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Señora Directora
DIRECCION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 379
Actuación : 412

Referencia : Expediente **No. 04109926**
Asunto : Solicitud de patente para: **"Composición veterinaria para animales pequeños".**
Solicitante : NORBROOK LABORATORIES LIMITED
Fecha de solicitud: Noviembre 2 de 2004.

I. RECURSO VÍA GUBERNATIVA

Yo, Luz Clemencia de PAÉZ, obrando como apoderada del solicitante en el asunto antes identificado, de acuerdo con la sustitución que me ha sido efectuada por el Doctor Emilio Ferrero, por el presente escrito atentamente manifiesto a usted que debidamente notificada de la Resolución **No. 14582** del 16 de Marzo de 2010, interpongo en su contra el recurso de **REPOSICIÓN**, para que sea revocada y en consecuencia se conceda el privilegio de patente de invención para **"COMPOSICIÓN VETERINARIA PARA ANIMALES PEQUEÑOS"** a favor de **NORBROOK LABORATORIES LIMITED.**

II. OPORTUNIDAD DE LA IMPUGNACION

La Resolución **No. 14582** del 16 de Marzo de 2010, fue notificada mediante edicto **No. 6114** desfijado el quince (15) de Abril de dos mil diez (2010), por lo que el término para interponer el recurso de reposición vence el veintidós (22) de Abril de dos mil diez (2010). En consecuencia, me encuentro dentro de la oportunidad legal.

III. LA DECISION RECURRIDA

3.1 Mediante la Resolución objeto de la presente impugnación, el Superintendente de Industria y Comercio decidió:

3.1.1 Negar el privilegio de patente para la invención **"COMPOSICIÓN VETERINARIA PARA ANIMALES PEQUEÑOS".**

3.2 En la parte motiva de la providencia, su Despacho concluye que el nivel inventivo de la presente solicitud de patente se ve afectado por los documentos EP0955063 (D1) y CH 663788 (D2) y se mencionan los siguientes fundamentos:

3.2.1 "Teniendo en cuenta el objeto de la solicitud, el cual es proporcionar composiciones acuosas inyectables de carprofeno que contienen poloxámero, es una derivación evidente del estado de la técnica, ya que una persona versada en la materia estaría en capacidad de tomar las composiciones inyectables desinflamatorias de D1 y cambiar el principio activo por carprofeno, del cual ya se conoce su acción desinflamatoria y analgésica, tal como lo enseña D2".

3.2.2 "Adicionalmente no se demuestran propiedades mejoradas o sorprendentes de las composiciones que se reivindican en comparación con las del estado de la técnica, debido a que si bien el solicitante asegura que las composiciones son estables a temperatura ambiente, D1 enseña que las composiciones de poloxámero-antiinflamatorio tienen una temperatura de transición entre 20-40°C y, por lo tanto, se puede asumir que tales composiciones también son estables a temperatura ambiente en forma de solución".

3.2.3 "El método de fabricación de las reivindicaciones 11-13, es un simple proceso de disolución y mezclado del principio activo en agua, que una persona versada en la materia lo encontraría obvio, ya que no tiene condiciones, ni procedimientos extraordinarios que permitan diferenciar tal procedimiento con los conocimientos usuales de preparación de soluciones de un técnico en formulación farmacéutica".

3.2.4 "De la lectura de la descripción no se evidencia que las composiciones son para aplicación intravenosa, solamente se relacionan composiciones de carácter inyectable que disminuyen la irritación local, por lo tanto, asegurar que las composiciones de la presente solicitud son inyección intravenosa no procede, pues no se encuentra sustento de ello en la descripción.

3.2.5 "Ahora bien, respecto a la estabilidad reclamada a lo largo del estudio de fondo se le pidió al solicitante que aportara resultados o estudios que demostraran que la composición es superior en comparación a lo conocido en el arte, pero el solicitante no aportó análisis comparativas que sustentaran el efecto técnico reclamado".

3.2.6 "Es preciso aclarar al solicitante, que de la manera como se reivindica el proceso de preparación de las composiciones reclamadas (poner juntas carprofeno o sus sales, poloxámero y agua)) no se diferencia en nada de un proceso usual de mezcla, el cual es conocido por un técnico en preparaciones inyectables".

3.2.7 "Así mismo, de la lectura de las reivindicaciones reclamadas se desprende que el poloxámero está con el solvente orgánico

(reivindicación 8), por lo tanto no existe diferencia real de lo reivindicado y lo enseñado por D1 ya que en ambos casos se mezcla el activo con un solvente y el poloxámero, para luego añadir agua. En conclusión, la supuesta diferencia existente con el procedimiento de fabricación de D1 no existe, ya que en ambos casos se mezcla activo con solvente orgánico para disolverlo y luego añadir agua".

IV. OBJETO DEL RECURSO

De acuerdo con los argumentos que expongo a continuación, solicito atentamente a su Despacho lo siguiente:

4.1. Revocar la Resolución No. Resolución **No. 14582** del 16 de Marzo de 2010.

4.2. Conceder el privilegio de patente de invención para **"COMPOSICIÓN VETERINARIA PARA ANIMALES PEQUEÑOS"** a favor de **NORBROOK LABORATORIES LIMITED.**

V. FUNDAMENTOS DEL RECURSO

Respetuosamente me permito disentir de lo expuesto por este Despacho en la Resolución objeto del presente recurso, ya que no compartimos la decisión del Señor Examinador con respecto a que la presente invención carece de nivel inventivo.

Lo anterior por las siguientes razones jurídicas y técnicas sobre las cuales respetuosamente solicitamos un pronunciamiento expreso de su Despacho:

5.1 Violación de los artículos 14 y 18 de la Decisión 486 de la Comisión de la Comunidad Andina:

De acuerdo con los artículos 14 y 18 de la Decisión 486:

> *"Artículo 14.- Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial."*(Subrayas fuera del texto)

> *"Artículo 18.- Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica"*

Ahora bien, el Manual para el Examen de Solicitudes de Patentes de invención en las Oficinas de Propiedad Industrial de los Países de la Comunidad Andina con respecto a la evaluación del nivel inventivo indica:

"La pregunta a contestar es si teniendo en cuenta el estado de la técnica en su conjunto existe alguna ***indicación que lleve a la persona versada en la materia a modificar o adaptar el estado de la técnica más cercano para resolver el problema técnico****, de tal forma que llegue a un resultado que estuviera incluido en el tenor de la(s) reivindicación(es)".* (Negrita fuera del texto).

La obligatoriedad de este Manual no reside en su fuerza normativa intrínseca sino en que el mismo es mencionado por el Tribunal de Justicia en reiterada jurisprudencia cuando interpreta estas normas de la Decisión 486 y por ende, cuando establece cómo debe adelantarse el estudio de nivel inventivo de una invfención.

Por lo tanto, teniendo en cuenta el carácter vinculante que tienen las interpretaciones prejudiciales del Tribunal para el Consejo de Estado y el hecho de que éste último revisa la legalidad de las decisiones adoptadas por su Despacho, consideramos que dicho Manual si resulta relevante para el estudio del nivel inventivo de la presente invención.

Así entonces, es posible concluir que para ser pertinentes en la la evaluación de nivel inventivo de la presente invención, los documentos citados **deberían** revelar sugerencias y enseñanzas claras para el técnico en la materia que lo lleven a obtenerla. Para este fin el Señor Examinador debe examinar si, de conformidad con dichos documentos (el estado de la técnica más próximo), una persona versada en la materia habría o no propuesto las características técnicas que distinguen la invención reivindicada y que permiten alcanzar los resultados obtenidos por dicha invención.

Para los anteriores efectos, el Examinador al realizar el examen de nivel inventivo debe verificar las enseñanzas del arte previo sin adoptar la posición de un inventor que se preguntaría cómo solucionar el problema técnico en cuestión. Lo anterior, por cuanto si se adopta la posición del inventor y se tiene conocimiento tanto del arte previo como de la forma como efectivamente se solucionó el problema técnico, la invención en todos los casos le resultará obvia y evidente.

A este respecto, consideramos pertinente poner de presente la cita del tratadista GOMEZ SEGADE, mencionada por el Tribunal de Justicia del Acuerdo de Cartagena en la sentencia proferida, dentro del Proceso No. 26-IP-99, el 23 de julio de 1999, en relación con el significado que la doctrina y la jurisprudencia le han dado a la característica de nivel inventivo.

> *"Tal y como lo establece GOMEZ SEGADE (obra citada) el inventor debe reunir los méritos que le permitan atribuirse una patente, sólo si la invención fruto de su investigación y desarrollo creativo constituyen "un salto cualitativo en la elaboración de la regla técnica", actividad intelectual mínima que le permitirá*

que su invención no sea evidente (no obvia) del estado de la técnica.".

Teniendo en cuenta el anterior planteamiento sobre lo que debe considerarse nivel inventivo, podemos concluir que una invención solamente cumplirá con este requisito cuando la misma haya resultado a través de estudio y experimentación (investigación) y constituya un adelanto tecnológico, materializado en un resultado inesperado (mejorado) y además no evidente para alguien del oficio normalmente versado en la materia.

Es así que el Tribunal de Justicia del Acuerdo de Cartagena, ha manifestado en reiterada jurisprudencia los elementos que debe tener en cuenta el examinador de patentes para establecer la existencia o no de NIVEL INVENTIVO en una invención:

"El nivel inventivo se configura con referencia a dos elementos que son:

a) el estado de la técnica y ,

b) la persona experta en la técnica en cuestión.

El estado de la técnica es el conjunto de elementos técnicos que se han hecho públicos antes de la fecha de presentación de la patente",

"El experto en la técnica es una figura ficticia a la que se recurre con el propósito de obtener un parámetro objetivo que permita distinguir la actividad verdaderamente inventiva de la que no es. Se tratará de una persona normalmente versada en el ámbito tecnológico a que se refiere el pretendido invento. Su nivel de conocimientos es más elevado en comparación con el nivel de conocimientos del público en general, pero no excede lo que puede esperarse de una persona debidamente calificada. Se busca la figura de un técnico de conocimientos medios, pero no especializados". (Sentencia del 21 de julio de 2000, proferida en el proceso 39-IP-2000, Magistrado Ponente Rubén HERDOIZA MERA).

Asimismo, es importante resaltar que si bien el resultado y/o los medios reivindicados en la invención objeto de análisis ya eran conocidos (estado de la técnica), la invención carecerá de nivel inventivo.

En efecto, se pone de presente que si al momento de efectuar el examen de nivel inventivo de una invención cualquiera, los medios usados para

la producción de dicho resultado son conocidos, este **solo** hecho no permitirá al examinador concluir que la invención carece de nivel inventivo y que por ende no puede ser objeto de patente de invención.

Precisamente esto resulta plenamente concordante con la posición reciente de esa misma Corporación, quien en sentencia del 28 de enero de 2010 en el Expediente No. 2004-00401, siendo Consejero ponente el Dr. Marco Antonio Velilla Moreno, señaló:

> "**[...] si el inventor para la creación en cuestión, extrajo elementos de anterioridades como componentes para su invento, no afectaría negativamente su altura inventiva, en la medida en que ésta se considere en su combinación y no en cada uno de los elementos extraídos para su estructura, tal como acertadamente lo analiza el Tribunal Andino en la interpretación solicitada e este proceso**" -se refiere a la sentencia de interpretación prejudicial del Proceso 198-IP-2006 -

De conformidad con el anterior artículo, se expone a continuación el por qué la presente invención posee nivel inventivo frente a los documentos D1 y D2, toda vez que para el presente caso la decisión de la División de Nuevas Creaciones fue expedida en contravención de la Ley, toda vez que se interpretó erróneamente el artículo 18 de la Decisión 486. Veamos:

5.1.1 Con respecto a las reivindicaciones de producto.

El problema planteado en la presente solicitud es la necesidad de composiciones acuosas inyectables de carprofeno que sean adecuadas para animales pequeños. Este problema fue el que precisamente solucionó la presente invención, toda vez que la misma proporciona composiciones inyectables que presentan efectos secundarios reducidos (estabilidad mejorada).

Ahora bien, D1 no divulga una composición que es una solución inyectable estable a temperatura ambiente, como la requerida por la presente solicitud. Sin embargo, no hay evidencia en D1 que demuestre que las composiciones allí reveladas sean estables a temperatura ambiente. En efecto, D1 revela composiciones SOL-GEL que pueden ser administradas a temperatura ambiente en forma líquida intramuscularmente y subcutáneamente. Sin embargo, dichas composiciones a temperatura corporal producen un gel de liberación lenta (Ver párrafo 0008 de la descripción en idioma original), a partir del cual los ingredientes activos son liberados de forma controlada.

