 50-64 and 65 and more years of life by sex. For the analysing of table data, standard methods of descriptive statistics were performed.

RESULTS

Structure of cerebro-vascular insult treated cases in Clinical Hospital Mostar according to the age and sex was shown in Table 1. A total of 393 cases were treated of brain insult in the period from 1998 through 2002 in Clinical hospital Mostar. Out of that number 196 (49.9%) were male patients, while 204 (51.9%) were female patients. Among both sexes a total of 16 cases (4.1%) were between 30 and 49 years old, 66 patients (16.8%) were from 50 through 64 years old, and waste majority of patients treated because of the brain insult were 65 and over. Among all male and female patients observing separately by gender as a cluster, majority of the treated male cases were in the oldest group of cases 73.5% (139 patients) as well as it was observed among female patients where registered percent in this group was even higher - 84.3% or 172 patients. The lowest number of cases according to the age structure was treated among younger patients: 30-49 years old among either sexes - 6.3% (or 12 cases) of male cohort, and only 1.9% (or 4 cases) of female cohort.

DISCUSSION

During the previous, recent period, population of Bosnia and Herzegovina was exposed to certain level of exposure to different risk factors influencing health status of individuals. This was particularly the case with the chronic non-communicable diseases, gastrointestinal problems as well as viral diseases. Psychosocial factors and changed life styles, dietary regime, psycho stresses and social and economic factors have linked with greater number of brain insult cases (11). Cardiovascular diseases are the leading cause of mortality Worldwide. Cerebro-vascular diseases are the leading cause of mortality among female population in neighbor countries in transition (Croatia), while the same diseases are in the second place among male population, just follow-

GENDER	NUMBER OF CASES				AGE			
	1998 - 2002		30 - 49		50 - 64		≥ 65	
	N°	%	N°	%	N°	%	N°	%
MALE	189	1000	12	6.3	38	20.1	139	73.5
FEMALE	204	1000	4	1.9	28	13.8	172	84.3
TOTAL	393	1000	16	4.1	66	16.8	311	79.1

TABLE 1. Frequency of hospital treated cerebro-vascular insult cases in West Herzegovina Canton from 1998 - 2002.

ing cardiovascular diseases (12). The same is characteristic for Bosnia and Herzegovina, and particularly for Federation of BiH (13). In West Herzegovina Canton from 1998 until 2002 total of 663 cerebro-vascular/brain insult patients were registered. Among those cases 393 (or 40.8% of total) were hospital treated patients. That percent is understandable because one percent of patients die before even reaching a hospital, one part is out-patient facility treated because as a part of primary health care, and centers for mental health and physical medicine and rehabilitation (e.g. in Ljubuski) (15) and the rest of the cases are going to a hospital. According to the available literature the greater part of hospital treated patients according to the age is after 50 year of life. That is confirmed in our study where 92.9% of patients were in that age group. We observed 79.1% patients senior then 65 years. If we look to the sex distribution, 48.1% were male, and 51.9% were female patients among hospitalized. This data is similar to other authors' findings (10). Our findings show that greater number of treated women were older then 65 - 84.3% comparing to 44.7% of men among all our hospitalized patients. According to the same findings, looking to the younger patients, that ratio is slightly different - 60.4% of men vs. 39.6% of treated women. That is understandable having in mind that women have some

hormone protection until the after of menopause, and this finding is matching the other authors' findings (4). Some authors' estimation considering age and brain insult is that in between 45 and 59 years of life should be about 46.0% of all brain insult cases (8). Brain insult incidence according to the literature until 45 years of life is 3% (8), while we found 4.1% in that age group among all our observed hospitalized patients. Calculated as an incidence rate per existing Canton population and per 1000 population it would be much lesser than the mentioned literature incidence rate - 0.180/1000. If we bear in minds that this patients were only hospital treated patients, without above mentioned out-patient clinics treated ones and lethal ones, that rate will be higher. Cerebro-vascular/brain insult as a last stage of cerebro-vascular diseases is among top leading mortality cause Worldwide, so research on risk factors for developing that disease is of the highest importance. Such a research study was done among 2,750 insured in FBiH using questionnaires and measurements. Among that population 48 (or 1.7%) were found to have brain insult. Regarding standard risk factors 15.5% were found to have hypertension, 15.8% hypercholesterolemia, and 5.9% diabetes. Similar results were found among 99 insured participants in this study from West Herzegovina Canton (14).

CONCLUSIONS

In this retrospective epidemiological study from 1998 - 2002, 393 brain insult patients were hospitalized from West Herzegovina Canton. The greater part of hospitalized patients age 65 and more were women; in the age group from 50 to 64 years majority were men, same as among patients from 30 to 49 which was the smallest patient cohort. Regarding objective of this study on frequency of hospitalization of cerebro-vascular insult cases West Herzegovina follows other neighboring countries in transition. More attention must be given to the prevention programs and activities.

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PLANT PIGMENTS (ANTIOXIDANTS) OF MEDICINAL PLANTS MALVA SILVESTRIS L. AND MALVA MOSCHATA L. (MALVACEAE)

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ABSTRACT

Qualitative-quantitative structure of plant pigments in wild plants *Malva silvestris* L. and *Malva moschata* L. (Malvaceae), which were collected in 20 locations in Sarajevo area and surroundings, was tested during spring and summer in 2003. Acetone extracts of both categories were made and rising paper-chromatography done for the purpose of qualitative analysis. Quantitative analysis was done by spectrophotometry. Chlorophyll a, chlorophyll b and xanthophylls presence was confirmed by separation of pigments from acetone extract of these plant species. Spectrophotometric analysis of acetone extracts showed these results (given in mg/L): chlorophyll a 2.384, chlorophyll b 0.213 and carotenoids 5.001. Data given in mg/g dry substance are: chlorophyll a 1.193x10⁻³, chlorophyll b 1.064x10⁻³, and carotenoids 5.18x10⁻³. Pigments structure (in mg/L) in species *Malva moschata* is 1.6 for chlorophyll, 1.4 for chlorophyll b and 0.384 for carotenoids. Data given in mg/g are: chlorophyll a 3x10⁻³, chlorophyll b 2.0x10⁻³, and carotenoids 1.3x10⁻³. Considering that species *Malva moschata* L. grows on ecologically clean soils as opposed to well-known medicinal species *Malva sylvestris* L. and considering the production of phytochemicals, phytochemical structure and pharmacological influence it can be considered very medical and be given advantage over this wider spread category.

KEY WORDS: chlorophyll, carotene, plant pigments, paper-chromatography, spectrophotometry, medicinal plant, edible wild plant, Bosnia and Herzegovina.

INTRODUCTION

Plant pigments have a very important role in the protection of photosynthetic system, as well as protection of plant organism itself from the negative influences of sun radiation. Basic pigments chlorophylls have a special defense role, especially chlorophyll a and b. Accessory pigments carotenoids are very important in that process (1). Nowadays, chlorophylls and carotenoids have a very important role in prevention and therapy of different diseases of human beings, including immune system, different forms of skin disease, and characteristic anti-oxidative influence is attributed to them. Chlorophylls show antimicrobial and deodorant activity, and they support vitamin B synthesis in organism. (2) Carotenoids are protectors of chlorophyll. They have an important role in strengthening of immune system, cardiovascular system, and act preventively against cancer (3). Otherwise, plant pigments are water insoluble. Extremely lipophilic chlorophyll appears in most of these plants in two chemically related forms as blue-green chlorophyll a and yellow-green chlorophyll b. Their ratio is most frequently 3:1. Chlorophylls fluoresce lively red in short wave ultraviolet light (4). Besides green chlorophylls in less quantity, liposoluble orange-red and yellow carotenoids are regular constituents of plastids in these plants. Those are unsaturated hydrocarbons, and in their chemical structure they are identical to tetraterpenes. Because of numerous conjugated double bonds, they are orange or red-colored lipophilic stains (lipochromes). β -carotene is the most frequent among them. Its chain ends form beta-ion rings, like in many others carotenoids (a-caroten). Xanthophylls are derivatives of carotene that contain oxygen. Lutein, which corresponds to beta-carotene, has one OH group on every ring. It does not act as vitamin, although it oxidizes even in darkness. Protective function is attributed to carotenoids of photosynthetic apparatus in regard to chlorophyll (5). For α and β -carotene, besides pro-vitamin activity, positive effects on immune system, cardiovascular disease was proven, and they reduce a risk from some forms of cancer, too (6). Types of plant species *Malva L.* family Malvaceae have been used as origin of medicinal substances from ancient times. But, most of them, like *M. sylvestris* and *M. moschata*, are widely used as healthy wild vegetable in preparation of wide dietetics spectrum. This activity, in great part, can be attributed to participation of basic and accessory pigments, which appear in genus *Malva* species in larger quantity (6). Both, flower, and leaf of *M. sylvestris* and *M. neglecta*

species are in official usage, and they prescribe by numerous pharmacopoeia. Those are German Pharmacopoeia (DAB 6 and DAB 7), then Pharmacopoeia Helvetica edition quinta and edition sexta (Bern 1933 and Bern 1971) and Ph.leg I and II. They are used like infusions for respiratory organs treatment - Species pectoralis and Species albae, and for skin disease treatment - Species emollientia. Concentration of anthocyanins is sufficient for achieving therapeutic effect in making infusion for cough and tonsillitis. Apart from that, French pharmacopoeia (Pharmacopoeia Française IX edition, Paris, 1974) lists *Malva alcea*, *Malva rotundifolia* and *Malva sylvestris* as officinal. It is important to mention that *Malva sylvestris* flos is the official drug in European pharmacopoeia (European Pharmacopoeia, fourth edition, Strasbourg, 2002), Czechoslovakian Pharmacopoeia Ph. B. II (Pharmacopoeia Bohemoslovénica, edition secunda, Praha, 1964) and in Polish Pharmacopoeia III (Ph. Pol.) II - Pharmacopoeia Polska III, PZWL, Warszawa, 1954). Basic metabolites in genus *Malva* species are: mucus, pectin, starch, sucrose, iridoid glycosides, vitamins, some tannin, mineral substances, fats, anthocyanine, ferments and others (6, 7, 8). Since *Malva sylvestris* is in the biggest part of its distribution, mainly grows in nitrified soils, which are frequently loaded with heavy metals, pesticides and other residues, its usage is limited in humane medicine. Therefore, it is important to find and test species with similar pharmacological/physiological effects, and which grow on ecologically favorable soils. One of those species is *Malva moschata* L. It finds its optimum in clear soils of mountain meadows, and it reaches high biomass production for the balanced collection and application in healthy phytopharmaceutical production. The basic goal of this work is to test qualitative and quantitative contents of plant pigments in *Malva sylvestris* species which grows on ecologically more burdened soils and *M. moschata* which grows on ecology more favorable soils, and after that estimate their potentials in modern dietetic and phytotherapy.