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Adicionalmente, mi representada desea poner de presente que el hecho que las composiciones poloxámero-anti-inflamatorias divulgadas en D1 presenten una temperatura de transición entre 20 a 40°C, no significa que dichas composiciones sean estables a temperatura ambiente en forma de solución. De hecho, la estabilidad de las composiciones necesita ser verificada a partir de estudios de estabilidad adecuados a varias temperaturas por al menos 6 a 12 meses. Sin embargo en D1, en la figura 4 simplemente se compara la estabilidad de los geles en relación con la resistencia de penetración de los mismos, los cuales comprenden diferentes concentraciones de Lutrol F68, F127 mas o menos 0.1% Carbopol (PAA) y no se menciona nada con respecto a la estabilidad en el tiempo y a distintas temperaturas de los activos en el gel.

De otro lado, D2 enseña sales de carprofeno que son útiles para la preparación de composiciones farmacéuticas analgésicas, antiinflamatorias y antirreumáticas. Los ejemplos 1-3 divulgados en D2 se refiere a la síntesis de sales de carprofeno. El ejemplo 4 se refiere a composiciones en tableta, cápsula y/o supositorio. A diferencia de la presente solicitud, ninguna de las composiciones allí reveladas presenta un polímero con carprofeno y no son inyectables. En efecto, todas las composiciones mencionadas en dicho documento presentan formas de dosis **sólidas**.

Ahora bien, es importante resaltar que para una persona versada en la materia no sería obvio reemplazar el ibuprofeno de D1 con carprofeno debido a que a pesar de que ambos son NSAIDs del grupo priopiónico, estos tienen diferentes propiedades.

Por ejemplo el Carprofeno es un ácido debil, con un pKa de 4.3 y un coeficiente de partición n-octanol/agua de 41 a un PH de 7.4; mientras el ibuprofeno tiene un pKa de 4.43 y un coeficiente de partición de 11.7 a un PH de 7.4. Ambas drogas poseen propiedades anti inflamatorias, analgésicas (sitios de acción periféricos y centrales) y antipiréticas.

En un contexto amplio, ambas drogas actúan disminuyendo la producción de prostaglandinas y tromboxano A2 de acido araquidónico por membranas de células dañadas.

Ahora bien, el Ibuprofeno es un NSAID no selectivo de COX. Asimismo, según estudios recientes las propiedades antiinflamatorias adicionales se deben a la modulación de la actividad de leucocito, producción reducida de citoquina, inhibición de radicales libres, transducción de señales u acción analgésica. Sin embargo, el carprofeno es un NSAID selectivo de COX-2 con una inhibición débil de las isoenzimas COX. Esta propiedad ha ayudado a obtener una licencia para el uso preoperatorio porque hay un riesgo reducido de que afecte el flujo de sangre renal durante un episodio de hipotensión en condiciones anestésicas.

Asimismo, el ibuprofeno puede ser un analgésico efectivo en caninos pero existe evidencia de que tiene un perfil de seguridad pobre en estas especies con riesgos inaceptables de ulceras gastrointestinales y fallas renales.

Un estudio realizado por Jackson et al ("Correlation of serum ibuprofen concentration with clinical signs of toxicity in three canine exposures"-Vet Hum Toxicol, 1991, Oct; 33(5): 486-8), anexo al presente memorial, evaluó la correlación entre la concentración de ibuprofeno en suero con signos clínicos de toxicidad en tres exposiciones caninas. No se observaron señales clínicas adversas o parametros de laboratorio anormales cuando las concentraciones de ibuprofeno en suero eran menores de 31mg/ml. Sin embargo, melena y niveles altos de nitrógeno ureico en sangre (azotaemia), se presentaron en animales con un a concentración de ibuprofeno en suero de 138 mg/ml.

Otro estudio realizado por Tsuchiya et al ("Early pathophysiological features in canine renal papillary necrosis induced by nefiracetam". - Toxicol Pathol, 2005; 33(5):561-9), anexa al presente memorial, observó las características pato fisiológicas en necrosis papilar renal en caninos (RPN) causada por nefiracetam en comparación con una droga de control (ibuprofeno a 50mg/kg/día por cinco semanas en perros sabueso). El ibuprofeno presento degeneración y necrosis en el intersticio renal papilar en la semana 5.

Asimismo, a partir de un caso de estudio de control realizado usando datos de la base de datos del **"National Animal Poison Control Center"** con el objetivo de caracterizar los factores de riesgo para ulceras gastro intestinales e insuficiencia renal aguda luego de la ingesta de ibuprofeno por el perro, se observaron diferencias significativas entre distintas razas de perro en términos de susceptibilidad a ulceras gastrointestinales. En particular, se encontró que el perro pastor alemán presenta un mayor riesgo de presentar efectos adversos y requiere de intervención agresiva incluso luego de la ingesta de pequeñas cantidades de ibuprofeno. Además, se encontró que el tiempo entre la ingesta y la intervención veterinaria fue critica tanto para repercusiones de insuficiencia renal aguda y gastrointestinal (Ver anexo: Poortinga and Hungerford. A case-control study of acute ibuprofen toxicity in dogs.-Prev Vet Med, 1998, May 1; 35(2): 115-24).

Las composiciones acuosas inyectables de la presente invención son para el suministro de analgesia en animales pequeños, especialmente animales de compañía, y en particular perros. Así entonces, teniendo en cuenta los argumentos arriba mencionados es claro que una persona versada en la materia buscando proporcionar una nueva preparación inyectable comprendiendo carprofeno para el suministro de analgesia en animales pequeños, y en particular en perros no se vería motivada a partir de las composiciones reveladas en D1, toda vez que las mismas contienen ibuprofeno, por cuanto dicho compuesto es toxico en perros y

por ende no solucionaría el problema técnico planteado en la presente solicitud.

Lo anterior, es aun más evidente si se tiene en cuenta que el ibuprofeno y el carprofeno actúan de forma muy distinta cuando son empleados como NSAID en formulaciones para perros. De tal manera que, no sería obvio para una persona versada en la materia reemplazar el ibuprofeno de D1 por carprofeno para obtener la presente invención.

En efecto, es importante resaltar que al ser empleado el carprofeno como ingrediente activo(antiinflamatorio) en las composiciones reveladas en D1 en lugar de iburpofeno, las composiciones obtenidas no seria adecuadas para ser administradas por inyección y , por tanto, no se obtendría la presente invención. Para demostrar lo anterior, se presentan a continuación los efectos obtenidos con composiciones de acuerdo con D1 al reemplazar el ibuprofeno por carprofeno:

- Formula.
Carprofeno 5%
Propilen glicol 20%
Copolimero A 22% Copolimero B 5%
Agua purificada q.s 100%.

En este ensayo, no se adicionó Cloruro de sodio ya que dicho compuesto tiene a aumentar la viscosidad de la solución. En todo caso, el producto ya era una pasta semi / gel a temperatura ambiente con excesiva formación de espuma lo cual lo hace inadecuado para la administración por inyección. Adicionalmente, el carprofeno no se disolvió completamente en esta formula.

De otro lado, el ejemplo 6 de D1 se repitió utilizando carprofeno en lugar de ibuprofeno.
- Formula.

Carprofeno 1%
Propilen glicol 20%
Copolimero A 15%
Copolimero B 10%
Agua purificada q.s 100%.

En este caso, en la composición obtenida el carprofeno no se encontraba en solución. La formulación formo espuma excesivamente incluso cuando se agito brevemente. Una formula de gel (como gel de manos) se obtuvo con burbujas de aire que difíciles de disipar. Una vez más, estas características de la formulación la harían inadecuada para su administración por inyección.

En contraste, la presente invención cuando es administrada subcutáneamente lleva a picos de niveles de plasma de carprofeno 2.5 a 3.5 horas luego de su administración (ver tabla 1 de la solicitud). Esto es critico debido a que el carprofeno inyectable es recomendado

para el control de dolor post operatorio. Como se menciona en la descripción, productos inyectables de carprofeno conocidos en el estado del arte comercialmente son conocidos como Rimadil inyectable el cual es adecuado para administración intravenosa (ver figura 1-comparación farmacocinética). Simismo, dicho producto se formula para el alivio del dolor e inflamación asociado con osteoartritis y para el control de dolor post operatorio asociado con tejido suave o cirugías ortopédicas en perros. Para el control de dolor post operatorio, Rimadil es administrado aproximadamente 2 horas antes del procedimiento.

Así entonces, es fundamental alcanzar los niveles circulantes de carprofeno rápidamente y no como se describe en D1 a través de un gel de liberación lenta. Tales composiciones, si se administran por vía subcutánea no alcanzarían los niveles terapéuticos en el tiempo requerido para una terapia eficaz. Por tanto, una persona versada no se vería motivada a reemplazar le ingrediente activo de las composiciones divulgadas en D1 por carprofeno con el fin de obtener la solución inyectable acá reivindicada, toda vez que se obtendrían composiciones de carprofeno de administración paranteral para caninos para el alivio del dolor post operatorio por medio de liberación lenta.

Finalmente, en forma respetuosa considero que en el presente caso el Examinador no pudo reconocer la actividad inventiva involucrada en el desarrollo, especialmente porque entendió en forma equivocada que al reemplazar el ingrediente activo de las composiciones reveladas en D1 se obtendrían la solución inyectable acá reivindicada y sus efectos ventajosos, cuando la anterioridad no se refiere al mismo tipo de composiciones que como las acá reivindicadas y no solucionan el problema técnico planteado.

Visto lo anterior, atentamente solicito a ese Despacho tener en cuenta los argumentos arriba mencionados y también los resultados de prueba adicionales que se presentan anexo al presente memorial, en los cuales se demuestra la estabilidad mejorada de las composiciones reivindicadas en la presente solicitud, de tal forma que al efectuar un un examen de nivel inventivo riguroso y de conformidad con las disposiciones del Manual para el Examen de Solicitudes de patente, se advierta la no-obviedad de la materia reivindicada en la presente solicitud.

Por lo expuesto anteriormente, no puede concluirse que antes de la fecha de prioridad reivindicada en la solicitud, resultara obvio para una persona versada en la materia obtener soluciones inyectables de carprofeno (de liberación rápida) estables, a partir de la combinación de las enseñanzas reveladas en los documentos D1 y D2.

5.1.2 Con respecto a las reivindicaciones de método.

D1 describe dos métodos para producir las composiciones:

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1) "El Proceso frío" (ver párrafo 0016 D1). Este método requiere poloxámeros para ser disueltos en agua desde 0 hasta 25°C. Componentes insolubles pueden ser disueltos en un solvente orgánico como etanol, i-propanol y propilenglicol, y luego puede ser combinado homogéneamente con la fase acuosa".

2) "El proceso caliente" (ver párrafo 0017 D1). En este método los copolímeros son disueltos como en el proceso frío pero usando una temperatura entre 60 a 100°C, y la solución es enfriada a 25°C.

En ambos procesos, agentes activos que no son solubles en agua son disueltos en solventes orgánicos antes de la mezcla con agua para formar composiciones inyectables.

A diferencia de lo anterior, sorprendentemente aunque el ibuprofeno y caprofeno tienen solubilidades en el agua parecidas, menor a 1mg/ml, en el proceso divulgado en D1 se debe disolver primero el ibuprofeno en un solvente orgánico, y posteriormente el agua es adicionada debido a que el ibuprofeno (al igual que el carprofeno) es insoluble en agua. Sin embargo, en la presente invención se ha encontrado inesperadamente que el carprofeno, polxámero y agua pueden ser adicionados simultáneamente a temperatura ambiente y ser mezclados hasta formar una solución homogénea. De modo que, dicho procedimiento se diferencia de los procedimientos usuales de preparación de soluciones, toda vez que se ha superado un prejuicio en el campo de la técnica a este respecto, lo cual es un indicio de su nivel inventivo en los términos del Manual Andino de Patentes.

5.2. Por lo tanto, queda así demostrado en forma definitiva, que la Resolución acá impugnada fue expedida con violación de los artículos 14 y 18 de la Decisión 486 y que en consecuencia la misma debe ser revocada y en su lugar se debe otorgar la solicitud de patente para la invención **"COMPOSICIÓN VETERINARIA PARA ANIMALES PEQUEÑOS"** a favor de **NORBROOK LABORATORIES LIMITED.** por cumplir con los requisitos de patentabilidad.