MATERIAL AND METHODS

MATERIAL

Testing was done on plant material of wild plants *Malva sylvestris* and *Malva moschata*. These plants belong to a wider spread family Malvaceae, order Malvales, subclass Dilleniidae, class Magnoliopsida and phylum Magnoliophyta (9).

METHODS

Methods of field work

Plant material was collected by hand in dry weather. Healthy specimens were collected in ecologically favorable soils. Surface parts of plant *Malva sylvestris* were collected in twenty locations in Sarajevo region. *Malva moschata* samples were collected from twenty locations on Igman mountain at altitude of 1000-1300 m in the summer and they were regularly conserved. This region of sampling is marked by coordinates 18° and 18°30' of east longitude and 43° 30' and 44° of north latitude.

Methods of laboratory work

Determination of qualitative quantitative pigments characteristics was done after extraction, by one-dimensional rising paper chromatography and by spectrophotometry (10, 11, 12).

Chloroplast pigments separating by method of chromatography

One-dimensional rising paper chromatography was used for determination of pigments. Chromatographic paper Whatman N°1 was used, and standard technique and reagents. Acetone extract of pigment was used as a sample. Eluent (medicinal gas-petroleum-ether: acetone-100:25:20) was used for separation. Time of separation was from 20 to 30 minutes. The distance from the start line corresponds molecular mass values in reverse order. Thus, the closest to the start line will be

- Chlorophyll b Mr = 907 (green-yellow fraction)
- Chlorophyll a Mr = 893 (light green fraction)
- Then above chlorophyll a in wider area xanthophylls place and their molecule masses range from Mr 552 for cryptoxanthene to Mr 600 for violaxanthene. Colors of this fraction are different nuances of yellow.
- Carotene fraction Mr ~336 yellow-orange color is the closest to the front line (12).

Spectrophotometric analysis of chlorophyll and carotenoids from acetone solution

Quantitative analysis of chlorophyll a and b and carotenoids is based on their extraction with acetone, followed by spectrophotometric analysis. Measure-

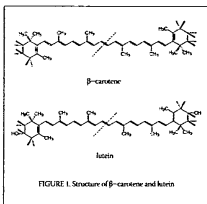


FIGURE 1. Structure of β -carotene and lutein

ments are done on wavelengths from 664 nm, because it is absorbance maximum for chlorophyll a, and 644 nm, because it is absorbance maximum for carotenoids. Measurements were done on spectrophotometer (Bansch & Lomb, General vertretung und Service - Stelle, Digitana AG 8003, Zurich). Reading of absorbance is on wave length (A) 664, 644 and 440 nm, and after that calculations are done:

- chlorophyll a = $0.284 \times A_{664} - 0.090 \times A_{644}$
- chlorophyll b = $21.426 \times A_{644} - 4.650 \times A_{664}$
- carotene = $4.645 \times A_{440} - 0.288 \times (a+b)$

where 0.284, 0.090, 21.426, 4.650, 0.288 are molar absorbance coefficients according to Westgren for absolute acetone and thickness of cuvette of 1 cm, and A-light absorption. Calculated values are in mg/L, and then we calculate raw substance in mg/g according to the following formula:

$$C_1 \times V \times R \\ C = \frac{\text{-----}}{G \times 1000}$$

where:

- C = content of certain fraction in mg/g of raw substance
- C₁ = concentration of pigments calculated according to formula (mg/L)
- V = extract volume
- R = extract dilution (if it exists)
- G = sample mass (dry or raw material)
- 1000 = factor of transforming of g into mg (12).

RESULTS

QUALITATIVE ANALYSIS

Chloroplast pigments separation by one-dimensional paper-chromatography
Paper chromatograms were obtained for every tested species. Identification of pigments was done based on characteristic color: chlorophyll b is light green fraction, chlorophyll a is dark green, and xanthophylls – yellow; and also on the bases of their position on chromatogram. Presence of chlorophyll a, chlorophyll b and xanthophylls was proved in analysed species of Malva genus. Chromatogram of cultivated carrot – *Daucus carota* L. var sativa was used as reference (Figures 2. and 3.).

QUANTITATIVE ANALYSIS

Spectrophotometric pigments analysis
Spectrophotometric analysis was done for quantitative analysis of pigments. Water was used as reference. Following results were obtained on the basis of measurements: (Table 1.). By entering these results in formula for pigments quantity calculation we got the values given in Table 2. Calculated values are in mg/L of acetone extract, and after that values are calculated according to given formula and they are in mg/g of dry mass for both species (Table 2.)

PIGMENT	MALVA SILVESTRI		MALVA MOSCHATA	
	mg/L	Mg/g dry mass	Mg/L	Mg/g dry mass
CHLOROPHYLL A	2.3807	$1.4934 \cdot 10^{-2}$	1.600	$8 \cdot 10^{-3}$
CHLOROPHYLL B	0.3100	$1.66 \cdot 10^{-3}$	1.419	$7.09 \cdot 10^{-3}$
CAROTENOIDES	1.07	$5.1834 \cdot 10^{-3}$	0.364	$1.82 \cdot 10^{-3}$

TABLE 2. Comparative spectrophotometrical analysis of plant pigments based on the mean values gotten from 20 samples

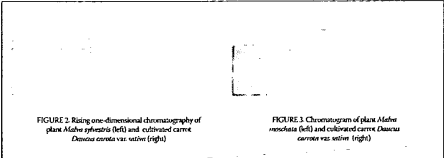


FIGURE 3. Chromatogram of plant Malva moschata (left) and cultivated carrot (Daucus carota var. sativa) (right).

PLANT SPECIES	CHLOROPHYLL A AND B RATIO		TOTAL CHLOROPHYLL AND CAROTENOIDES RATIO
	A/B	B/A	
MALVA SILVESTRI	2.3	26.1	
MALVA MOSCHATA	1.2	3.1	

TABLE 3. Quantitative perspectives between wine pigments

total chlorophyll and carotenoides ratio is significantly different. In normal circumstances it is usually 5:1. But, that proportion in plant *Malva sylvestris* is 2.6:1, and in plant *Malva moschata* is extremely high 8.3:1 (2). Chlorophyll b, as dark green fraction, was separated nearest to the start, and chlorophyll a as light green fraction, and we compared it with the same fraction separated in chromatogram of cultivated carrot as a reference. Chlorophyll presence was confirmed in both categories.

POTENTIAL FOR USAGE IN MODERN PHYTOTHERAPY

Common mallow *Malva sylvestris* is wider spread plant. It has been favorite food and medical plant in the Balkans peninsula from ancient times. It is particularly appreciated as vegetable. Fresh leaves contain about 130 mg% vitamin C and as much as 18 mg% carotene. Leaves contain considerable quantity of iron and calcium. Therefore, they are considered the healthiest vegetable in this area (12). Leaves and flowers of *Malva sylvestris* were used for the treatment of difficult diseases of respiratory tract, including malignant diseases of this system.

Decoct of this plant is effective agent against middle ear inflammation (13, 14, 15). This species has proven to be specially healthy food in soldiers feeding in nature, and in the cases of mass catastrophe as well (16, 17, 18). In *Malva moschata* species, till now, no systematic studies of nutritive and pharmacological active substances was done. But, it is supposed that this species contains similar substances like the wider spread species and that it can be used in the same purpose. *Malva sylvestris* usually grows on nitrified ground, by roads, near traffic arteries, on soils, which can be loaded with heavy metals, pesticides and other residuum with unfavorable influence on human health. Because of that this species from natural soils has or may have limited usage. Plant species *Malva moschata*, grows far from human settlements, in mountain or sub-mountain zone, by edges of meadows in the zone of coniferous forests and mixed deciduous and conifer forests, on the grounds which are natural and unburden with organic and inorganic pollutants. Therefore, this species has excellent possibilities in preparation of different nutritive and phytotherapeutics in relation to majority if species of *Malva* genus and it has big comparative advantage in this respect.

CONCLUSION

Wild medicinal plant species *Malva sylvestris* and *Malva moschata* contain different plant pigments or antioxidants. Chlorophyll a, chlorophyll b and xanthophylls presence was verified by separation of pigments from acetone extract of these plant species. Spectrophotometric analysis of acetone extract showed these results (given in mg/L): chlorophyll a $2.386 \cdot 10^{-2}$, chlorophyll b $0.312 \cdot 10^{-2}$ and carotenoides $1.07 \cdot 10^{-2}$. Data given in mg/g dry substance are: chlorophyll a $1.4934 \cdot 10^{-2}$, chlorophyll b $1.66 \cdot 10^{-3}$ and carotenoides $5.1834 \cdot 10^{-3}$. Pigments content (in mg/L) in category *Malva moschata* is 1.6 for chlorophyll a, 1.419 for chlorophyll b, and 0.364 for carotenoides. Data given in mg/g are: chlorophyll a $8 \cdot 10^{-3}$, chlorophyll b $7.09 \cdot 10^{-3}$ and carotenoides $1.82 \cdot 10^{-3}$. There is high potential for application of plant pigments in the prevention and modern therapy therefore main and accessory plant pigments play important role as antioxidants and as anticancer means. Species *Malva moschata* has more advantages as an effective antioxidant means than other plant species *Malva sylvestris*.

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EMBRYOGENESIS OF THE RAT TELENCEPHALON - A MORPHOLOGIC AND STEREOLOGIC ANALYSIS

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ABSTRACT

Comparative researches of borderline between telencephalon neuroepithelium and its surrounding mesenchyme in successive early developing stages lack in literature. The aim of this investigation was to carry out systematic morphologic and stereologic analyses of rat telencephalon in early developmental stages. We analysed serial sections of rat embryonic brain from the 1st (E12) to the 15th (E15) day of gestation. The surface densities (S_V) of an external mesenchymal surface, an internal mesenchymal surface and a neuroepithelial (ventricular) surface were examined stereologically and compared. The surface density of the external mesenchymal surface was the biggest at E12 (4.05mm⁻²) and the least at E15 (1.87mm⁻²) - postnatally. The surface density of the internal mesenchymal surface was the biggest at E12 (4.05mm⁻²) and the least at E15 (1.87mm⁻²) - postnatally. The surface density of the neuroepithelial surface was the biggest at E12 (3.31mm⁻²) and the least at E15 (1.87mm⁻²) - postnatally. Our stereological estimates give objective numerical proof of significant morphogenetic changes in telencephalon shape described by morphologic analyses. The major advantage of stereological methods is the possibility to carry out the estimation procedures in specified, well-defined brain regions or layers.

KEY WORDS: rat telencephalon, development, stereology.

INTRODUCTION

Development of the human telencephalon begins by enclosing of rostral neuroporus at the cranial end of embryonic neural tube (24 days of development; crown-rump length 2.5-3.5 mm). It is the beginning of cortex development. Developmental process that leads to final forming of telencephalic hemispheres could be named as morphogenesis of the brain cortex. Changes of external and internal telencephalic shape are results of complex histogenetic events and internal forming of telencephalic vesicles wall (1). Morphogenesis and histogenesis of rat brain are much more faster than in human with regard of 24 days rat development. Rat embryonic neural tube is already completely closed at the cranial end at 12th day of pregnancy (E12) (2,3). The forebrain changes from a relatively simple tubular structure with thin walls surrounding a large ventricular system to a thick-walled brain with a highly convoluted but reduced ventricular system (4). Comparisons about brain development are based on observations carried out on ontogenetic successive stages. Microscopic analysis of brain together with analysis of serial sections and model reconstruction of them gives information about shape of some telencephalic parts and basic relations between them (5-8). Three-dimensional reconstructions of the normal rat embryonic neocortex on days E15, E17, E19 and E21 show that the neocortical ventricular zone (VZ) shrinks rapidly in the medial direction during cortical morphogenesis (7). Cell proliferation and migration, growth of immature neuronal processes (dendrites and axons) and forming of cytoarchitectonic layers may be light microscopic analysed. Altman and Bayer (8) studied cellular compartmentation in the germinal matrices (primary neuroepithelium and a subventricular zone - SVZ) of the rat cerebral cortex at embryonic developmental stages. Between E12 and E15 the rat cortical germinal matrix consists only of the primary neuroepithelium. SVZ has formed in the early ventrolateral aspect of the cortex by E16. It grows in depth for several days but the neuroepithelial depth decreases. In order to understand the overall organization of the neocortex, Marin-Padilla (6) studied cortical development of various mammalian embryos using the Golgi method and introduced a new cytoarchitectonic theory of the phylogenetic evolution of the mammalian neocortex. The neocortex starts its development with a primary plexiform layer in the telencephalon, which precedes and is essential for the formation of the cortical plaque. Layer I and the sub-layer derived from this primary plexiform layer which

represents the primitive cortical organization shared with reptiles and amphibians. The other cortical layers (II-VI) derived from cortical plaque which is an innovation in mammals. During the development of the cortical plaque migration, early differentiation and morphological/functional maturity of the neurons occur. Light microscopic researches incompletely showed subsequent events in pia mater development and relation of some layers of mesenchymo-neuroepithelial interface. There are doubt opinions about origin of delicate layer on the brain surface called pia mater (1). Some authors maintain pia develops from mesodermal layer which surrounds telencephalon (10), while the others consider that the origin of pia mater is neuroectoderm developing from rostral part of neural groove (11). However, most authors agree with claim that each tissue taking part in forming meninges, have to pass mesenchymal stage without regard to its origin. It is necessary to collect data from different fields of research for complete analysis of every single stage of brain development. Modern methods of quantitative analysis are from stereology field. Stereology is the methodology which provides meaningful quantitative descriptions of real three-dimensional glob structure from measurements of two-dimensional images sampled from the glob (12). Efficient unbiased stereological methods of quantitative analysis of different regions in CNS have been developed since 1984, and are superior to the conventional basic methods previously used (6). The usefulness of stereological principles is established for estimation of neuron and synapse number and size, synapse gradients, neuron point patterns in three-dimensional space and capillary surface area (13,14,15,16,17,18,19,20,21,22). Neuronal migration occurs early during embryonic development (23). Control of neuronal migration involves different cell populations and multiple molecular mechanisms (cell-cell adhesion, interaction with extracellular matrix protein, neurotransmitter release, growth factors availability). Various forms of cell migration in the embryonic telencephalon of mammals were investigated using a combination of several molecular biological techniques (24,25). Systematic comparative stereological researches of borderline surfaces of mesenchyme and neuroepithelium in early developing stages of rat telencephalon lack in literature. The aim of this investigation was to analyse the earliest stages of the developing rat telencephalon morphologically and quantitatively and to compare sizes of neuroepithelial and mesenchymal surfaces.

MATERIAL AND METHODS

Rat brains used in this study were obtained from Fisher inbred rats with accurately timed pregnancies. The investigation was carried out on serial sections of five embryonic rat heads in each examined day of development. Gestation was considered to have begun early in the morning when sperm was found in the vaginal smear. The following 24 hours were designated E1. Embryos were isolated from the uterus of a pregnant female under anesthesia on E12 to E15. Fixation was performed by immersion of embryos in 1% glutaraldehyde and 1% paraformaldehyde in 0.13M sodium phosphate buffer. The whole telencephalon was embedded in Epon-Araldite. Serial 1 µm plastic sections of telencephalon were stained with toluidine. The stereologic analysis was performed using the semicircular test system L 36 at objective magnification x10. The sections were used systematically and intermittently. Number of samples for each embryo was calculated according to DeHoff (12). By means of standard stereological procedures were calculated surface density (Sv) of external mesenchymal surface (m/ext), surface density of the mesenchymo-neuroepithelial borderline (m/int) and surface density of neuroepithelial ventricular surface (e/int). Referent space was telencephalon area of the forebrain (Fig.1). The results are presented as the mean ± SD. Statistical differences between two means were determined by Student's t-test. P<0.05 was considered statistically significant.

RESULTS

E12: neural tube is completely closed in its cranial part. Rostral part of prosencephalon shows slightly widenings (telencephalic vesicles origin) which appeared as paired, slightly expressed lateral prominences of neuroepithelial wall (Fig.2a). Between telencephalic vesicles origin and in direction to caudal there is origin of primitive ventricles. Lateral ventricles are in telencephalic vesicles area. In mesenchymal wrapper of neuroepithelial origin starlike cells partly touching each other with their cytoplasmic processes. Primitive blood vessels are in parallel position with the neuroepithelial surface. Surface density of mesenchymal external surface was $(4.05 \pm 0.27)/mm^2$, surface density of mesenchymo-neuroepithelial borderline was $(4.02 \pm 0.15)/mm^2$ and surface density of ventricular neuroepithelial surface of was $(3.31 \pm 1.09)/mm^2$. E13: rostral part of prosencephalon shows already well

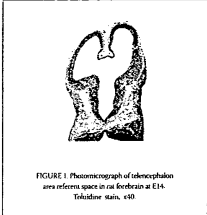


FIGURE 1. Photomicrograph of telencephalon area showing upper rat forebrain at E14. Toluidine stain, x40.

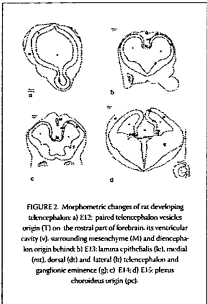


FIGURE 2. Morphometric changes of rat developing telencephalon at E12: paired telencephalic vesicles origin (1) on the rostral part of forebrain, its ventricular cavity (2) surrounding mesenchyme (3) and telencephalic origin behind (4). E13: lateral neuroepithelial (5) medial (6), dorsal (7) and lateral (8) telencephalic and ganglionic eminence (9). E14: E15 plus choroid plexus origin (10).

expressed and divided areas of telencephalon. Paired lateral well expressed prominences are telencephalic vesicles—origin of future hemispheres (Fig.2a). Central area between paired telencephalic vesicles is telencephalon impar. Its neuroepithelial wall is very thin, particularly in lamina epithelialis area. Some parts of neuroepithelial telencephalon wall are more thickened in comparing with the other, so they can divide on basal, basolateral, dorsal and medial telencephalon. Particularly, basal area of telencephalon named ganglionic eminence is

thicken. It is corpus striatum origin. At E13 there is already penetration of blood vessels from surrounding mesenchyme to neuroepithelial wall of telencephalon. All examined surface densities were significantly higher at E12 comparing to E13 ($p<0.0005$). E14: telencephalic vesicles are more prominent in comparing to day before (Fig.2c). At basolateral parts of telencephalic vesicles are very thick, neuroepithelial wall protrudes to ventricular cavity, and lumen of ventricle becomes folded. Telencephalon impar and surrounding mesenchyme are positioned in front of ganglionic eminence. Telencephalic vesicles spread above telencephalon impar to rostral direction. There aren't any statistic differences between surface densities examined at 13th and 14th day of development ($p>0.05$). E15: telencephalic vesicles are growing in their basal, lateral and dorsal parts. In area of telencephalon impar mesenchyme and a thin neuroepithelial wall spread in direction to telencephalic ventricular cavity in fingerlike shape processes. It is the origin of plexus choroideus (Fig.2d). Surface density of external mesenchymal surface is significantly lower ($p<0.005$). Internal neuroepithelial surface is significantly lower ($p<0.01$) but surface density of mesenchymo-neuroepithelial borderline is

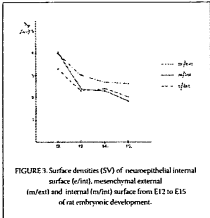


FIGURE 3. Surface densities (SV) of neuroepithelial internal surface (fint), mesenchymal external surface (fext) and internal (fint) surface from E12 to E15 of rat embryonic development.

EMBRYONIC DAY	SV _{fint}	SV _{fext}	SV _{fint}
E12	4.05 ± 1.37	4.02 ± 1.15	3.31 ± 1.07
E13	2.38 ± 1.15	2.29 ± 0.96	2.32 ± 1.09
E14	2.31 ± 0.85	2.26 ± 0.82	2.39 ± 0.84
E15	1.87 ± 1.18	1.69 ± 1.02	2.01 ± 1.30

TABLE 4. Quantitative proportions between some pigments

not different in comparing to a previous day ($p>0.05$). Average surface densities of mesenchymal-external surface, mesenchymo-neuroepithelial borderline and ventricular neuroepithelial surface from E12 to E15 of rat telencephalon development are shown on Table 1. and Figure 3.