VI. PETICIÓN

Por todo lo anterior, solicito a ese Despacho lo siguiente:

6.1. Que al considerar las razones precedentes se revoque la decisión contenida en la Resolución No. **14582** del 16 de Marzo de 2010, en cuando resuelve negar el privilegio de patente de invención.

6.2. Que se otorgue el privilegio de patente de la solicitud para: **"COMPOSICIÓN VETERINARIA PARA ANIMALES PEQUEÑOS"** a favor de **NORBROOK LABORATORIES LIMITED.**

CAVELIER
ABOGADOS

VII. NOMBRE Y DIRECCION

El nombre y la dirección de la sociedad recurrente son los siguientes:

NORBROOK LABORATORIES LIMITED
Newry, Country Down BT35 6JP,
Reino Unido

VIII. NOTIFICACIONES

Atentamente manifiesto a usted que recibiré notificaciones en la Secretaria de su Despacho o en mi oficina de abogado situada en la Carrera 4ª. No. 72-35, piso 4°, de esta ciudad de Bogotá, D.C.

IX. ANEXOS.

- Copia autenticada del memorial de sustitución de poder del Doctor Emilio FERRERO a la Doctora Luz Clemencia de PAÉZ.
- Datos de estabilidad de solución de Carprofeno inyectable para animales pequeños.
- Estudio de solución inyectable de Carprofeno: Efecto de la concentración de la droga y el polímero en la estabilidad.
- Jackson et al.Correlation of serum ibuprofen concentration with clinical signs of toxicity in three
- Poortinga and Hungerford. A case-control study of acute ibuprofen toxicity in dogs.-Prev Vet Med, 1998, May 1; 35(2): 115-24.
- Tsuchiya et al.Early pathophysiological features in canine renal papillary necrosis induced by nefiracetam. - Toxicol Pathol, 2005; 33(5):561-9).
- Ficha técnica Rimadil.

Señora Directora,

Luz Clemencia de Paez

LUZ CLEMENCIA DE PAEZ
C.C. 35.456.344 de Usaquén
T.P.A. No. 23.555

COLO-14456-841-002-7
MGM\MGM\ID-169684\WPC14456
No. M-2010-169684\MGM

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NOTARIA SEXTA DE BOGOTA

RECONOCIMIENTO Y PRESENTACION PERSONAL

Bogotá, D.C. 22 ABR 2010

Ante el

NOTARIO SEXTO (E) DEL CIRCULO DE BOGOTA D.C.

Compareció (eron) Luz Clemencia Suarez de Paez

Quien(es) se identificó(aron) con la(s) C.C. 35416544 TP 23555

Y declaró(aron) que la(s) firma(s) que aparece(n) en el presente documento es(son) la(s) suya(s) y que el contenido del mismo es cierto.

En constancia se firma esta diligencia.

Luz Clemencia Suárez

TP #23555 CSJ

REPÚBLICA DE COLOMBIA
Mabel Andrea Rodríguez D.
NOTARIA (E)
NOTARÍA SEXTA DE BOGOTÁ

178 178

CAVELIER
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Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. S. D.

Poderdante : **NORBROOK LABORATORIES LIMITED**

Poder conferido el : 27/09/2004

Yo, EMILIO FERRERO, obrando como apoderado principal del poderdante arriba mencionado, mediante el presente escrito sustituyo el poder a mi otorgado a la doctora LUZ CLEMENCIA DE PAEZ, identificada con cédula de ciudadanía No. 35.456.344 y Tarjeta Profesional N°23.555, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil.

Señora Jefe,

EMILIO FERRERO
T.P.A. N° 31.929
C.C. N° 438.019 de Usaquén

Acepto:

LUZ CLEMENCIA DE PAEZ
T.P.A. N° 23.555
C.C. No. 35.456.344 de Usaquén

COMO NOTARIA SEXTA DEL CIRCULO DE BOGOTA D.C. HAGO CONSTAR QUE LA FOTOCOPIA COINCIDE CON EL ORIGINAL QUE ME FUE PRESENTADO A LA VISTA
BOGOTA D.C. REPUBLICA DE COLOMBIA
22 ABR 2010
NOTARIO ENCARGADO
REPUBLICA DE COLOMBIA
Mabel Andrea Rodriguez
NOTARIA (E)
NOTARIA SEXTA DE BOGOTA

(3)

NOTARIA SEXTA DE BOGOTA
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C. 03 NOV 2009
Ante mi AMPARO QUINTERO ARTURO
NOTARIA SEXTA DEL CIRCULO DE
BOGOTA, D.C.
Compareció(eron) Emilio Ferrero Williamson Luz Clemencia Suárez de Páez
Quien(es) exhibió(eron) la(s) C.C.
438019 T.P. 31.927
35456344 T.P. 23.555
Y declaró(aron) que la(s) firma(s) que aparece(n) en el presente documento es(son) la(s) suya(s) y que el contenido del mismo es cierto.
En constancia se firma esta diligencia.

Emilio Ferrero
Luz Clemencia Suárez

REPUBLICA DE COLOMBIA
Amparo Quintero Arturo
NOTARIA
NOTARÍA SEXTA DE BOGOTÁ

COMO NOTARIA SEXTA DEL CIRCULO DE BOGOTA D.C. HAGO CONSTAR QUE LA FOTOCOPIA COINCIDE CON EL ORIGINAL QUE HE TENIDO A LA VISTA BOGOTA D.C. REPUBLICA DE COLOMBIA
22 ABR 2010
NOTARIO ENCARGADO

REPUBLICA DE COLOMBIA
Mabel Andrea Rodríguez
NOTARIA (E)
NOTARÍA SEXTA DE BOGOTÁ

TABLA 1

Datos de estabilidad de solución de Carprofeno inyectable para animales pequeños.

Lote No: 5395-94 a 30 ± 2°C, 60 ± 5% RH

Parámetro	Inicial	3 Meses	6 Meses	9 Meses	12 Meses	18 Meses
Apariencia	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido
pH	7.5	7.5	7.5	7.5	7.5	7.5
Contenido Carprofeno	5.08	5.14	5.10	5.05	5.03	4.96
Productos de degradación Total:	0.1@1.85RRT 0.1%	0.3@1.48RRT 0.3%	0.1@1.47RRT 0.3 @1.54RRT 0.1@1.62RRT 0.1@1.85RRT 0.6%	0.1@0.56RRT 0.1@1.53RRT 0.1@1.88RRT 0.3%	0.1@ 1.54RRT 0.1@ 1.84 RRT 0.2%	0.3@ 1.50RRT 0.1@ 1.85RRT 0.1@ 2.04RRT 0.5%
Alcohol Bencílico	1.07	1.08	1.07	1.09	1.10	1.10
SFS	0.0008%w/v	ND	ND	ND	ND	ND
Integridad de sello	Cumple	Cumple	Cumple	Cumple	Cumple	Cumple
SOR	0	0.03	0.08	0.11	1.79	-0.04
Jeringabilidad	Pasa	Pasa	Pasa	Pasa	Pasa	Pasa
Esterilidad	Pasa	NR	NR	NR	NR	NR
Endotoxinas Bacterianas	Pasa	NR	NR	NR	NR	NR
Volumen de llenado	105%	NR	NR	NR	NR	NR

NR: No Requerido
ND: No Detectado

TABLE 2

Datos de estabilidad de solución de Carprofeno inyectable para animales pequeños.

Lote No: 5404-92 a 30 ± 2°C, 60 ± 5% RH Inverted

Parámetro	**Inicial**	**3 Meses**	**6 Meses**	**9 Meses**	**12 meses**	**18 meses**
Apariencia	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido
pH	7.3	7.5	7.5	7.5	7.5	7.5
Contenido Carprofeno	4.84	4.87	4.78	4.80	4.84	4.77
Productos de degradación Total:	0.1@1.48RRT 0.1%	0.3@1.47RRT 0.3%	0.1@1.47RRT 0.3 @1.55RRT 0.1@1.85RRT 0.5%	0.1@0.57RRT 0.1 @1.51RRT 0.2%	ND	0.3@ 1.49RRT 0.1@ 1.85RRT 0.4%
Alcohol Bencílico	0.97	0.953	0.939	0.959	0.947	0.973
SFS	0.001%w/v	ND	ND	ND	ND	ND
Integridad de sello	Cumple	Cumple	Cumple	Cumple	Cumple	Cumple
SOR	2	0.03	0.09	0.13	1.56	-0.34
Jeringabilidad	Pasa	Pasa	Pasa	Pasa	Pasa	Pasa
Esterilidad	Pasa	NR	NR	NR	NR	NR
Endotoxinas bacterianas	Pasa	NR	NR	NR	NR	NR
Volumen de llenado	105%	NR	NR	NR	NR	NR

NR: Not Required
ND: None Detected

TABLE 3

Datos de estabilidad de solución de Carprofeno inyectable para animales pequeños. P-CARPSA-030

Lote No: 5174-91 at 30 ± 2°C, 60 ± 5% RH (Inverted)

Parámetro	Inicial	3 Meses	6 Meses	9 Meses	12 Meses	18 meses	24 meses
Apariencia	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido
pH	7.4	7.4	7.4	7.4	7.4	7.5	7.5
Contenido Carprofeno	4.81	4.77	4.86	4.76	4.86	4.77	4.86
Productos de degradación Total:	0.1@ 1.88 0.1	0.1@ 1.88 0.1	0.1@1.41 0.3@ 1.51 0.1@1.86 0.5	0.1@1.88 0.1	0.1@ 1.55 0.1@ 1.85 0.2	0.3@1.51 0.1@1.89 0.4	0.1@ 1.51 0.1@ 1.88 0.2
Alcohol Bencílico	0.99	0.944	0.961	0.946	0.936	0.932	0.945
SFS	Cumple	ND	ND	ND	ND	ND	ND
Integridad de sello	Cumple	Pasa	Pasa	Pasa	Pasa	Pasa	Pasa
Specific Optical Rotation	0.1	0.2	-0.6	0.04	0.15	0.07	-0.05
Jeringabilidad	Pasa	Pasa	Pasa	Pasa	Pasa	Pasa	Pasa
Esterilidad	Pasa	NR	NR	NR	NR	NR	Pasa
Endotoxinas bacterianas	Pasa	NR	NR	NR	NR	NR	NR
Volumen de llenado	104%	NR	NR	NR	NR	NR	NR

NR: Not Required
ND: None Detected

TABLE 4

Datos de estabilidad de solución de Carprofeno inyectable para animales pequeños.

Lote No: 5395-94 at 40 ± 2°C, 75 ± 5% RH Inverted

Parámetro	Inicial	3 Meses	6 Meses	9 Meses	12 meses
Apariencia	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido
pH	7.5	7.5	7.5	7.5	7.5
Contenido Carprofeno	5.08	5.09	5.08	5.01	Cumple 5.04
Productos de degradación Total:	0.1@1.85RRT 0.1%	0.3@1.48RRT 0.3%	0.3@1.52RRT 0.1@1.9RRT 0.4%	0.1@0.56RRT 0.2@1.56RRT 0.1@1.88RRT 0.4%	0.3 @1.52RRT 0.1@1.84RRT 0.4%
Alcohol Bencílico	1.07	1.08	1.08	1.09	1.09
SFS	0.0008%w/v	ND	ND	ND	ND
Integridad de sello	Cumple	Cumple	Cumple	Cumple	Cumple
SOR	0	0.02	0.10	0.05	1.60
Jeringabilidad	Pasa	Pasa	Pasa	Pasa	Pasa
Esterilidad	Pasa	NR	NR	NR	NR
Endotoxinas bacterianas	Pasa	NR	NR	NR	NR
Volumen de llenado	105%	NR	NR	NR	NR

NR: Not Required
ND: None Detected

TABLE 5

Datos de estabilidad de solución de Carprofeno inyectable para animales pequeños.

Lote No: 5404-92 at 40 ± 2°C, 75 ± 5% RH Inverted

Parámetro	**Inicial**	**3 Meses**	**6 Meses**	**9 Meses**	**12 meses**
Apariencia	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido
pH	7.3	7.5	7.5	7.5	7.5
Contenido Carprofeno	4.84	4.82	4.78	4.80	4.83
Productos de degradación Total:	0.1@1.48RRT 0.1%	0.3@1.47RRT 0.3%	0.2@1.47RRT 0.2@1.55RRT 0.1@1.85RRT 0.5%	0.1@0.57RRT 0.1@1.52RRT 0.2%	0.1@1.54RRT 0.1%
Alcohol Bencílico	0.97	0.950	0.948	0.957	0.949
SFS	0.001%w/v	ND	ND	ND	ND
Integridad de sello	Cumple	Cumple	Cumple	Cumple	Cumple
SOR	2	0.03	0.13	0.11	1.94
Jeringabilidad	Pasa	Pasa	Pasa	Pasa	Pasa
Esterilidad	Pasa	NR	NR	NR	NR
Endotoxinas bacterianas	Pasa	NR	NR	NR	NR
Volumen de llenado	105%	NR	NR	NR	NR

NR: Not Required
ND: None Detected

TABLE 6

Datos de estabilidad de solución de Carprofeno inyectable para animales pequeños. P-CARPSA-030

Lote No: 5174-91 at 40 ± 2°C, 75 ± 5% RH (Inverted)

Parámetro	Inicial	3 Meses	6 Meses	9 Meses	12 Meses
Apariencia	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido	Una solución clara e incolora a amarillo pálido
pH	7.4	7.4	7.5	7.5	7.5
Contenido Carprofeno	4.81	4.78	4.81	4.74	4.78
Productos de degradación Total:	0.1@ 1.88 0.1	0.2@ 1.49 0.1@1.88 0.3	0.1@ 1.41 0.3@1.52 0.1@ 1.87 0.5	0.1@ 1.88 0.1	0.1@ 1.55 0.1@ 1.85 0.2
Alcohol Bencílico	0.99	0.944	0.956	0.945	0.934
SFS	Cumple	ND	ND	ND	ND
Integridad de sello	Cumple	Pasa	Pasa	Pasa	Pasa
Rotación optica especifica	0.1	0.02	-0.6	0.10	0.14
Jeringabilidad	Pasa	Pasa	Pasa	Pasa	Pasa
Esterilidad	Pasa	NR	NR	NR	NR
Endotoxinas bacterianas	Pasa	NR	NR	NR	NR
Volumen de llenado	104%	NR	NR	NR	NR

NR: Not Required
ND: None Detected

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Carprofeno – Efecto de la concentración de la droga y el polímero en la estabilidad.

Lote No (composición)	Contenido Carprofeno %w/v		
	Inicial	Condiciones de almacenamiento (luego de 3 semanas)	
		25 °C, 60 % RH	40 °C, 75 % RH
P14098			
Carprofen 0.25%w/v			
L-arginine 0.155%w/v			
Lutrol 0.5%w/v	0.242	0.255	0.251
Benzyl Alcohol 1.0%v/v			
SFS 0.25%w/v			
WFI q.s. to 100%v/v			
P14099			
Carprofen 0.25%w/v			
L-arginine 3.1%w/v			
Lutrol 0.5%w/v	0.243	0.242	0.245
Benzyl Alcohol 1.0%v/v			
SFS 0.25%w/v			
WFI q.s. to 100%v/v			
P14102			
Carprofen 0.25%w/v			
L-arginine 0.155%w/v			
Lutrol 12.0%w/v	0.248	0.250	0.251
Benzyl Alcohol 1.0%v/v			
SFS 0.25%w/v			
WFI q.s. to 100%v/v			
P14107			
Carprofen 0.25%w/v			
L-arginine 0.155%w/v			
Lutrol 20%w/v	0.240	0.245	0.244
Benzyl Alcohol 1.0%v/v			
SFS 0.25%w/v			
WFI q.s. to100%v/v			

Conclusión:
Si la concentración de carprofeno esta dentro del rango de 0.25% a 5%, se obtienen formulaciones viables y estables.
En la presencia de 0.25% carprofeno, el Lutrol puede ser usado hasta 20% (P14107) y se obtienen formulaciones viables y estables.

Correlation of Serum Ibuprofen Concentration with Clinical Signs of Toxicity in Three Canine Exposures

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ABSTRACT. The clinical signs and serum concentrations of ibuprofen are reported for 3 canine exposures. No adverse clinical signs or abnormal laboratory parameters were observed when serum ibuprofen concentrations were less than 31 ug/mL. Melena and 38 mg blood urea nitrogen/dL (normal 7-26 mg/dL) were present in an animal with a serum ibuprofen of 138 ug/mL.

The increased availability of ibuprofen in the home is apparently associated with an increase in canine exposures (1). This agent became available in 1984 in over-the-counter preparations containing 200 mg ibuprofen/tablet. It was previously only available in prescription preparations. The Hennepin Regional Poison Center was consulted on 32 canine ibuprofen exposures in 1989, and 37 times during the first 3/4 of 1990 (Jackson TW: Hennepin Regional Poison Center, 1989 and 1990 Annual Reports, personal communication). Other Centers have reported similar data (1-3). experimental and field cases of ibuprofen poisoning in dogs have previously been reported (4,5). Since serum concentrations of ibuprofen were not reported in the previous field case, we report here 3 canine exposures with such data.

CASE REPORTS

Case 1

A 10-y old, 11 kg, male Terrier-cross dog was brought to a veterinary clinic for neutering. The owner reported that within the last 12 h the dog started vomiting a black tarry liquid. The owner admitted administering one 200 mg ibuprofen tablet (ADVIL, Whitehall) 3 times daily for the previous 4-5 d. This regime resulted in a dose of 53 mg/kg/day. The animal was hospitalized for the hematemesis and potential for progression of the toxicity.

Lactated Ringers Solution (LRS) was administered on the first day at a rate of 83 ml/h via an indwelling jugular catheter. Additionally, a preparation (DIATHAL, Schering) containing dihydrostreptomycin sulfate, procaine penicillin G, diphenamil methyl sulfate, and chlorpheniramine maleate was given at a dose of 0.073 ml/kg im every 12 h for 3 treatments. Nothing was given per os. The only abnormal parameter in a clinical chemistry profile was a mildly elevated blood urea nitrogen (BUN) or 38 mg/dl (normal = 7-26 mg/dl). The animal had a serum ibuprofen concentration of 138.0 ug/ml at that time. Hematemesis was observed approximately every 4-6 h during the first 24 h of hospitalization.

On the second day of hospitalization, the vomiting stopped. The BUN decreased to 26 mg/dl and the serum ibuprofen concentration was 27.5 ug/ml. Although water was offered, LRS was continued at a rate of 83 ml/hr. Approximately 12 h after the vomiting stopped, small amounts of food were offered.

The third day of hospitalization, the iv catheter was removed and the dog was discharged on no medication. The owners were instructed to feed small, frequent meals. The serum ibuprofen concentration at discharge was 8.3 ug/ml.

Four days following discharge (day 7), the dog returned for a follow-up examination. BUN and serum ibuprofen concentrations were 16.3 mg/dl and 5.5 ug/ml, respectively. No significant findings were revealed by urinalysis, and the dog has remained asymptomatic for 12 mo.

Case 2

The owner of a 7-kg wirehair fox terrier dog contacted a veterinarian after discovering that the dog had ingested 1 or two 800 mg tablets of ibuprofen (MOTRIN, Upjohn) at some time during the previous 6 h. This resulted in a po dose of approximately 114-228 mg/kg. On the advice of the veterinarian, the owner induced vomiting at home with hydrogen peroxide. No tablet fragments were found in the

vomitus. Following consultation with the poison center, the dog was hospitalized due to the potential for toxicity. The dog was asymptomatic at this time.

On day 1 of hospitalization, LRS was infused at a rate of 35 ml/hr via an iv catheter. Two capsules of activated charcoal (280 mg each) were administered po. The serum ibuprofen concentration was 62 ug/ml 5 h post-ingestion. On day 2, the dog was active, had adequate urine output, and did not demonstrate hematemesis or melena. Fluid therapy was continued. On day 3, no adverse effects were observed. The dog was alert and active, had a good appetite, and had adequate urine output. The iv catheter was removed and 250 ml of LRS were administered sc. The serum ibuprofen concentration was 3.8 ug/ml 36-h post-ingestion. Prior to discharge, another 250 ml of LRS was given sc.

Case 3

An 8-mo-old, 12 kg, male samoyed dog ingested approximately four 800 mg ibuprofen tablets. The calculated dose was 267 mg/kg. After contacting the emergency veterinary service, the owner induced vomiting at home within 2 h of ingestion. Neither tablet fragments nor blood was seen in the emesis. The owner transported the dog to an emergency clinic.

Physical examination revealed a hyperactive, panting dog. The temperature was 102.0 F (normal 99-100°F), heart rate was 168 beats/min (normal 100-130 bpm), mucous membranes were pink, lungs were clear, heart sounds were normal, and no urine was expressed from the bladder. LRS was infused via an iv catheter. Forty-two g of activated charcoal were given po as a slurry. Eight hours after hospitalization, the dog was alert and producing adequate urine; however he had intermittently vomited small amounts of food and activated charcoal. On the advice of the poison center, the veterinarian continued fluid therapy and monitored for melena, vomiting and hematemesis. The dog continued to vomit every 15 to 20 min for 3 h.

By day 2, the vomiting had stopped and urine output was adequate. The serum ibuprofen concentration was 30.7 ug/ml 21-h post-ingestion. The dog was discharged with instructions to let the dog drink small amounts of water and observe it for further vomiting.

Upon arrival home, the dog ingested the previous day's vomitus which may have contained ibuprofen tablet fragments. The animal was transferred to the regular veterinarian where apomophine was administered with good results. Urine output was adequate. The dog was discharged and recovered uneventfully.

DISCUSSION

The therapeutic indications and mechanisms of ibuprofen action are reviewed elsewhere (6,12). Ibuprofen is used in human and veterinary medicine for its anti-inflammatory, analgesic, and antipyretic activity. It acts by reversibly inhibiting cyclo-oxygenase, an enzyme involved in the synthesis of eicosanoids (7,8), which are mediators of inflammation, pain, and fever. In addition, eicosanoids promote gastric mucus production (prostacyclin I_2) and maintain renal perfusion in hemodynamically compromised animals (prostacyclin E_2) (6). The toxic effects of cyclo-oxygenase inhibitors have been associated with these functions.

Doses, clinical signs, and laboratory data have previously been reported for cases of canine ibuprofen exposures. Toxic effects have been reported in dogs ingesting single doses of 50 (1), 100 (13) and 125 (4) mg ibuprofen/kg body weight. Vomiting, diarrhea and melena have been reported in experimental female dogs receiving 16 mg/kg/day for 26 w (4). Any ibuprofen exceeding the therapeutic dose of 5 mg/kg bid in dogs may induce toxicity (5).

Following po administration, ibuprofen is readily absorbed (4). Peak plasma concentrations in the dog are reached 90 min following po administration. The half-life of ibuprofen in dogs is 3.6 to 5.4 h following a po dose of 5 mg/kg (14). The comparable value in humans is 2 h (15). The elimination half-life in dogs or humans following overdose is not known. The long plasma half-life in dogs is apparently due to an inability to adequately metabolize ibuprofen. For example, 4 ibuprofen metabolites have been detected in the plasma of rabbits, 2 in humans, 3 in rats, and none in dogs (4).

Reported signs of ibuprofen toxicity in dogs include anorexia, vomiting, diarrhea, ataxia, melena, polyuria, polydipsia, and decreased level of consciousness. Laboratory studies have revealed increases in BUN and creatinine, and decreases in hemoglobin concentrations (1). Gross postmortem examinations have revealed gastric ulcers or erosions and intestinal inflammation (4).

General, symptomatic and supportive treatment for ibuprofen toxicosis in dogs has been recommended (13). General treatment includes emetics, adsorbents and cathartics. Symptomatic and supportive therapy is primarily aimed at the gastrointestinal effects. Cimetidine and ranitidine are H_2 receptor antagonists that reduce gastrointestinal irritation, ulceration and hemorrhage. Sucralfate minimizes injury to the gastric mucosa. Magnesium or aluminum hydroxide is an antacid. Fluid therapy may be used to correct and/or prevent hypovolemia.