DISCUSSION

Unbiased stereological methods have been applied for determining real changes in cell number because of disparity between a density and the total number as a quantitative measure (12). Numerous investigators of CNS by means of data about the number of neurons and their synapses developed meaningful animal models of normal development and aging, neuronal connectivity and neurodegenerative diseases (2,4,25,26,27,28,29,30,31,32,33). The major advantage of stereological methods is the possibility to carry out the estimation procedures in specified, well-defined brain regions or layers (14,25,26,32,37). Quantitative results of our research of rat telencephalon development show that sizes of mesenchymal and neuroepithelial bordering surface decrease with increasing of telencephalon origin and its surrounding mesenchyme. Surface density of mesenchymal external borderline is the biggest at E12. At E13 it suddenly decreases and is the smallest at E14. Surface density of internal (ventricular) neuroepithelial surface also is the biggest at E12 and the smallest at E15 but it shows minor increase at E14. Surface density of mesenchymo-neuroepithelial borderline is the biggest at E12 and suddenly decreases at E13. It reaches the smallest value at E15 similar to external mesenchymal borderline surface. With regard that the surface density is quantitative indicator of surface size in unit of organ volume, we consider that our stereological examines objectively, numerically give proof of significant morphogenetic changes of external and internal shape of telencephalon described by morphologic analyses. At E12 all examined surfaces are relatively the biggest in compare to the later developmental stages, with regard to that telencephalic neuroepithelial wall and surrounding mesenchyme are the smallest at that time. There is an increase of neuro-

epithelial quantity by progressing of morphogenesis and histogenesis. Our morphologic findings are consistent suggest that rat cortical germinal matrix consists only of a primary neuroepithelium between E12 and E15 (8). At E15 a thick ventricular zone is a major component of the neocortex (7). Mesenchymo-neuroepithelial borderline is of a special significance because of their inductive interaction. At E13 when blood vessels penetrated from mesenchyme to neuroepithelium, the size of their borderline suddenly decreases. From E13 to E14 slightly increase of ventricular neuroepithelial surface density is possible to explain with the most intensive telencephalic morphogenetic changes observed at these stages of development. The elongated medial structure of telencephalon impar appears at E13. With thickening of basolateral telencephalon some parts of neuroepithelial wall protrude to the ventricular cavity and ventricular lumen becomes folded at E14. The result is the increasing of internal neuroepithelial surface. One of the early events in the establishment of regional diversity in brain is the subdivision of the forebrain into

the cerebral cortex and underlying basal ganglia. Fishell et al. (28) visualized cell movement within the VZ of the dorsal and basal regions of the E15 murine telencephalon. Cell dispersion was restricted at the border between the cortical VZ and the lateral ganglionic eminence, the basal telencephalic VZ. Noyt et al. (34) examined a short range signal that restricted movement of cells between the proliferative zones of the dorsal and basal telencephalon. They found the boundary which isolates these respective environments through either a contact dependent or a short-range diffusible mechanism. The dramatic shrinkage of the neocortical part of VZ is caused by a rapid growth of the basal ganglia in dorsomedial direction from primordia in the ventrolateral telencephalon which obliterates the contiguity between the lateral ventricle and neocortical primordium. As the result the neocortical VZ is displaced dorsomedially (2). We concluded that the ventricular lumen and internal surface decrease at E15 because of sudden growth of ganglionic eminence.

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HIGHER MORTALITY RISK OF LUNGS CARCINOMA IN VINEYARD SPRAYERS

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ABSTRACT

This study investigated mortality rate of lung carcinoma in professional vineyard sprayers. Clinical investigation was performed in 187 professional vineyard sprayers who had been exposed to the inhalation of the particles of Bordeaux mixture for 24 years on average. Bordeaux Mixture is used for prevention against mildew attacking vineyards. The control group was composed of 187 inhabitants of the same area who did not have any contact with the mentioned substance. A cytological investigation of the sputum specimens obtained from 104 tested inhabitants was performed. The sputum specimens were stained with standard haematoxylin-eosin method and also with special method (rubanic acid) to prove the incidence of copper granules in macrophages. The findings show a considerable statistical difference in the frequency of occurrence of lung carcinoma between the group of vineyard sprayers smokers and the control group (37% vs 27% p=0.01). The risk of lung carcinoma in the vineyard sprayers was three times higher compared to the risk of smokers in the control group, with a statistical probability of 0.15 in the scope from 1.16-RR<2.28. Mortality risk of lung carcinoma in the professional vineyard sprayers is significantly higher (p<0.01) compared to the risk in the control group.

KEY WORDS: lung carcinoma, vineyard sprayers

INTRODUCTION

Pimentel and Marques described the case of pulmonary disease in vineyard workers and they named it "vineyard sprayer's lung". It is an occupational disease caused by inhalation of the copper sulphate solution neutralised by hydrated lime (1). The changes in the respiratory system are caused by inhalation of the pesticide known as Bordeaux mixture (1:2.5 copper sulphate solution) which is used for spraying vineyards as a protection against mildew (2). The roentgenogram of vineyard sprayer's lung is similar to that of asbestosis, with a tendency to develop into diffuse interstitium fibrosis. Inhalation of the particles of Bordeaux mixture for a long period causes frequent chronic respiration symptoms as cough, chest constrictions and dyspnoea, along with ventilation disorder with dominant restrictive-obstructive changes (3,4). Experimental research, carried out on the animals exposed to the inhalation of Bordeaux mixture, caused the interalveolar trapping of macrophages, formation of granulomas and multiplication of collagen, elastic and reticular fibres in the interstitium (5). Plamenac and collaborators described alveolar macrophages with copper formations in the cytoplasm found with the workers engaged in vineyard spraying (6). Pulmonary biopsy in vineyard sprayers often showed the presence of inflammatory changes of bronchi and interstitium. Taking into the consideration clinical picture of the disease appearing after longer exposition to copper sulphate, Vilard found the relation between pulmonary lesions and the development of pulmonary carcinoma (a). So far professional risk of increased incidence of respiratory changes in vineyard sprayers has not been described extensively in medical literature as this type of study demands aimed cohort research through longer period. Monitoring the health in vineyard workers during a longer period encouraged us to undertake the investigation with a special focus on the mortality rate of pulmonary carcinoma in professional vineyard sprayers.

SUBJECTS AND METHODS

The investigation was performed among the vineyard sprayers who worked in the largest vineyards in Botovo and Blato (51 000 hectares of vineyards) in the Hercegovina-Neretva Canton and West Herzegovina Canton, Bosnia and Herzegovina. Bordeaux mixture is used for spraying the vineyards in the whole area. All subjects were men in the age of 51.3 on average. They were divided into two groups. The first group was composed of 187 workers who were for about 24 (SD16) years engaged

in vineyard spraying. The control group was composed of 187 workers who did not get in touch with Bordeaux mixture. They all had 23 (SD 5.3) years of work experience. In this longitudinal prospective study we monitored the occurrence of lungs carcinoma in both groups in two periods, first from 1983-1992 and second from 1993-2002. We also investigated influence of smoking on the incidence of lungs carcinoma in both groups. For these reasons each group was divided into sub groups of smokers and non-smokers. Taking into account professional exposure to pesticide, personal record of a patient, work experience, systematic clinical examination and chest roentgenograms all the subjects suspected to have lungs carcinoma were sent to pulmonary department for further diagnostic and therapeutic treatment. For each patient with confirmed diagnosis of lungs cancer further therapy was performed (surgery, radiological therapy or combined chemotherapy) and his health condition was monitored until death. We made cytological analyses of sputum specimens randomly taken from 52 vineyard sprayers as well as from the control group. The only criteria for the selection had been that none of the subject had any respiratory disease of any aetiology in his case history. Sputum specimens were obtained by morning cough on three consecutive days. Only expectorated material contained pulmonary macrophages was accepted as sputum. After fixation in 75% alcohol, the sputa were embedded in paraffin and the section were stained with haematoxylin-eosin, tested for iron by Turnbull method and for copper with rubenic acid and benzidine.

RESULTS

In the group of 121 vineyard sprayers non-smokers, during the investigation 12 vineyard sprayers died of lungs carcinoma (mortality rate 99.17%). During the first investigation period (1983-1992) 4 sprayers died (mortality rate 33.95%) and during the second period (1993-2002) 8 sprayers died (mortality rate 66.11%). In the control group of 121 workers non-smokers, 3 workers died of lungs carcinoma (mortality rate 24.79%). In the period from 1983-1992, 1 worker died (mortality rate 8.26%) and in the period from 1993-2002, 2 workers died (mortality rate 16.54%) in Table 1. In the group of 66 vineyard sprayers smokers 15 workers died. Five workers died in the first investigation period (mortality rate 75%) and 10 workers died in the second period (mortality rate 151.51%). In the control group of 66 smokers, 5 subjects died from lungs carcinoma (mortality rate 75.76%). Two subjects died in the first period of investigation (mortality rate 30.30%) and 3 workers died

TABLE 1. Mortality of lungs carcinoma in professional vineyard sprayers

SUBJECTS	AGE	NUMBER OF SUBJECTS	NUMBER OF DEATH FROM 1983-1992	NUMBER OF DEATH FROM 1993-2002	TOTAL NUMBER OF DEATH SUBJECTS FROM 1983-2002
VINEYARD SPRAYERS	36-56				
NON-SMOKING		121	4 (3.30%)	8 (6.57%)	12 (9.87%)
MORTALITY RATE PER 1000					
SMOKERS		66	5 (75.76%)	10 (151.51%)	15 (227.27%)
MORTALITY RATE PER 1000					
CONTROL GROUP	36-65				
NON-SMOKING		121	1 (0.82%)	2 (16.52%)	3 (2.47%)
MORTALITY RATE PER 1000					
SMOKERS		66	3 (45.45%)	5 (75.76%)	8 (121.21%)
MORTALITY RATE PER 1000					

TABLE 2. Incidence of cytological findings in professional vineyard sprayers' sputa

SUBJECTS	AGE	NUMBER OF SUBJECTS	SPUTA WITH ABNORMAL COLUMNAR CELLS	SQUAMOUS METAPLASIA WITH OR WITHOUT MACROPHAGES	ECGNO PHILIA	RESPIR. SPINALS	SALIVA BLACK OF EXPECTORATIONS
VINEYARD SPRAYERS	36-65						
NON-SMOKERS		52	36 (69%)	11 (21%)	2 (3%)	4 (7%)	5 (9%)
SMOKERS		19	19 (100%)	7 (37%)	2 (10%)	2 (11%)	4 (21%)
CONTROL GROUP	36-65						
NON-SMOKERS		31	6 (19%)	0 (0%)	0 (0%)	1 (3%)	1 (3%)
SMOKERS		17	13 (76%)	2 (12%)	1 (6%)	2 (12%)	3 (18%)

in the second period (mortality rate 45.45%). Mortality rate of vineyard sprayers smokers was significantly higher (122.27%) than in the control group (75.76%). The results of monitoring show that there is a significant statistical difference in respect to the lungs carcinoma rate ($X^2=6.55, p<0.05$) between the investigated group of workers engaged in vineyard spraying non-smokers and the control group of the workers non-smokers. There is a significant statistical difference in the height of relative risk of incidence of lungs carcinoma in the vineyard sprayers non-smokers compared to the control group ($RR=4.15, p<0.05$). Relative risk of lungs cancer occurrence in the workers non-smokers engaged in vineyard spraying with Bordeaux Mixture is on average four times higher ($1.16<RR<13.8$) than in the control group. The results show that there are significant statistical differences in respect to the occurrence of lungs carcinoma between the group of vineyard sprayers smokers and the control group ($X^2=4.77, p<0.05$). The risk of

lungs carcinoma was three times higher in the vineyard sprayers than in the control group of smokers with a statistical probability of 95% in the range $1.16<RR<7.78$. RESULTS OF CYTOLOGICAL INVESTIGATIONS We made a cytological analysis of sputum specimens obtained from 52 randomly selected vineyard sprayers. We also analysed the same number of sputum specimens obtained from the control group in Table 2. We investigated 52 vineyard sprayers between the ages 36-65, 33 of them were non-smokers and 19 smokers. The table shows increased sputum expectoration in both groups of vineyard sprayers non-smokers and smokers compared to the control group. Microscopic examination of the sputum in the non-smokers group showed the following abnormalities: abnormal columnar (irritated) cells in 36 (70%) cases, squamous metaplasia of respiratory epithelium was found in 13 cases - in 11 cases (51%) without atypical, in 2 (6%) cases with