Each of the animals in the cases reported were exposed to greater than 50 mg ibuprofen/kg body weight. The dogs' doses were 53 mg/kg/day for 4-5 d (Case 1) and single doses of 114-228 mg/kg or 267 mg/kg (Cases 2 and 3, respectively). Although emetics may have subsequently reduced the available dose, absorption of ibuprofen was confirmed in each case via its detection in the serum. Significant ibuprofen was absorbed despite

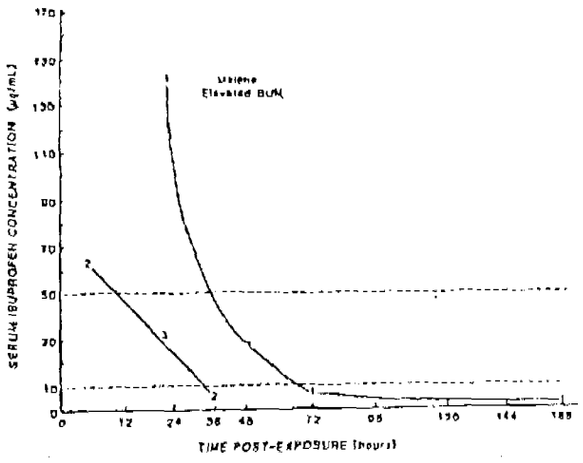

Figure 1. Serum ibuprofen concentrations relative to time post-exposure.

effective emetic therapy within 6 h (Case 2) and 2 h (Case 3). This is in agreement with the previously reported speed of absorption following po administration (4). Only in Case 1 were adequate numbers of time points available to discern a pharmacokinetic pattern. A biexponential pattern was observed when ibuprofen dosing was discontinued (Fig 1).

Hematemesis, emesis and melena were observed in 2 of the cases and a mild elevation of BUN was observed in Case 1. At no time was creatinine elevated. Hemoglobin was not determined in any instance.

The ibuprofen concentration in serum correlated well with clinical signs and the clinical chemistry values, but not with the exposure dose. No clinical signs were present in the dogs when their serum ibuprofen concentrations were less than 31 ug/mL. An increased BUN and melena were present at the time the serum ibuprofen was 138 ug/mL. Although a concentration of 62 ug ibuprofen/mL serum was observed without concurrent clinical signs, it was measured within 5 h of ingestion, so cyclo-oxygenase activity may not yet have been inhibited or may not have been inhibited long enough to significantly reduce PGI_2 and PGE_2 in the stomach and kidney, respectively. Sustained serum ibuprofen concentrations between 10 and 50 ug/mL are reportedly necessary to provide therapeutic relief in humans from inflammation.

CONCLUSION

Serum ibuprofen concentrations may be used to confirm absorption of the drug following an unknown exposure or an unknown dose of ingestion, to evaluate the success of emesis or adsorptive or cathartic therapy, and to monitor symptomatic therapy in the dog. Dogs with hematemesis, melena and elevated BUN from ibuprofen ingestion may recover uneventfully with symptomatic treatment. Serum ibuprofen concentrations greater than 130 ug/mL may fall to less than 10 ug/mL within 48 h in dogs given symptomatic treatment. Despite exposure to high doses of ibuprofen, dogs receiving agressive treatment may remain asymptomatic.

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US PESTICIDE STANDARDS TO PREVAIL IN TRADE AGREEMENT

The White House stated that in negotiating the North American Free Trade Agreement with Mexico (NAFTA), "We will maintain our right to impose stringent pesticide, energy conservation, toxic waste and health and safety standards... We will design and implement an integrated border environmental plan to address air and water pollution, hazardous wastes, chemical spills, pesticides and enforcement." In pressing for an extension of fast track authority, President Bush stated in letters to members of Congress, "The fast track in no way limits the ability of Congress to review any agreement negotiated, including the Uruguay Round or an NAFTA. If Congress is not satisfied, it retains the unqualified right to reject whatever is negotiated. But refusing to extend the fast track would end negotiations before they have even begun and relinquish a critical opportunity for future economic growth."

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Early Pathophysiological Features in Canine Renal Papillary Necrosis Induced by Nefiracetam

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ABSTRACT

To ascertain the early pathophysiological features in canine renal papillary necrosis (RPN) caused by the neurotransmission enhancer nefiracetam, male beagle dogs were orally administered nefiracetam at 300 mg/kg/day for 4 to 7 weeks in comparison with ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), at 50 mg/kg/day for 5 weeks. During the dosing period, the animals were periodically subjected to laboratory tests, light-microscopic, immunohistochemical, and electron-microscopic examinations and/or cyclooxygenase (COX)-2 mRNA analysis. In laboratory tests, a decrease in urinary osmotic pressure and increases in urine volume and urinary lactate dehydrogenase (LDH) level were early biomarkers for detecting RPN. Light-microscopically, nefiracetam revealed epithelial swelling and degeneration in the papillary ducts in week 7, while ibuprofen displayed degeneration and necrosis in the papillary interstitium in week 5. In immunohistochemical staining with COX-2 antibody, nefiracetam elicited a positive reaction within interstitial cells around the affected epithelial cells in the papillary ducts (upper papilla) in week 7, and ibuprofen positively reacted within interstitial cells adjacent to the degenerative and/or necrotic lesions in week 5. Ultrastructurally, nefiracetam exhibited reductions of intracellular interdigitation and infoldings of epithelial cells in the papillary ducts, whereas ibuprofen showed no changes in the identical portions. Thus, the early morphological change in the papilla brought about by nefiracetam was quite different from that elicited by ibuprofen. By the renal papillary COX-2 mRNA expression analysis, nefiracetam exceedingly decreased its expression in week 4, but markedly increased it in week 7, suggesting an induction of COX-2 mRNA by renal papillary lesions. These results demonstrate that the epithelial cell in the papillary ducts is the primary target site for the onset of RPN evoked by nefiracetam.

Keywords. Renal papillary necrosis; dog; nefiracetam; ibuprofen; cyclooxygenase-2.

INTRODUCTION

Renal papillary necrosis (RPN) is known to be caused by chronic use of mixed analgesics or NSAIDs (Alden and Frith, 1991; Bach and Thanh, 1998; William et al., 1996), 2-bromoethylamine hydrobromide (Bach et al., 1991; Sabatini, 1984) or D-ormaplatin (Kolaja et al., 1994). Nevertheless, advances in understanding the pathogenesis of RPN have still been slow. As for NSAID-induced RPN, although direct cytotoxic action and/or ischemic injury through inhibition of the vasodilatory effects of renal prostaglandins (PGs) as a result of cyclooxygenase (COX) inhibition have been reported as important contributing factors, the entire mechanism remains unclear (Alden and Frith, 1991; Khan and Silverman, 1999; Rocha et al., 2001; Sabatini, 1988; Whiting et al., 1999).

Nefiracetam (*N*-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide, Figure 1) is a novel pyrrolidone derivative possessing nootropic effects. In pharmacological studies, nefiracetam has facilitated cognitive function in a wide variety of animal models. In brief, this compound has reduced amnesia produced by scopolamine (Sakurai et al., 1989) and apomorphine (Nabeshima et al., 1994). Nefiracetam has also been found to attenuate amnesia produced by the neurotoxin AF64A (Abe et al., 1994), electrolytic lesions of the basal forebrain (Nishizaki et al., 1998), and hypoxia (Hiramatsu et al., 1992), and further has improved the cognitive performance of aged rats (Nabeshima, 1994). In pharmacokinetic studies, nefiracetam was extensively metabolized, and more than twenty metabolites were observed in serum, urine, and tissues (Sellers et al., 2004). In previous long-term toxicological investigations with rats, dogs, and monkeys (Hooks et al., 1994; Jindo et al., 1994; Kajimura et al., 1994; Tsuchiya et al., 2003), degeneration and/or necrosis in the renal papilla was observed only in dogs given 300 mg/kg/day of nefiracetam for 8 to 11 weeks. This alteration was provoked by the inhibition of renal PG synthesis resulting from relatively high penetration and retention of the nefiracetam metabolite M-18 into the canine renal papilla. In laboratory tests, a decrease in urinary osmotic pressure and increases in urine volume and urinary lactate dehydrogenase (LDH) level were observed at the early phase (Tsuchiya et al., 2003).

In the present study, to ascertain the early pathophysiological features in RPN induced by nefiracetam, dogs were orally administered nefiracetam at 300 mg/kg/day for 4 to 7 weeks in comparison with ibuprofen, an NSAID, at 50 mg/kg/day for 5 weeks. Periodic laboratory tests including urine volume, urinary osmotic pressure, and urinary LDH were performed to reconfirm the biomarkers for the onset of RPN. At the same time, the renal morphological aspects were investigated by light- and electron-microscopic examinations

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Abbreviations: RPN, renal papillary necrosis; NSAIDs, nonsteroidal anti-inflammatory drugs; COX, cyclooxygenase; PG, prostaglandin.

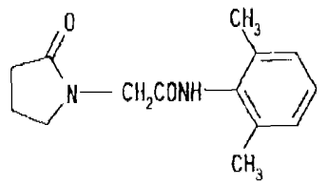

FIGURE 1.—Chemical structure of nefiracetam.

and immunohistochemical staining with COX antibodies in conjunction with COX-2 mRNA expression analysis.

MATERIALS AND METHODS

Chemicals

Nefiracetam was synthesized in the Akita Factory of Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Ibuprofen was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were the highest grade available from commercial sources.

Animal Treatment

A total of 22 male LRE strain or Nosan beagles (8.9–12.6 kg) at approximately 17 to 19 months of age were used. Dogs were housed individually under controlled conditions at a temperature of 21–25°C, a relative humidity of 40–80%, a lightning cycle of 12 hours (from 8:00 to 20:00 each day), and with 15 or more changes of filtered air/hour. Commercial diets were given ad libitum, and the animals were allowed free access to water except on the sampling day for urine or serum. They were divided into 6 groups as follows. Group 1, intact control group (Nos. 1, 2 and 3); Group 2, nefiracetam treatment group at 300 mg/kg/day for 4 weeks (Nos. 4, 5, 6, and 7); Group 3, nefiracetam treatment group at 300 mg/kg/day for 5 weeks (Nos. 8, 9, 10, and 11); Group 4, nefiracetam treatment group at 300 mg/kg/day for 6 weeks (Nos. 12, 13, 14, and 15); Group 5, nefiracetam treatment group at 300 mg/kg/day for 7 weeks (Nos. 16, 17, 18, and 19) and Group 6, ibuprofen treatment group at 50 mg/kg/day for 5 weeks (Nos. 20, 21, and 22). In this study, the first dosing week was designated as week 1. Periodic laboratory tests were performed in dogs from groups 1 (nontreated control), 5 (nefiracetam for 7 weeks), and 6 (ibuprofen for 5 weeks) before the commencement of treatment and weekly thereafter. At the end of the respective treatment periods, the dogs were killed by exsanguination from the axillary artery under pentobarbital anesthesia (25 mg/kg, iv, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and the bilateral kidneys were removed.

Laboratory Tests

Urinalysis and serum biochemistry were performed once before treatment and weekly during the treatment period. Urine was collected by using a collecting tray for approximately 17 hours (overnight). During collection, the dogs were denied food and water, and urine was kept cool on ice. After centrifugation (4°C, 1500 rpm, 10 minutes) of collected urine, approximately 5 mL of the supernatant was filtered through a membrane filter (0.8 μm, DISMIC-25cs, ADVANTEC, Tokyo, Japan). A 2.5-mL sample of the filtered supernatant was applied on the disposable column PD-10 (Sephadex G-25 M, Amersham Biosciences UK Ltd., Buckinghamshire, UK) and 3.5 mL of 0.0625 M Tris-HCl (pH 6.8) was used for elution. In fresh urine, osmotic pressure was measured with an osmometer (model 3C2, Advanced Instruments, Inc., Norwood, MA, USA). Creatinine (CRE) content in the filtered urine supernatant was measured with a CRE-KAINOS (KAINOS Laboratories, Inc., Tokyo, Japan). LDH in the eluted solution was determined with a LDH CII test (Wako Pure Chemical Industries, Ltd.). CRE values were used for the correction of LDH level. Approximately 3.5 mL of blood was collected from the cephalic vein into a tube containing polymer gel as a serum separating agent, and serum was separated by centrifugation (4°C, 3000 rpm, 15 minutes). Serum CRE and urea nitrogen (UN) were measured by a Hitachi 7350 auto-analyzer (Hitachi Ltd., Tokyo, Japan).

Light Microscopy

The left kidney was removed, fixed in 10% neutral buffered formalin (Wako Pure Chemical Industries, Ltd.), embedded in paraffin wax, sectioned at 5-μm thickness, stained with hematoxylin and eosin (H&E) and alcian-blue, and examined microscopically (Table 1). The renal papilla included the upper papilla and papillary tip.