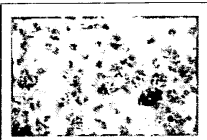


FIGURE 1. Copper granules were noted in the cytoplasm of the macrophages (halophages). (HE, 400X)



FIGURE 2. There are eosinophils and respiratory spirals in the sputum of smokers. (HE, 300X)

atypical metaplastic cells in 33 (20%) cases copper granules were noted in the cytoplasm of the macrophages (halophages) (Figure 1); eosinophilia were found in 4 (11%) cases; respiratory spirals in 5 (15%) cases. In the sputum of smokers the following findings were noted: abnormal (irritated) columnar cells were found in 19 (100%) cases; squamous metaplasia was found in 13 cases, in 7 (17%) cases there were no signs of atypia, while in 5 (60%) cases atypical metaplastic cells were found; halophages were found in 14 (54%) cases; eosinophilia in 2 (11%) cases; respiratory spirals in 4 (21%) cases. (Figure 2) The control group was composed of 52 subjects in the age range 36-65. There were 35 smokers and 17 non-smokers. The following findings were evident in the group of non-smokers abnormal (irritated) cylindrical cells in 4 (11%) cases; eosinophilia and respiratory spirals were found in 1 case (3%). Squamous metaplasia and halophages were not evident within the group. In the group of smokers the following findings were evident: 15 (88%) subjects had abnormal (irritated) cylindrical cells; squamous metaplasia was found in 3 cases, in 2 cases (13%) without atypical and in 1 case (6%) with atypical metaplastic cells; eosinophilia was found in 2 (11%) cases; respiratory spirals were noted in 3 (18%) cases; chalcuses were not found in this group of subjects.

DISCUSSION

Morphological, clinical-radiological and functional changes in the lungs of vineyard sprayers are well known. Besides general symptoms, the patients also had the symptoms related to the respiratory system, in the beginning interpreted as a consequence of development of chronic bronchitis or tuberculosis, with ha-

ematological findings almost within normal limits. Koch bacillus was not noted in the consecutive sputum that the patients produced and their tuberculin tests were negative. The applied therapy that included antibiotics and antituberculous did not result with a complete regression of the disease. In some patients the radiological picture showed the presence of tumorous lung shadows, which was the indication for biopsy. In these cases pathohistological findings included: the desquamation of pneumocytes, formation of the predominantly histiocytic granulomas in the septa, individual and/or multiplied that gradually developed into fibrohyaline nodules similar to those found in silicosis (1,2,3). There are the quotations in medical literature about the effects of Bordeaux mixture on the respiratory system that include changes in the pulmonary interstitium and bronchial epithelium. Numerous foci of non-specific bronchopneumonia, with inflammatory cells and macrophages indicated the affection of bronchial parenchyma, which was proved by the experiments on animals (3,5). At present some authors are of the opinion that copper is the aetiological agent responsible for changes in the interstitium, i.e. functional changes that occur in the cases of longer exposure to its inhalation (4,5). Cytological changes of the respiratory tract found in the sputum analysis are the proof of severe lesions of cylindrical epithelium in professional vineyard sprayers. A high percentage of the sputum specimens contained desquamated, abnormal columnar bronchial cells that showed a whole spectrum of degenerative changes from non-specific irritation to severe cellular degenerative changes of alveolophoria type. The occurrence of numerous respiratory spirals, squamous metaplasia of the cells in the bronchial epithelium and atypical cells

indicated that long-term inhalation of the pesticide of copper sulphate might increase the risk of bronchogenic carcinoma (6,7). This study revealed enhanced expectoration of sputum in vineyard sprayers, which indicates the irritation effects of inhaled substance (8,9). The investigation of cytological changes in respiratory epithelium of the sputum specimens showed considerable deviations compared to normal sputum specimens. More frequent occurrence of squamous metaplasia and abnor-

mal cylindrical cells in non-smokers and smokers group of vineyard sprayers compared to the control group indicates chronic irritation effect of used substance-copper, which was constantly evident within pulmonary macrophages in the sputum of vineyard sprayers (10,11). There are quotations in medical literature on higher risk of lung carcinoma caused by long exposure to the inhalation of the granules of copper sulphate (6,12,13), which is in accord with the findings of our investigation.

CONCLUSION

Our results show a considerably higher mortality of lung carcinoma in professional workers engaged in vineyard spraying compared to those who were not exposed to the inhalation of mentioned substance. This also applies to the group of vineyard sprayers non-smokers. In this group the tobacco smoke is excluded as a possible aetiological factor responsible for lung carcinoma.

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NITRIC OXIDE IN GENTAMICIN-INDUCED ACUTE TUBULAR NECROSIS IN RATS

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ABSTRACT

Gentamicin is still widely used in clinical practice in spite of its renal toxicity. The role of nitric oxide (NO) in that process is not completely elucidated. The aim of this study was to investigate the relationship between plasma level of NO and the histopathological changes of kidney in acute tubular necrosis (ATN) induced by gentamicin in rats. Study was carried out in Albino-Wistar rats, both sexes (n = 18), average body weight 300 ± 20 g, divided in two equal groups: control and gentamicin group. The control group was injected with 0.9 % NaCl i.p., and gentamicin group was injected with gentamicin in the dose of 80 mg/kg/day i.p. in a period of 5 consecutive days. NO plasma level was determined by the production of nitrate and nitrite using classical colorimetric Griess reaction. Kidney specimens were stained with hematoxylin-eosin (H-E) and Periodic acid-Schiff (PAS) stain. Semi-quantitative histological analysis was used for the evaluation of the level of kidney damage. Both, the plasma NO level and the level of kidney damage were statistically higher in rats with gentamicin-induced ATN in comparison to the control group. In spite of that the correlation between plasma NO level and the level of kidney damage was not found. The role of plasma level NO in gentamicin induced ATN in rats could possibly indicate on the role of NO in renal damage caused by gentamicin.

KEY WORDS: nitric oxide, gentamicin, acute tubular necrosis

INTRODUCTION

Aminoglycosides, including gentamicin, are still widely used in the treatment of gram negative infections. However, marked nephrotoxicity limits the use of these drugs. The gentamicin renal toxicity is apparently related to its accumulation in the proximal convoluted tubules. Cell death may be due to lysosomal dysfunction, interference with mitochondrial respiration or damage to cell membrane ionic pumps. It is not known, however, whether the gentamicin induced some cytotoxic products and/or factors resulting from exposure to gentamicin modulate ATN (1). Nitric oxide (NO) could be involved in this process. NO diffuses rapidly across membranes and transmit a signal over many cells (2). NO is involved in the regulation of many physiological processes, as well as in the pathophysiology of a number of diseases (3). NO is synthesized enzymatically from L-arginine in numerous tissues and cells by three structurally distinct isoforms of the enzyme, nitric oxide synthase (NOS). The special role has inducible (iNOS) isoform. When it is induced by endotoxins and/or cytokines it generates high, sustained levels of NO. These elevated levels of NO can cause cellular cytotoxicity and tissue damage (4). In the last few years, a series of *in vivo* and *in vitro* studies have begun to reveal a close relationship between NO and proximal renal tubules and a significant role of NO in proximal tubule physiology and pathophysiology. It is still controversial whether the proximal tubules produce NO under basal conditions. However, numerous evidences suggest that the proximal tubules be constantly exposed to NO, which might include NO from non-proximal tubule sources. When challenged with variety of stimuli, including toxic agents and hypoxia, the structures of the proximal tubule are able to produce large quantities of NO (5). A wide range of insults including ischemia, sepsis and nephrotoxic agents (gentamicin) can lead to the renal failure. Results of numerous studies have shown that NO plays important role in development of acute renal failure (ARF) as well as chronic renal failure (CRF) (6). Pharmacological studies of NO's role in renal failure have produced confusing and contradictory results (6, 7, 8, 9). Because of that the aim of this study was to compare the plasma level of NO and the histopathological changes of kidney tissue in Wistar rats with gentamicin-induced acute tubular necrosis.

MATERIALS AND METHODS

ANIMALS

Study was carried out in Albino-Wistar rats, both sexes (n=16), average body weight 300 ± 20 g. Experiments were performed with the permission of the local Ethic Committee. All animals were allowed one week of adaptation period before beginning of the experiment. Standard rat chow and tap water were given *ad libitum*. Animals were divided in two equal groups: control and gentamicin group and housed in standard cages.

EXPERIMENTAL PROTOCOL

The control group (n=8) was injected intraperitoneally (i.p.) with 0.9 % NaCl for a period of 5 consecutive days. The gentamicin group was injected intraperitoneally with gentamicin in the dose of 80 mg/kg/day in a same period. The injections were carried out between 9.00 and 9.30 a.m. to minimize the circadian variation seen in gentamicin-induced nephrotoxicity (10).

SURGICAL PROCEDURE

The animals were sacrificed 24 hours after the last injection of gentamicin. The animals were anesthetized with ether and the front wall of the abdominal cavity was removed. Blood for the plasma NO level measurement was collected from the bifurcation of the aorta. The kidneys were removed immediately after that, vertically divided into two sections and fixed in 10% formalin and then embedded in paraffin wax.

NO

The plasma level of NO was determined by the production of nitrate and nitrites using classical colorimetric Griess reaction (11). Absorbency was measured at 546 nm. The results were expressed as $\mu\text{mol/dm}^3$.

HISTOPATHOLOGY

Sections of kidney were cut and stained with hematoxylin-eosin (H-E) and Periodic acid-Schiff (PAS). The light microscopic of the kidney sections was done according to Houghton et al. (12). The changes were limited to the tubulointerstitial areas and were graded as follows: 0=normal; 1= areas of focal glomerular/epithelial cell degeneration and granular debris in the tubular lumina with or without evidence of desquamation in small foci (< 1% of total tubule population involved by desquamation); 2= tubular epithelial necrosis and desquamation easily seen but involving less than half of cortical tubules; 3= more than half of the proximal tubules showing necrosis and desquamation.

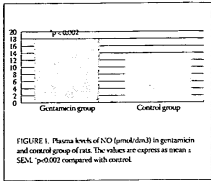


FIGURE 1. Plasma levels of NO (µmol/dm³) in gentamicin and control group of rats. The values are expressed as mean ± SEM. $p < 0.002$ compared with control.