Immunohistochemical Staining of COX-1 or COX-2 in the Renal Papilla

A section from the left kidney was deparaffinized, and an antigen was retrieved with Dako Proteinase K solution (Dako Cytomation Co., Ltd., Kyoto, Japan). After incubation with 3% hydrogen peroxide for 5 minutes, the sections were preblocked with Nonspecific Staining Blocking Reagent containing 0.25% casein and carrier protein (Dako Cytomation Co., Ltd.), and then were incubated with a primary antibody overnight at 4°C. As COX-1 and COX-2 primary antibodies, ovine COX-1 polyclonal rabbit antiserum (No. 160108,

TABLE 1.—Distribution of dogs and number of tissue blocks and sections in the experiment.

Dose	Intact control	Nefiracetam at 300 mg/kg/day				Ibuprofen at 50 mg/kg/day
Treatment period	7 weeks	4 weeks	5 weeks	6 weeks	7 weeks	5 weeks
Light-microscopic examinations						
Number of dogs	3	3	4	4	4	3
Immunohistochemical staining						
Number of dogs	2	2	2	2	2	2
Electron-microscopic Examinations						
Number of blocks	10/dog	10/dog	10/dog	10/dog	10/dog	10/dog
Number of semithin sections	3–6/dog	3/dog	3/dog	3/dog	3–10/dog	3–10/dog
Number of ultrathin sections	1–2/dog	None	None	None	1–2/dog	2/dog

Cayman Chemical Co., Ann Arbor, MI, USA) and murine COX-2 polyclonal rabbit antibody (No. 160106, Cayman Chemical Co.), respectively, were used. These antibodies were diluted 1:400 with 0.1% Tween-Tris buffer. Immunoreactive complexes were detected via an LSAB 2 Systems-HRP (Dako Cytomation Co., Ltd.) and visualized with diaminobenzidine (DAB, Dako Cytomation Co., Ltd.), which reacts with peroxidase to give a brown reaction product (Table 1). The sections were counterstained briefly with hematoxylin. All control sections were treated with no primary antibody.

Electron Microscopy

In the present study, since apparent morphological alterations were light-microscopically noted in week 7 for nefiracetam and in week 5 for ibuprofen, electron-microscopic examination was performed only for these points. A tiny portion from the left kidney (papillary section) was removed, fixed in 3% glutaraldehyde and 2% osmic acid, dehydrated with alcohol, and embedded in epoxy resin. Thick sections were made and stained with 1% toluidine blue for light microscopic survey. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a transmission electron microscope (H-500, Hitachi Ltd., Table 1).

Quantitative Real-Time RT-PCR Analyses of Papillary COX-2 Gene Expression

In the present work, inasmuch as the early morphological features in RPN brought about by nefiracetam were quite different from those by ibuprofen, serial changes in the renal papillary COX-2 mRNA expression were assessed only in nefiracetam-treated groups. Total RNA was extracted from an approximately 40 mg portion of the papilla from the right kidney with an Absolutely RNA RT-PCR Miniprep Kit (Stratagene, La Jolla, CA, USA), which includes DNase treatment to remove genomic DNA. Extracted total RNA was converted to cDNA with cocktails consisting of 1× PCR buffer (Applied Biosystems, Foster City, CA, USA), 5 mM $MgCl_2$, 1 mM dNTP mixture (Toyobo Co., Ltd., Osaka, Japan), 1U/μL RNase inhibitor (Toyobo Co., Ltd.), 2.5 U/μL Random Primer (Takara Bio Inc., Shiga, Japan), and 0.125 U/μL AMV Reverse Transcriptase XL (Takara Bio Inc.). The following primers and TaqMan probes were designed by using a Primer Express (Applied Biosystems) for the detection of each target gene sequence. Real-time PCR data and analyses were collected in Applied Biosystems 7700 Sequence Detection System instruments. The amount of each gene target is normalized to an endogenous control (18S rRNA, 20 × Pre-Developed TaqMan Assay Reagents, Applied Biosystems), and then expressed as the ratio to the mean control values (each value in a treated dog/the mean value of 3 control dogs).

The following primers and probes were used for PCR. Forward primer, 5'-CTG TGG GCC AGG AGG TCT T-3'; Reverse primer, 5'-CAC TCT GTT ATG CTC CCG CAG-3'; TaqMan probe, 5'-TGC CTG GTC TGA TGA TGT ATG CCA CC-3'.

Statistical Analyses

The quantitative laboratory data in groups 1 (intact control), 5 (nefiracetam: 7-week treatment) and 6 (ibuprofen: 5-week treatment) are represented as the group mean ± standard deviation (SD) at each sampling point, and following analyses were carried out: (1) A paired *t*-test compared with the predose value in each group was applied, and (2) A 2-group comparison between the intact control group and each treatment group was carried out as follows. The homogeneity of the variance between two groups was analyzed by F-test at each sampling point. If the variance was homogeneous, Student's *t*-test was subsequently used. If the variance between the groups was not homogeneous, then Aspin-Welch's *t*-test was used.

The COX-2 mRNA expression data in the group 1 and nefiracetam-treated groups 2, 3, 4, and 5 (corresponding to 4-, 5-, 6-, and 7-week treatment, respectively) are represented as the group mean ± SD. Then, the aforementioned 2-group comparison between the intact control group and each treatment group was performed. A 2-tailed p-value less than 5% was considered to be statistically significant. EXSAS ver. 6.10 (Arm Corporation, Osaka, Japan), a software package, was used for these analyses.

Animal Welfare

All experimental procedures were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Pharmaceutical Co., Ltd.

Results

Laboratory Tests

In dogs receiving nefiracetam, decreased urinary osmotic pressure and a tendency of increasing urine volume were observed in weeks 6 to 7. Urinary LDH tended to be higher from week 5. Serum UN showed somewhat higher values than that in the predose or control group throughout the study period without any remarkable increase in serum CRE. In dogs given ibuprofen, a decrease in urinary osmotic pressure, increases in urine volume and urinary LDH and a slight increase in serum UN were sporadically noted in weeks 1 to 5 (Figure 2). All alterations seen in ibuprofen were earlier and severer than those in nefiracetam.

Light Microscopy

In dogs given nefiracetam, no treatment-related changes were observed by week 6. In week 7, however, epithelial swelling of the papillary ducts (upper papilla) was seen in 3 of 4 dogs with increased eosinophilic-staining intensity in the cytoplasm of epithelial cells (Table 2, Figure 3C). Degeneration and desquamation of epithelial cells was sometimes noted (Figure 3C, inset). The alcian-blue staining intensity was comparable to the control throughout the dosing periods (Figure 3D). Mineralization of the papilla without detectable change in interstitial cells was noted in 2 of 4 dogs (Table 2). In dogs receiving ibuprofen, degeneration and necrosis of the interstitium in the papillary tip were noted in 2 of 3 dogs (Table 2 and Figure 3E); however, no remarkable changes were noted in the remaining papillary ducts. The alcian-blue staining intensity was markedly decreased in the degenerative and/or necrotic areas of the papillary interstitium (Figure 3F). Mineralization of the papilla was seen in 1 of 3 dogs (Table 2).

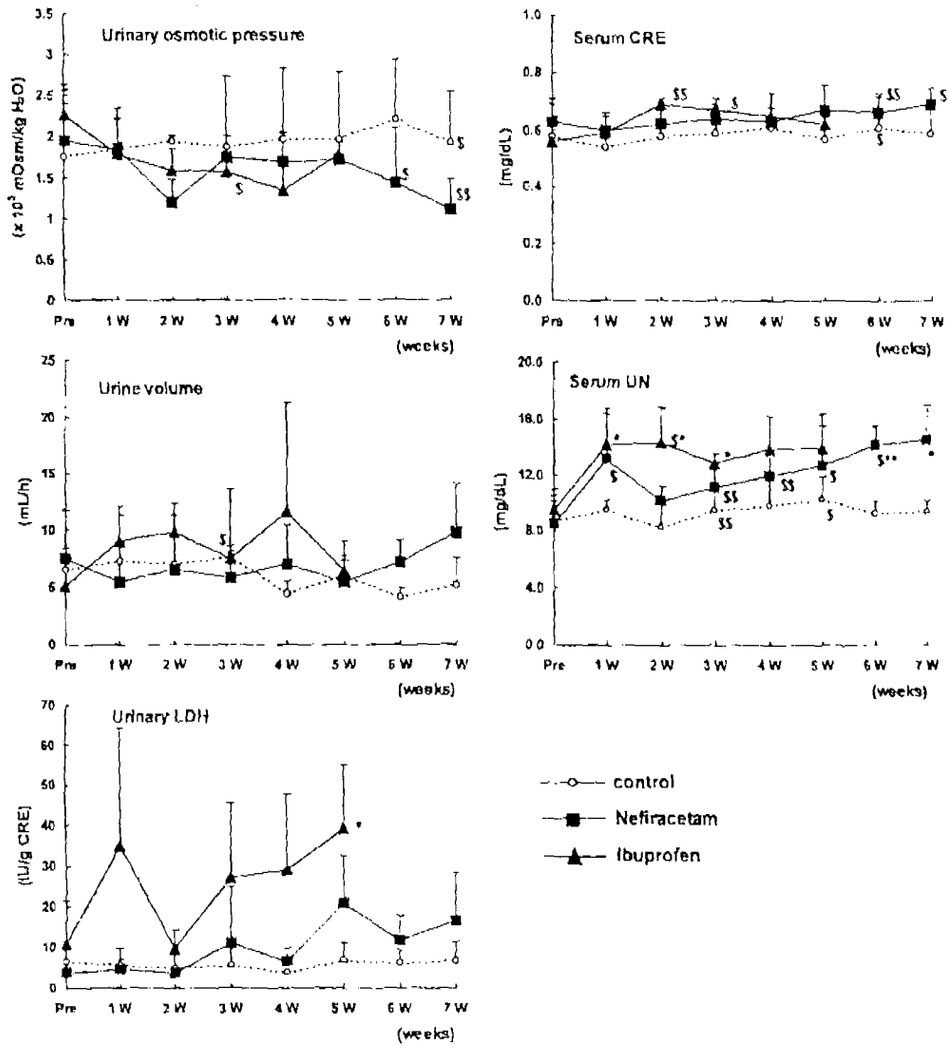

FIGURE 2.—Urinary osmotic pressure, urine volume, urinary lactate dehydrogenase (LDH), serum creatinine (CRE), and serum urea nitrogen (UN) values in male dogs receiving repeated oral administration of nefiracetam at 300 mg/2 kg/day for 7 weeks or ibuprofen at 50 mg/kg/day for 5 weeks. Pre: preadministration. Values are shown as the mean ± SD (n = 3–4) $^{*}p < 0.05$, significantly different from the control (Student's t-test). $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, significantly different from the prevalue (Paired t-test).

Immunohistochemical Staining of COX-1 or COX-2 in the Renal Papilla

In the COX-1 staining, there were no significant differences among the control, nefiracetam, and ibuprofen groups (Table 2). In the COX-2 staining, the immunoreaction in control dogs was seen in the cytoplasm of interstitial cells in the papillary tip (Table 2 and Figures 4A and 5). In dogs receiving nefiracetam, though the immunoreaction was within the cytoplasm of interstitial cells in the papillary tip by week 6, similarly as in the control, interstitial cells around affected epithelial cells (upper papilla) were positively reacted in week 7 (Table 2 and Figures 4B and 5). In dogs given ibuprofen, a marked positive immunoreaction was seen within interstitial cells adjacent to the degenerative and/or necrotic areas of interstitium in the papilla (Table 2 and Figures 4C and 5).

Electron Microscopy

In control dogs (animal Nos. 1 and 3), epithelial cells of the papillary ducts showed a cuboidal appearance with the nucleus located in the center of the cell, and short microvilli were seen on the luminal surface. The lateral plasma membrane was interdigitated, and the basal membrane displayed infoldings. Small numbers of mitochondria and vacuoles were noted in the cytoplasm (Figure 6A). In dogs receiving nefiracetam (animal Nos. 18 and 19), epithelial cells of the papillary ducts showed swelling and the microvilli

TABLE 2.—Light-microscopy and *immunohistochemistry* of the kidney in male dogs receiving repeated oral administration of nefiracetam or ibuprofen for 4 to 7 weeks

Dose	Control			Nefiracetam at 300 mg/kg/day															Ibuprofen at 50 mg/kg/day		
Treatment period	7 weeks			4 weeks				5 weeks				6 weeks				7 weeks				5 weeks	
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Light microscopy																						
Papillary duct (upper papilla)																						
Swelling, epithelia	—	—	—	NP	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	
Degeneration, epithelia	—	—	—	NP	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—
Papillary tip																						
Degeneration/necrosis, interstitium	—	—	—	NP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	++	+
Decrease in alcian-blue staining intensity, interstitium	—	—	—	NP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	++	+
Papilla																						
Mineralization	—	—	—	NP	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—
Immunohistochemistry in the papilla																						
COX-1																						
Collecting duct, epithelia, cytoplasm	+	N	+	N	+	+	N	+	+	N	N	+	+	N	N	N	N	+	+	N	+	+
Ducts, epithelia, cytoplasm, tip of papilla	—	N	—	N	—	—	N	+	+	N	N	+	—	N	N	N	N	—	+	N	+	+
Transitional epithelium, cytoplasm, *pelvis*	+	N	+	N	+	+	N	+	+	N	N	+	+	N	N	N	N	+	+	N	+	+
COX-2																						
Interstitial cells, tip of papilla	+	N	+	N	+	+	N	+	+	N	N	+	+	N	N	N	N	—	—	N	—	—
Interstitial cells, upper papilla	—	N		N	—	—	N	—	—	N	N	—	—	N	N	N	N	+	+	N	—	—
Interstitial cells, around degenerative/necrotic lesions	—	N		N	+	—	N	—	—	N	N	—	—	N	N	N	N	—	—	N	++	++

—: no change; +: slight; ++: moderate. NP: not examined because the papilla was not present. N: not examined.

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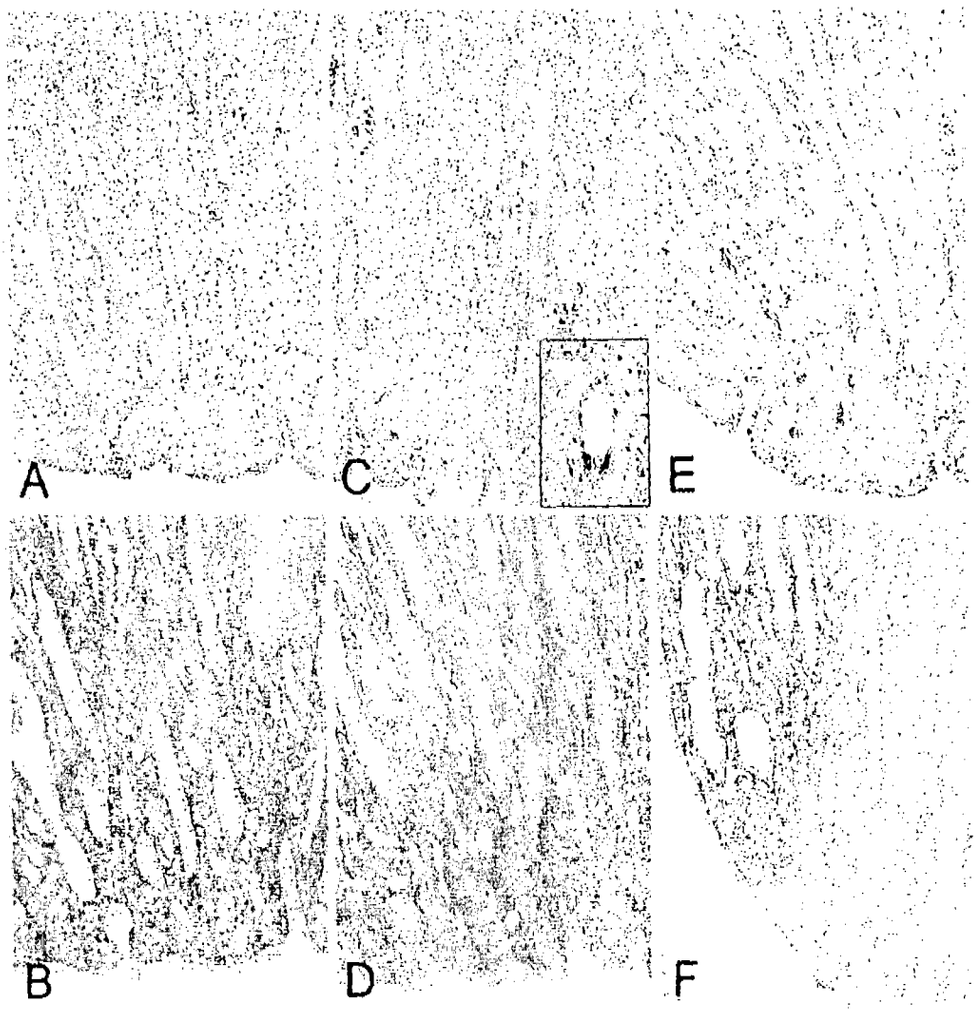

FIGURE 5.—Light micrograph of the renal papilla from male dogs receiving repeated oral administration of nefiracetam at 300 mg/kg/day or ibuprofen at 50 mg/kg/day. (A, C, and E) H&E staining. (B, D, and F) Alcian-blue staining. (A–B) Intact control in week 7, ×95. (C–D) Nefiracetam in week 7, ×95. Epithelial swelling of the papillary ducts was seen. Inset: degeneration of epithelial cells (higher magnification), ×170. (E–F) Ibuprofen in week 5, ×95. Degeneration and necrosis of interstitium in the papilla were noted.

were decreased or absent. Membranous blebs were noted in the luminal surface. Intracellular interdigitation and infoldings in both lateral and basal membranes were inconspicuous. These epithelial cells revealed sparse cytoplasm containing large vacuoles (Figure 6B). In dogs given ibuprofen (animal No. 21), no remarkable changes in the epithelial cells of the papillary ducts were noted.

COX-2 mRNA Expression in the Renal Papilla

Nefiracetam exceedingly decreased COX-2 mRNA expression in week 4, but it recovered to the basal control level in weeks 5 to 6. Afterward, COX-2 mRNA markedly increased in week 7 (Figure 7).

DISCUSSION

In our previous study (Tsuchiya et al., 2003), nefiracetam caused degeneration and/or necrosis in the renal papilla only in dogs administered nefiracetam at 300 mg/kg/day for 8 to 11 weeks; namely, decreases in urinary osmotic pressure were observed from week 5, followed by increases in urine volume and urinary LDH from week 8. The initial renal morphological change was necrosis of ductal epithelial cells in the papilla in week 8. In the present work, to ascertain the earlier pathophysiological features in RPN, male beagle dogs were given orally 300 mg/kg/day of nefiracetam for 4 to 7 weeks in comparison with 50 mg/kg/day of ibuprofen, an NSAID, for 5 weeks, and laboratory tests, light-microscopic, immunohistochemical, and electron-microscopic

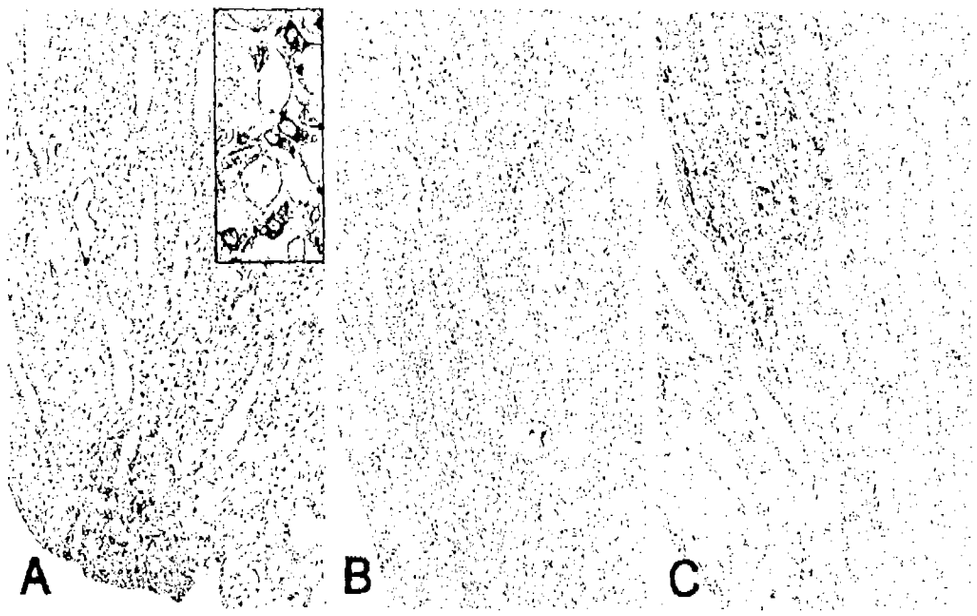

FIGURE 4.—*Immunohistochemical* staining of COX-2 in the renal papilla from male dogs receiving repeated oral administration of nefiracetam at 300 mg/kg/day or ibuprofen at 50 mg/kg/day. Anti-COX-2, LSAB method. (A) Intact control in week 7, ×140. Positive reaction was noted in the cytoplasm of interstitial cells in the papillary tip. *Inset*: higher magnification, ×860. (B) Nefiracetam in week 7, ×140. Interstitial cells around affected epithelial cells in the papillary ducts showed positive reaction. (C) Ibuprofen in week 5, ×140. Strongly positive reaction was seen in interstitial cells adjacent to degenerative and/or necrotic interstitium in the papilla.

examinations, and/or COX-2 mRNA analysis were periodically performed.

In laboratory tests, decreased urinary osmotic pressure and increased urine volume in weeks 6 to 7 of nefiracetam treatment were essentially consistent with those obtained in week 8 of the previous study (Tsuchiya et al., 2003), but an increased urinary LDH level was observed earlier (week 5). Alternatively, these parameters were reconfirmed to be useful early biomarkers to detect the onset of RPN evoked by nefiracetam. Ibuprofen also elicited decreased urinary osmotic pressure, increased urine volume and increased urinary LDH, although there was a great interindividual variance. The onset and severity of these changes by ibuprofen were earlier and stronger than those by nefiracetam, which would be closely linked with necrosis of the interstitium in the papillary tip observed only in the ibuprofen group as mentioned below.

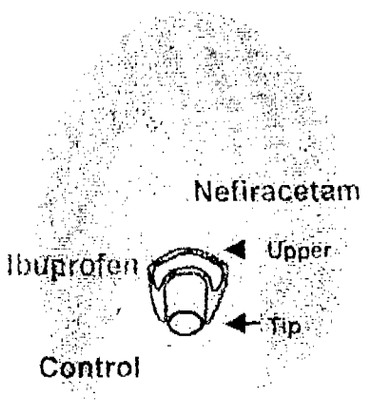

FIGURE 5.—Schematic area showing positive immunoreactions. The area surrounded by a black line (control), blue line (nefiracetam), or red line (ibuprofen). Arrowhead: the upper papilla, arrow: the papillary tip.