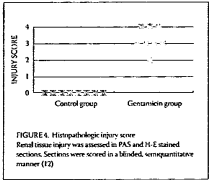


FIGURE 4. Histopathologic injury score. Renal tissue injury was assessed in PAS and H-E stained sections. Sections were scored in a blinded, semiquantitative manner (12).

tion, but intact tubules are easily identified and a complete or almost complete proximal tubular necrosis.

STATISTICAL ANALYSIS
Values are presented as mean ± SEM. Differences between the groups were tested by Student's *t*-test. Differences were considered significant for $p < 0.05$.

RESULTS

NO
The plasma level of NO in rats with gentamicin-induced ATN and in control group are shown in Figure 1. Gentamicin group had significantly higher plasma NO level compared with the control group ($p < 0.002$).

HISTOPATHOLOGY
The specimens of kidney taken from the gentamicin administered rats showed extensive tubular damage (Figure 3, A and B). The necrotic areas were observed particularly in the superficial cortex. Desquamated proximal tubular epithelial cells were widely observed in these necrotic areas. Brush-border membranes of almost all cells were disrupted. In the intact proximal tubular epithelial cells the presence of vacuoles were observed. Kidney specimens from control animals showed the normal structure of healthy tubules with abundant luminal brush-border membranes (Figure 3, A and B).

SEMIQUANTITATIVE HISTOLOGY
Kidney tissue sections were scored in a blinded semiquantitative manner based on histological changes (12) (Figure 4). In the gentamicin group, three were grade 4, four were grade 3 and one was grade 2. In the control group all specimens of kidney tissue were not changed (grade 0).

DISCUSSION

The numerous studies (1,3,14,15,16) describe experimental toxic ATN caused by uranyl-nitrate, mercury chloride, radiocontrast or nephrotoxic agents such as aminoglycosides. These studies were conducted with the purpose for clarification of underlying pathophysiological mechanisms and for identification of new therapeutic strategies. Acute renal tubular damage induced by toxic agents leads to different morphological changes in certain structures of kidney. Proximal tubules are the part of the nephron in which gentamicin induced the primary damage of various degrees. The histopathological changes could be less marked in intensity and limited only to smaller areas with focal granular vacuolar degeneration and granular debris within tubule's lumen or can also be manifested by completely necrotic proximal tubule (14). Most of the studies so far, have shown that, in the pathogenesis of toxic renal injury, NO could have certain role. It is not still, completely certain how, and to what extent, this gas contributes to the development of tubular damage (2). Our results showed that the plasma level of NO risen significantly in rats with ATN caused by gentamicin. The gentamicin dose in our study was 50mg/kg/day for 5 consecutive days and it was sufficient for induced ATN although previous reports suggested higher doses of gentamicin (14,15,17). The presence of ATN was confirmed by histological findings of kidney in gentamicin group of animals. Morphological changes in kidney specimens in our study were similar to the ones observed by Erdem et al. (14,15) who investigated protective effects of different substances in gentamicin-induced acute tubular necrosis in rats. Results of our histopathological analysis have shown marked necrosis of cortical tubules in the gentamicin group. Proximal tubular cells were transferred into an

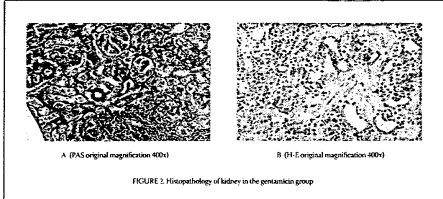


FIGURE 3. Histopathology of kidney in the gentamicin group.

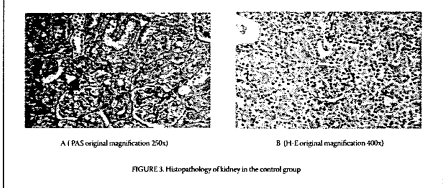


FIGURE 3. Histopathology of kidney in the control group.

amorphous mass. In certain distal tubules smaller quantity of amorphous content was observed, while in others that amorphous content filled out whole lumen of the tubules. Epithelium of distal tubules was affected by early dystrophic - necrobiotic changes (swelled cells, and around the nuclei was observed light ring). Certain proximal tubules in cortico-medullary zone had normal epithelium and cellular nuclei, while in adjacent tubules multiple PAS positive granules were observed within the cytoplasm. Some of the proximal tubules, located closer to corticomedullary zone, had partially damaged brush border. Kidney specimens from control animals showed the normal structure of healthy tubules, with abundant luminal brush-border membranes. The observed rise of NO plasma level is in accordance with results of Yangsiawe and co-workers (13), who reported increased NO plasma level in rats with mercury-chloride induced ATN. Similar results were obtained by Chatterjee co-workers (18), who also observed significant increase of plasma NO level in renal

ischemia /reperfusion induced ATN. These findings could indicate that increased production of nitric oxide by different kidney cells and structures might also play a role in the progression of gentamicin-induced acute tubular necrosis through the exacerbation of proximal and partially distal tubule epithelial cell damage. Orida and Lai (4) have stated that iNOS is expressed constitutively along the renal tubule, with the greatest expression around the medullary thick ascending loop of Henle and also within cells of proximal tubules. Our hypothesis is that gentamicin as an toxic agent in our study model have triggered the iNOS expression, so that increased plasma concentration of NO and consequent renal cells damage and necrosis might be mainly due to induced expression of iNOS and excessive production of NO under that conditions. We believe that the use of inhibitors of iNOS in our further studies will offer explanation for possible role of NO in renal damage caused by gentamicin.

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KEY WORDS: therapeutic flexible bronchoscopy, cystic fibrosis.

INTRODUCTION

Cystic fibrosis or mucoviscidiosis is an autosomal recessive disease which is manifested as a multisystemic disease of mostly exocrine glands. The majority of CF patients also have a chronic lung disease, pancreas insufficiency and an increased level of chloride in the sweat (over 60 mmol/l). The gene responsible for cystic fibrosis is located at 7q 31.2. By its gene product, it is marked as a CFTR gene (cystic fibrosis transmembranous regulator). It is a protein that functions as a chloride channel regulated by a cAMP that controls the flow of chloride through the cell membrane. Radiologically, the changes we find are bronchiectatic. The therapy is life-long (inhalator corticosteroids ICS Flutotide, inhalation with garamycin, steroids, tobramycin and 0.9 % NaCl) and antibiotic therapy (cefazidim, gentamycin, ceftriaxson, azitromicin). Physical therapy: massage vibratione to remove as much as possible of the mucous plug from the bronchial tree. In oil resolvable vitamins with pancreatin is a life long therapy of the digestive tract.

THE CASE REPORT OBJECTIVE

This is a case report about a 10-year-old girl with cystic fibrosis, who has been treated with therapeutic flexible bronchoscopy. A ten-year-old girl K.A. was repeatedly hospitalized at the Pediatric Clinic of the Kosovo University Hospital in Sarajevo due to respiratory insufficiency. The diagnosis was originally made when she was 4 years of age. The early development anamnestical data are normal. Family history: her younger sister also has CF. Anamnestically a respiratory deterioration occurred in February 2001. The patient had already been treated conservatively, but she was still very sick, so she was hospitalized. Therapeutic flexible bronchoscopy was performed with a broncho-alveolar lavage in general anaesthesia. On admission: a 10.5-year-old girl, BW 10.5 kg, BL 131 cm, afebrile, tachidyspnoic with perioral cyanosis, pale. Auscultatory findings on the lungs: crepitations, bilaterally. Chest X ray shows a diffuse-like spot shadows with bronchiectasie. Laboratory findings: ABG- respiratory acidosis, poor oxygen saturations 68 %.

DISCUSSION

The case report describes a 10-year-old girl with cystic fibrosis, who was hospitalized at our clinic. The girl had been under the longaecef conservative therapy. Therapeutic flexible bronchoscopy was performed in general

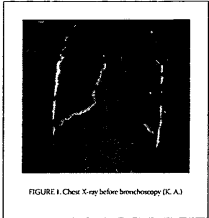


FIGURE 1. Chest X-ray before bronchoscopy (K.A.)

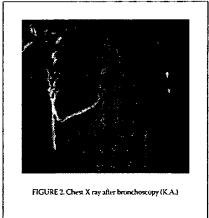


FIGURE 2. Chest X ray after bronchoscopy (K.A.)

anaesthesia. "Local bronchoscopy findings by aspiration of tracheo-bronchial trunucus it was found hyperemia and a lot of mucous sticky secretion inside of tracheobronchial tree, especially middle lobe right side, lingual and basals part of the lungs. It was performed broncho-alveolar lavage and given steroids on the place of changed inflamed, mucous membrane of the bronchi. It was also given pulmozyme to destruid mucous and make better spontaneous expectorations. Control chest x ray was performed and it was better. The auscultatory findings on the lungs turned out to be better. The infection control has been enabled, as well as spontaneous expectorations.

CONCLUSION

The treatment of children with cystic fibrosis is very complex, and besides the conservative treatment it also requires therapeutic flexible bronchoscopy. CF children must undergo regular control in order to control the infection and avoid spreading bacterial infection. (a) Local findings of the tracheobronchial tree is very important and wall of the bronchi must be control because of huge amount of sticky mucus.

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SERTRALINE AND ALPRAZOLAM IN THE TREATMENT OF PANIC DESORDER

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ABSTRACT

A compared, 12 week, placebo controlled study, with fixed dose, outpatient study of patients diagnosed with panic disorder with and without agoraphobia according to ICD-10, was conducted to evaluate the efficacy and safety of sertraline and alprazolam. The study included 40 patients, divided in two groups. We evaluated number of ICD-10 defined panic attacks, agoraphobia and anticipatory anxiety. All patients were aged 18 year and older and were randomized to either sertraline or alprazolam. Sertraline applied in fixed doses of 40 mg/day and alprazolam in doses 1-1.5 mg/day significantly reduced the frequency of panic attacks in panic disorder patients, reduced symptoms of agoraphobia and anticipatory anxiety.

KEY WORDS: panic disorders, SSRI, benzodiazepine.

INTRODUCTION

Panic disorder implied possible efficiency of the selective serotonin reuptake inhibitor (SSRI) in the treatment (1). Lifetime prevalence in the USA and Europe is 3%. Very often this disorder affects young population (2). Sertraline is a specific and potent, highly selective SSRI which can be used in the treatment of panic disorder with or without agoraphobia (3,4). Generally, panic disorders and panic attacks affect approximately 11% of the general population. This study examines the effects of prior benzodiazepine treatment, alprazolam compared to the current treatment with sertraline among patients with panic disorder with or without agoraphobia. It is well known that the majority of patients with panic disorder are treated with benzodiazepines, but the effectiveness of this therapy is insufficient, especially in cases where agoraphobia and anticipatory anxiety were present (5).

PATIENTS AND METHODOLOGY

This study involved 40 patients treated at the out-patient department with diagnosed panic disorder with or without agoraphobia according to the ICD-10 criteria.

All patients were older than 18. They were divided into the two groups. First group of 20 patients was treated with 20 mg/day of sertraline, and second (20 patients) with 1-1.5 mg/day of alprazolam over 12 weeks period. In this research a questionnaire was applied that consisted of basic social and demographic data, Hamilton Anxiety Rating Scale (HAM-A), and Hamilton Depression Rating Scale (HAM-D), as well as questionnaire about the side effects of the applied medications.

RESULTS

Table 1. presents gender structure of the sample. We can notice that there is no statistically significant difference, although there are slightly more women in the group treated with sertraline. Table 2. displays the age structure of the sample. We can notice that the most dominant age group is 30-39 for those treated with sertraline, and 40-49 for those treated with alprazolam. There is no statistically significant difference between the groups. Table 3. presents the mean values of age in both groups, which does not differ significantly. The mean age in the group of patients treated with sertraline is 35 years, while in the group of patients treated with alprazolam is

GENDER	MALE	MEDICATION		TOTAL
		SERTRALINE	ALPRAZOLAM	
		N	N	
		8	10	18
		%	%	%
		40.0	50.0	45.0
		N	N	N
		12	10	22
		%	%	%
		60.0	50.0	55.0
		20	20	40
		%	%	%
		100.0	100.0	100.0

TABLE 1. Patients according to gender Chi square=45.55 p=0.05

AGE	30-39 YEARS	MEDICATION		TOTAL
		SERTRALINE	ALPRAZOLAM	
		N	N	
		7	7	14
		%	%	%
		35.0	35.0	35.0
		9	5	14
		%	%	%
		45.0	25.0	35.0
		2	4	6
		%	%	%
		10.0	20.0	15.0
		2	4	6
		%	%	%
		10.0	20.0	15.0
		30	30	40
		%	%	%
		100.0	100.0	100.0

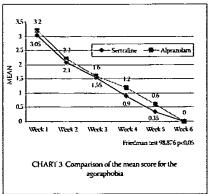
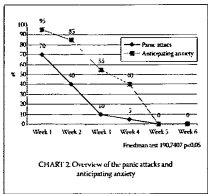
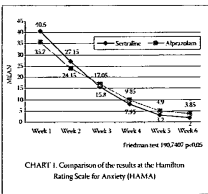
TABLE 2. Patients according to age Chi square=24.65 p=0.05

MEDICATION	N	MEAN	STANDARD DEVIATION	STANDARD ERROR MEAN
SERTRALINE	20	35.00	9.285	2.068
ALPRAZOLAM	20	36.65	11.123	2.487

TABLE 3. Overview of the average age
Lever test for quality of variance: $F(1,38)=9.405$

APPLIED MEDICATION		WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6
SERTRALINE	MEAN	25.35	17.35	10.20	5.25	3.20	1.00
	STANDARD DEVIATION	8.725	6.633	5.245	4.982	4.839	1.622
ALPRAZOLAM	MEAN	27.65	20.00	12.70	6.05	3.40	1.25
	STANDARD DEVIATION	8.677	6.407	5.131	4.872	3.085	1.489
TOTAL	MEAN	26.60	18.67	11.45	5.65	3.30	1.12
	STANDARD DEVIATION	8.685	6.577	5.224	4.638	4.029	2.078

TABLE 4. Comparison of the results at the Hamilton Rating Scale for Depression (HAM-D)
Friedman test: $F(5,38)=9.028$ p<0.05



36.65 years. Table 4. presents comparison of the results at the Hamilton Rating Scale for Depression (HAM-D) between the two groups, where we can see depressive symptoms decreased at the end of the second week that in the group of patients treated with sertraline (p<0.05). At the Chart 1. results of the Hamilton Rating Scale for Anxiety are presented. It is noticeable that in the group of patients treated with SSRI, beside the higher initial scores, there was a significant decrease of anxiety symptoms after the second week, compared to alprazolam. Group treated with alprazolam shows less effects of the medication in terms of relieving anxiety symptoms after the third week. Chart 2. shows the frequencies of panic attacks and anticipating anxiety. It is noticeable that in the group of patients treated with SSRI already during the second week, among 30% of patients panic attacks disappeared, while anticipating anxiety disappeared in 10% of cases. After the fourth week Panic attacks and anticipating anxiety completely withdrew. (p<0.05). It is noticeable in the Chart 3. that fears sig-

nificantly decreased among patients treated sertraline and alprazolam, although patients treated with sertraline felt the effect of the medication somewhat sooner, or to be more precise during the third week (p<0.05).

CONCLUSIONS

Patients with panic disorder with or without Agoraphobia treated with SSRI (sertraline) in fixed dose of 50 mg/day showed reduction of panic attacks compared to the patients on Benzodiazepine (alprazolam) treatment in dose of 0.1-0.3 mg/day. With this we can conclude that the sertraline is efficient in the treatment of panic disorder with or without Agoraphobia.

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INTRAOSSEOUS INJECTION
OF CLINDAMYCIN
PHOSPHATE INTO THE
CHRONIC APICAL LESION
OF LOWER MOLAR -
A CASE REPORT

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ABSTRACT

Periapical disease is the result of bacteria, their product, and the host response to them. Early histological studies of diseased periapical tissue have not been able to demonstrate viable bacteria in the lesions studied. Recent reports indicate that many of periapical lesions are indeed infected before and after endodontic treatment. The validity and applicability of the microbial delivery overcome many disadvantages that we see with systemic drugs. In this case report we presented a novel approach of managing chronic diffuse periapical lesion of lower molar based on specific selection of intracanal medicament in combination with direct periapical injection. We used bacterial culturing and antibiotic sensitivity test to select specific intracanal medicament. In addition we presented an intrasosseous injection technique to locally deliver the selected medicament directly into the periapical lesion. Our findings are encouraging and promising. The validity and applicability of the technique needs to be tested in a well controlled clinical trial.

KEY WORDS: periapical disease, root canal therapy, microbiology, intrasosseous injection, local antimicrobial delivery, clindamycin

INTRODUCTION

Periapical disease is the result of bacteria, their product, and the host response to them. Early histological studies of diseased periapical tissue have not been able to demonstrate viable bacteria in the lesions studied (1). More recent reports indicate that many of these lesions are indeed infected before and after endodontic treatment. In 1992 Wayman studied 58 cases of periapical lesion (2). He cut these lesions in half and examined one half histological and cultured the other half. In only 8 of 58 cases could he demonstrate bacteria histologically. However, when the other half lesion was cultured 51 of 58 cases were positive. He found 133 isolates, of which 87 were strict anaerobes, 37 were facultative anaerobes and only 9 were aerobes. Two showed that 88% or 14 of 16 periapical granulomas were positive for bacteria when they were cultured. Barkhodar and Desouza (3) also found bacteria in granulomas and cystitis. For many clinicians calcium hydroxide is the most commonly used intracanal medicament, although the effectiveness of many other intracanal antimicrobials, such as metronidazole antibacterial gel (4), Idermix with tetracycline (5), clindamycin-impregnated fiber (6), antibiotic mixtures of ciprofloxacin, metronidazole, minocycline (7), has been proven effective. Calcium hydroxide as an intracanal medicament has many advantages (8,9), but latest evidence demonstrated that enterococci and fungi, commonly found in cases of endodontic failure are highly resistant to calcium hydroxide (10).

AIM

The primary aim of this case report is to evaluate the effectiveness of using microbial culturing and antibiotic sensitivity testing to select specific antimicrobial medicaments to manage endodontic infections. A secondary aim is to evaluate the effectiveness of the selected antibiotic as an intracanal medication in combination with intrasosseous injection directly into the periapical lesion

MATERIAL AND METHODS

CLINICAL PRESENTATION

The patient (age 32, gender female, race white) came to our office with very slight symptoms in a region of left lower second molar. Clinical and radiographic examination showed coronal microleakage, dental decay, infected pulp space, separation with large apical radiolucent lesion. Root canal system

was infected as a result of microleakage and consequently dental decay and bacterial flora was transferred from root canal towards periapical tissue.

Specimen collection and microbiological procedure

After clinical and X-ray diagnose of asymptomatic left mandibular second molar with diffuse periapical lesion we did access preparation by sterile burs, restoration and complete removal of soft, carious dentin using sterile burs without water spray. ISO 1/2/3 K files used to scale off dentin debris from the root canal walls. Specimen collected by K-files is directly inoculated in liquid thioglycolate broth with paraffin oil, for further primary and secondary microbiological procedure (Figure 1.). These procedures included: direct microscopy examination of the specimen, showing size, shape, morphology, and Gram staining of the present bacteria. During aerobic procedure specimen from thioglycolate broth is inoculated in the blood agar, McConey and Sabourad (Figure 2.). For anaerobic procedure another agar plate is necessary with 4% homin and placed discs. Anaerobic plates were in jar with Gas-pac system, closed for 48-72 hours at 37°C in incubator (Figure 3.). Findings of bacteria around discs and far from discs determined the result of antibiotic susceptibility test. All isolated Streptococcus spp. during microbiological procedure were sensitive to clindamycin.

Conventional endodontic treatment

After completing mechanical instrumentation of two root canals of mandibular second molar we used oxidizing agent, 3% hydrogen peroxide as root canal irrigant. According to the antimicrobial susceptibility test obtained, as an intracanal medicament a sterile pharmaceutical formulation (with the proper rheological properties) for the root canal treatment was prepared and used. Antimicrobial agent clindamycin phosphate, as an intracanal medicament, was used in concentration of 3%, incorporated in the formulation containing benzyl alcohol, sodium hydroxide, hydroxyethylcellulose, propylene glycol and water for injection. The temporary filling was intact, without leaking, or contamination, sealed hermetically, without exudate, or foul odor from the root canals. Next step was complete obturation of the root canals using cement sealer and gutta-percha cone. All procedures were done with respect to ethical standards regulated by Helsinki Declaration.

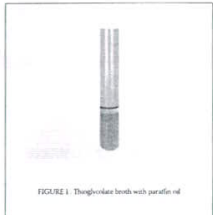


FIGURE 1. Thoroughly clean both with paraffin oil.



FIGURE 2. Amoxicillin plate - Streptococcus agalactiae.

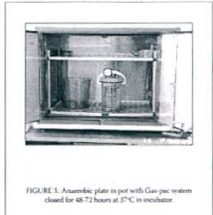


FIGURE 3. Amoxicillin plate in pot with Gas-pac system closed for 48-72 hours at 37°C in incubator.

With assigned form of patient consent we proceeded with the treatment and local antimicrobial drug delivery.

The use of intranasal injection

In our next appointment patient received regional anesthesia (Figure 4). The point of intranasal penetration was attached gingiva, area between the two roots of the second lower molar. We used special equipment, the perforator attached to a slow speed hand piece was advanced through anesthetized gingiva and bone until penetration through the cancellous bone is experienced (Figure 5) (11, 12). The perforator was removed, the short 8 mm needle was inserted through the perforation into the cancellous space and 1.8 ml of clindamycin phosphate solution was delivered slowly (Figure 6).

CLINICAL OUTCOME

We monitored response to treatment on sequential periapical radiographs using a portable transmission densitometer (Portable densitometer X-Rite 331, X-Rite Ltd., United Kingdom). We took periapical images at the beginning of the endodontic treatment, during obturation of the root canal two- and six months later. A change in periapical lesion was detected by X-Rite 331 densitometer showing improvement in periapical healing process. Measured optical density units at the beginning of the endodontic treatment and two months later showed difference of 0.16 ODU, suggesting improvement in healing process of the affected tissue. Figures 7, 8 and 9 present periapical images obtained at the beginning of the endodontic treatment, after completed obturation and six months later.

DISCUSSION

In this case report we used bacterial culturing and antibiotic sensitivity to select specific intracanal medicament, in addition we presented an intranasal injection technique to deliver the selected medicament directly into the periapical lesion. The combined use of the selected antibiotic as intracanal medicament as well as direct injection into the periapical area enhanced the healing and resolution of the lesion as demonstrated on the sequential periapical radiographs. The intervention is completely painless since we had to apply conventional mandibular block, before we used supplemental intranasal injection technique for local application of clindamycin phosphate. Clindamycin is derived from Strepto-



FIGURE 4. Class mandibular block.



FIGURE 5. The perforator attached to a slow speed hand piece, advanced through gingiva and bone.

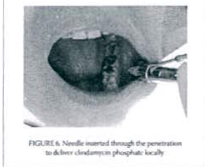


FIGURE 6. Needle inserted through the penetration to deliver clindamycin phosphate locally.



FIGURE 7. First periapical image of lower molar.



FIGURE 8. Periapical image after completed obturation.



FIGURE 9. Periapical image after six months.

myces lincolnensis (13). The mechanism of action, like the macrolide class, is inhibition of protein synthesis by binding to the 50S ribosomal subunit. It has a wide distribution, but does not cross the blood-brain barrier. The drug is concentrated in the bone, respiratory tissues, mucus, and saliva. At lower concentrations the drug is

bacteriostatic, but bacteriocidal at higher concentration. Resistance is mediated by decreased permeability of the drug into the cell and alteration of binding sites on the 50S ribosome subunit. The spectrum of action is primarily against gram-positive and gram-negative bacteria and anaerobes (especially B. fragilis). Clindamycin

phosphate has been shown to be effective, among others in the treatment of periodontal abscess, periodontitis, and bone infections when caused by susceptible anaerobic bacteria or susceptible strains of gram positive bacteria such as Streptococci, Staphylococci and Pneumococci. It possesses both a bacteriostatic and a bactericidal action. Clindamycin affected microbial cultures show biphasic steady state generation curves. An

initial (phase I) generation of the clindamycin affected microbes is followed by an ultimate (phase II) generation at the same dose level. The dependence of the apparent generation rate constant on drug concentration yields a sigmoidal curve, which is coincident by a potency factor for the phase I and phase II generations of clindamycin affected microbes and suggests a common mechanism of action for both generation phases.

CONCLUSIONS

In previously reported cases of conventional endodontic treatment of lower molars with chronic diffuse periapical pathology the outcome of treatment has been slow and questionable. In this case report we presented a novel approach of managing these lesions based on specific selection of intracanal medicament in combination with direct periapical injection. Our findings are encouraging and promising. The validity and applicability of the technique needs to be tested in a well controlled clinical trial.

ACKNOWLEDGMENT

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COLLATERAL CIRCULATION IN HUMAN HEART

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ABSTRACT

The aim of the investigation was to demonstrate different types of collaterals of coronary arteries using the method of coronary angiography and injection-corrosion method. The investigations were carried out on 30 human cadaveric hearts from the Department of Anatomy, and 30 angiograms of patients from the Cardiology Department of Clinics Centre in Sarajevo. Clinical investigations were retrospective and prospective on patients that were treated in hospital, and on patients that just arrived in hospital (based on findings of coronary angiography). The results show the existence of different types of collaterals: intercoronary and intra-coronary. We established collaterals in a case with occlusion of the right coronary artery and left coronary artery in which better development of collaterals was established.

Our patients were classified in two groups:
1) Patients with good collaterals and good left ventricular function;
2) Patients with poor collaterals and impaired left ventricular function.
On the anatomical material we found different types of collaterals as well.
Our results show that coronary angiography is useful diagnostic method for the demonstration of coronary collaterals.

KEY WORDS: coronary arteries, collaterals, injection-corrosion method, angiography

INTRODUCTION

The collaterals of human coronary arteries have always attracted the attention of anatomists, pathologists, surgeons, as well as experts in many clinical disciplines. The occurrence of coronary diseases has increased recently so much that it stimulates researchers to become acquainted with collaterals of coronary arteries. Its real significance is expressive in cases with occlusion or stenosis of coronary arteries: angina, myocardial infarction, congenital cardiovascular malformations etc. (1,2,3,4,5). Therefore, the aim of the investigations was to demonstrate different types of collaterals of the coronary arteries in normal condition and the conditions of coronary disease using the method of coronary angiography and injection corrosion method. On the other side, these investigations are important because of contrast opinions that are given in literature concerning coronary arteries collaterals. According to some authors collaterals exist and they are functional (6,7,8,9,10). The others think that collaterals exist, but that they are insufficient for collateral circulation and only develop in pathological conditions (11). Ischemia changes of the heart, variations of coronary arteries and the collaterals have been studied by many authors: Hadzidimovic, Werner, Pohl, Seiler, Kamenica, Satic, Rockstroh, Rapps, Holmvang, Billinger, Meyer and others.

MATERIAL AND METHODS

Our investigations were carried out on 30 human hearts (regardless of sex or age), some of which were without microscopically visible pathological changes, and on 30 angiograms of patients with occlusion or stenosis of coronary arteries. The investigations on the hearts without visible pathological changes were undertaken at the Department of Anatomy of the School of Medicine in Sarajevo, whereas the data regarding the hearts with signs of coronary disease were obtained in cooperation with the Cardiology Department of Clinics Centre in Sarajevo. Clinical investigations were retrospective and prospective on patients that were treated in hospital, and on patients that just arrived to hospital (based on findings of coronary angiography). Their ages ranged from 45-72 years. Diagnoses were different: chronic stable angina and atypical angina, myocardial infarction, arterial hypertension. Various methods were applied in our investigations: injection-corrosion and coronary angiography. In injection-corrosion method, violet in two different colours was injected in both of coronary arteries and blue substance in veins. After successful

injection the hearts were placed into 30% HCl solution and then rinsed in a weak jet of water. Soft parts of the hearts decayed and came off, whereas filled blood vessels stayed preserved because they are not subject to corrosion. With this method we examined coronary arteries and veins as well as their collaterals. We also applied coronary angiography method. A catheter was inserted into the femoral artery. We used the catheters by Judkins for both right and left coronary artery. Omnipaque was introduced as a contrast. Both arteries were X-rayed in two radiographic projections: RAO (right anterior oblique) and LAO (left anterior oblique). For all patients we applied left ventriculography for the evaluation of ejective fraction. Using this method we expected the existence of collaterals in occlusion or stenosis of coronary arteries (coronary disease). On the angiograms we analyzed collaterals as "good" and "bad" based on diameter. The investigations were done with respect to ethical standards regulated by Helsinki Declaration.

RESULTS

The results show the existence of different types of collaterals: intracoronary (between the branches of the same coronary artery) and intercoronary (between two coronary arteries). We established collaterals in a case with stenosis or occlusion of the right coronary artery as well as of the left coronary artery and its branches in which better development of collaterals has been established. On the anatomical material we found different types of collaterals using injection-corrosion method (Figure 1,2). Our patients were classified into two groups (based on findings of coronary angiography):

- patients with good collaterals and good left ventricular function (Figure 3, 4, 5, 6)
- patients with good collaterals and impaired left ventricular function (Figure 7, 8, 9, 10).

DISCUSSION

Although many authors (1,6,8,9) wrote about ischemia changes of the heart, variations of coronary artery and their collaterals, even today there are many different and, what is more, contradictory opinions in the literature about the role of collateral circulation in revascularization in the field with a damaged blood vessels. So, one group of authors (4) consider the role of coronary arteries in that sense minimal, whereas the other authors (2,3,5) find their role important but not examined enough. Our investigations confirmed the existence of the coronary arteries collaterals, both on the hearts with-



FIGURE 1. Intracoronary collaterals, left coronary artery injection corrosion

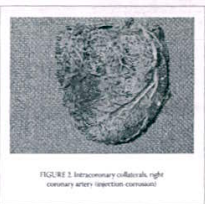


FIGURE 2. Intracoronary collaterals, right coronary artery injection corrosion

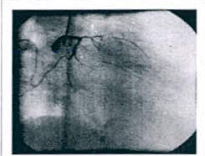


FIGURE 3. RAO left coronary arteriogram
1. Proximal occlusion of the left anterior descending branch
2. Minimal changes on the circumflex branch

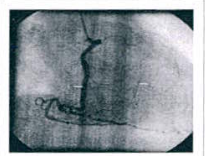


FIGURE 4. RAO right coronary arteriogram
1. Right coronary artery dominant
2. Distal part LAD has been filling septal branches of the RCP



FIGURE 5. RAO left ventricle (diastole)



FIGURE 6. RAO left ventricle (systole)

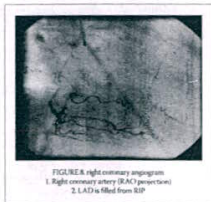
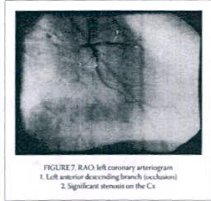
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out visible pathological changes and the ones with visible pathological changes - coronary disease (n=11). Clinical investigations showed two groups of patients: with good collaterals and good left ventricular function and with good collaterals and impaired

left ventricular function. In the first group EF (ejective fraction) was 60%, in the second one EF was 35%. The valuation of collaterals were based on diameter - "good" collaterals with diameter more than 1 mm, and "bad" collaterals with diameter less than 1 mm. (3).

CONCLUSION

Our results show that the injection corrosion method and coronary angiography are useful methods for the demonstration of coronary collaterals in the normal and pathological condition. In patients with coronary artery disease angiographic evaluation of collaterals is a good diagnostic method and has potential clinical value. Collateral function is better in patients with good left ventricular function (ejective fraction 60%) than in those with impaired left ventricular function (ejective fraction 35%). This result would be expected but was not shown clear using angiographic method. The present angiographic study assesses the relation of collateral and regional myocardial function. "Good" collaterals with diameter more than 1mm have important role in protection of left ventricular function (EF=60%) but the situation is not the same for "bad" collaterals with diameter less than 1mm (EF=35%).



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