Light-microscopically, in dogs receiving nefiracetam, no changes were seen in the kidney by week 6. In week 7, however, epithelial swelling and degeneration of the papillary ducts appeared accompanied with a positive immunoreaction to COX-2 within interstitial cells around the affected epithelial cells in the upper papilla. Ultrastructurally, epithelial swelling, impaired microvilli, and blebbing on the luminal surface were noted with reductions of intracellular interdigitation and infoldings in both lateral and basal membranes. In contrast, treatment with ibuprofen resulted in degeneration and necrosis of the interstitium in the papillary tip in week 5; however, no remarkable changes were observed in the remaining papillary ducts. In the COX-2 staining, unlike the case with nefiracetam, ibuprofen showed a positive reaction within interstitial cells adjacent to degenerative and/or necrotic areas in the papillary tip. Ultrastructurally, no changes in epithelial cells of the papillary ducts were noted. Thus, these data implied that the early

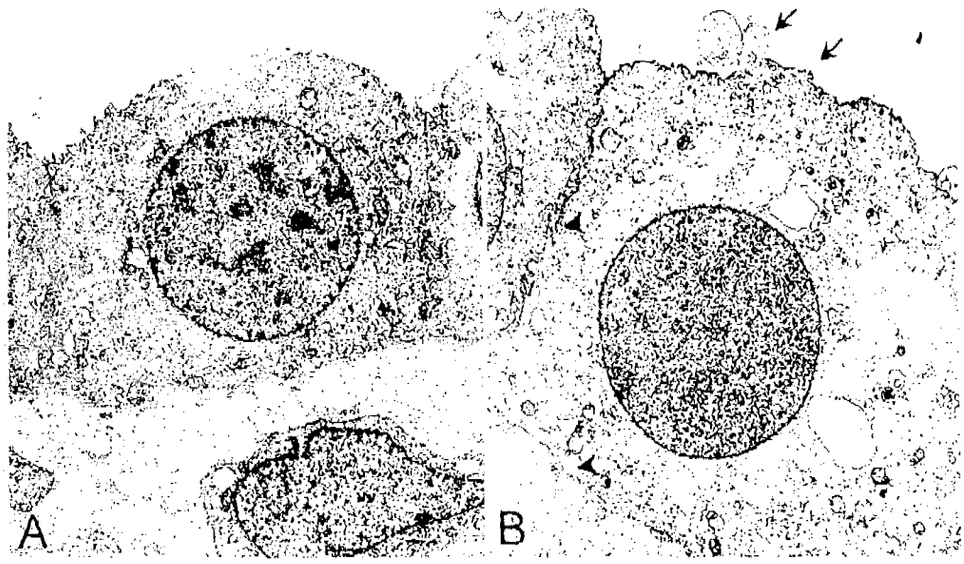

FIGURE 6.—Electron micrograph of the papillary ducts from male dogs receiving repeated oral administration of nefiracetam at 300 mg/kg/day. Glutaraldehyde-osmium fixation. (A) Intact control in week 7, ×6500. Normal structures of epithelial cells were seen. (B) Nefiracetam in week 7, ×6500. Epithelial cells of the papillary ducts showed swelling and decreased microvilli. Membranous blebs were noted on the luminal surface (arrow). Interdigitation and infoldings in both lateral and basal membranes were inconspicuous (arrowhead).

morphological alteration in the papilla brought about by nefiracetam was quite different from that by ibuprofen. Mineralization in the papilla observed in both nefiracetam and ibuprofen treatment may suggest the preexistence of damaged regions. Cellular swelling, loss of microvilli, and blebs seen in the electron-microscopic examination of nefiracetam-treated animals may be indicative of reversible injuries under the hypoxic or ischemic conditions (Cotran et al., 1999). Since dogs given nefiracetam showed severe hypoactivity, of which some lay on their side (Kashida et al., 1996), the continued hypoxic states due to repeated treatment seemed to be a trigger to evoke papillary epithelial swelling.

As for the COX-1 staining, there were no significant differences among the control, nefiracetam, and ibuprofen groups. Generally, COX-1 has been reported as a constitutive isoform having housekeeping functions, whereas COX-2 is an inducible isoform induced by inflammatory stimuli or growth factors (Hao et al., 2000). More recent studies have suggested that COX-2 is also expressed in the renal medulla and is the major isoform contributing to medullary PG generation in vivo, especially in dehydrated subjects (Khan et al., 1998; Neuhofer et al., 2004). Sellers et al. (2004) have further shown that COX-1 is the main isoform involved in the synthesis of renal PGs in non-human primates and humans while both COX-1 and COX-2 contribute to the synthesis of renal PGs under basal conditions in rats and dogs. Hence, COX-2 was confirmed to play more important roles than COX-1 in the progression of RPN in dogs given nefiracetam.

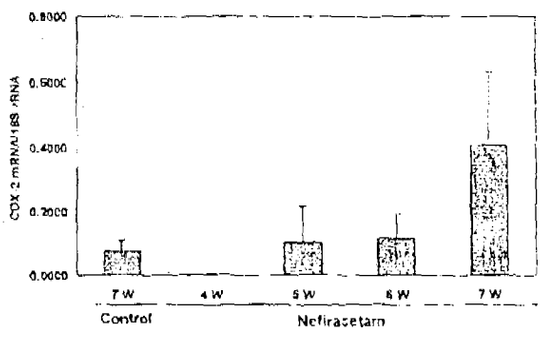

FIGURE 7.—COX-2 mRNA expression of the renal papilla in dogs receiving repeated oral administration of nefiracetam at 300 mg/kg/day. Values are shown as the mean ± SD (n = 3–4).

The renal papillary COX-2 expression in nefiracetam-treated dogs decreased exceedingly in week 4, but returned to the basal control level in weeks 5 to 6. In week 7, however, COX-2 mRNA markedly increased as compared with the control. Therefore, it was considered that nefiracetam depressed COX-2 mRNA expression at the early phase, followed by a compensating increase, and then marked upregulation was seen in response to the progression of renal injuries with continuous treatment. This assumption was partly supported by the fact that the positive immunoreaction to COX-2 was increased in the interstitial cells around the affected epithelial cells of the upper papilla in week 7, suggesting an induction of COX-2 mRNA.

It has been reported that the epithelia promote organ homeostasis by restricting the flow of ions and solutes between cells across the epithelial cells, and the intercellular junctional complexes such as the tight junction, adherens junction, and desmosomes are important to maintain the cell-cell contact (Collares-Buzato et al., 1998; George et al., 2004; Johnson, 2005). Bachmann and Kriz (1982) have demonstrated that the

epithelia (especially when salt secretion is stimulated) exhibit an elaborate "intracellular membranous labyrinth" created by cellular interdigitation and infoldings of the basolateral membrane. Goto et al. (2003) have previously shown that the nefiracetam metabolite M-18 reduces transepithelial electric resistance (TER) in the primary cultured uroepithelial cells of canine urinary bladder, and M-10 and M-18 displayed a deformation of uroepithelial cells and a slight reduction of the ZO-1 band, which is an essential protein associating with the tight junction. Likewise, we have reported that the concentration ratio of M-18 in the papilla to medulla or cortex is remarkably higher in dogs than in rats and monkeys, and that M-18 possessed inhibitory effects on PG synthesis in canine renal papillary slices (Tsuchiya et al., 2003). By taking into account these previous findings (Kashida et al., 1996; Tsuchiya et al., 2003), we propose the following mechanism underlying RPN evoked by nefiracetam: the reductions of intracellular interdigitation and infoldings at both lateral and basal membranes in epithelial cells of the papillary ducts may initially inactivate the paracellular transport. This is followed by decreased cellular integrity, and then M-18 penetration and retention into the renal papilla occur. M-18 inhibits renal papillary PG synthesis, and certain dogs finally incur ischemic injury due to decreased PG synthesis. Further investigation after 7-week treatment will be required to support the above suggestion.

In conclusion, the earliest morphological event in RPN evoked by nefiracetam is quite different from that by ibuprofen. The epithelial cells in the papillary ducts are considered to be the primary target site for the onset of RPN evoked by nefiracetam.

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A case–control study of acute ibuprofen toxicity in dogs

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Abstract

A case–control study used data in the National Animal Poison Control Center database to characterize risk factors for gastrointestinal ulceration and acute renal failure subsequent to the acute ingestion of ibuprofen in the dog. For gastrointestinal ulceration (GIU) subsequent to ibuprofen ingestion, four factors differentiated the 116 cases from the 93 controls. Risk of GIU was lower for dogs where the time from ingestion to intervention was known as opposed to missing (adjusted odds ratio (aOR) = 0.12, $p = 0.0001$). Risk of GIU was also lower for the Labrador breed (aOR = 0.22, $p = 0.004$). Risk of GIU was higher for each unit of the logarithm of time to intervention (aOR = 2.63, $p = 0.0002$) and for the German Shepherd breed (aOR = 5.67, $p = 0.14$). For acute renal failure (ARF) subsequent to ibuprofen ingestion, two factors differentiated the 80 cases from the 64 controls. Risk of ARF was lower for dogs where the time from ingestion to intervention was known as opposed to missing (aOR = 0.15, $p = 0.0001$). Risk of ARF was higher for each unit of the logarithm of time to intervention (aOR = 2.16, $p = 0.01$). Although this study failed to describe a dose–response relationship, it appears that there are significant breed differences in susceptibility to GIU subsequent to ibuprofen exposure. Time to intervention was critical for both GIU and ARF outcomes. Dogs, particularly German Shepherds, ingesting even small amounts of ibuprofen, may need to be managed aggressively. © 1998 Published by Elsevier Science B.V.

Keywords. Ibuprofen; Dog; Ulceration; Renal failure; Case–control studies

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1. Introduction

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) that has been available in an over-the-counter form in the United States since 1984 (Kore, 1990). It is the second most popular over-the-counter pain reliever in the United States, with annual sales in excess of US$400 million per year (Wilke, 1995; Olson, 1994). As ibuprofen has become more widely prescribed and readily available, the National Animal Poison Control Center has seen a corresponding increase in the number of calls related to canine exposures to these medications (Buck et al., 1990). Ibuprofen has become the most common generic drug generating calls to the NAPCC for both dogs and cats (Buck et al., 1990). It is reported to have a narrow range of safety in dogs with repeated dosing. Signs of intoxication include vomiting, diarrhea, hematemesis, melena, anorexia, depression, and azotemia (Conlon, 1991). These reflect the two main syndromes of toxicity: gastric ulceration and acute renal failure (Kore, 1990).

Published reports of ibuprofen toxicosis in the dog deal only with the clinical signs and the dose ingested (Adams, 1969; Spyridakis, 1986; Scherkel and Frey, 1987; Conlon, 1991; Wood, 1990; Godshalk, 1992; Behrend, 1996) Other potential risk factors, (such as age, weight, breed, sex and time or type of intervention) have not been analyzed.

The objective of this study was to determine which risk factors increase the likelihood of gastric ulceration or acute renal failure in canine cases of ibuprofen exposure reported to the NAPCC. Identification of factors associated with increased or decreased risk of gastrointestinal ulceration or acute renal failure secondary to ibuprofen exposure will allow veterinarians to make recommendations for therapeutic and prophylactic management.

2. Materials and methods

2.1. Study population

The study population for this project included all canine ibuprofen exposures reported to the National Animal Poison Control Center (NAPCC) from 1992–1995. The NAPCC is a non-profit service associated with the American Society for the Prevention of Cruelty to Animals (ASPCA). It was the first veterinary poison center in North America. Since 1978, it has provided advice to animal owners and conferred with veterinarians about poisoning exposures. The NAPCC maintains a computer database of over 250 000 cases and is currently adding to this database at a rate of 25 000 cases per year. The NAPCC has received calls concerning more than 650 cases of non-steroidal anti-inflammatory drug exposures each year. Information regarding these exposures is stored in an Informix® Structured Query Language database.

2.2. Selection of case and control animals

Case definitions were developed for two syndromes: gastrointestinal ulceration (GIU), and acute renal failure (ARF). Gastrointestinal ulceration cases were defined

based on signs reported to the NAPCC by either the owner or the attending veterinarian. A gastrointestinal scale (similar to an index used in a retrospective NSAID study involving humans; Trewin, 1994), was developed to assign weights to various clinical signs. Melena and hematemesis were each assigned a weight of 3. Abdominal pain and anemia were each assigned a weight of 2. Vomiting, diarrhea, lethargy, weakness, collapse and anorexia were each assigned a score of 1. To qualify as a GIU 'case', a dog had to achieve a minimum score of 3.

A similar method of case definition was used to select cases with signs of acute renal failure. Based on criteria for hospital-acquired renal failure in dogs (Behrend, 1996), we created a 'Nephropathy Index'. An elevated serum creatinine measurement (greater than 2.0 mg/dl) was assigned a value of 3, while an elevated Blood Urea Nitrogen (BUN) measurement (greater than 23 mg/dl) was assigned a value of 2. An elevated serum phosphorus measurement (greater than 7.0 mg/dl) was also assigned a weight of 2 for our renal failure index. In order to be defined as an ARF case, an animal must have been assigned an index of at least 3. This case definition allowed us to discount mild increases in creatinine that may have been due to pre-renal causes rather than renal causes.

Follow-up calls must have occurred between 48 and 96 h for all animals in this study to confirm the presence of clinical signs and to determine how long the clinical syndrome persisted. Dogs which were exposed to ibuprofen and free of clinical signs at follow up were used as gastrointestinal controls. Renal controls had to undergo similar diagnostic rigor as the renal cases. To serve as ARF controls, dogs must have been exposed to ibuprofen and free of clinical signs with normal BUN, serum creatinine and serum phosphorus values at 48 to 96 h post-exposure.

2.3. Risk factors characterized

Both cases and controls were characterized based on several risk factors. These risk factors included dose of ibuprofen ingested, dose of ibuprofen absorbed, time from ingestion to intervention, breed of dog, weight of dog, age of dog, and sex of dog.

Dose ingested was analyzed as mg of drug per kg of body weight and as total mg ingested by each dog. Both the actual dose and the logarithm of the dose were tested in separate models. Since the NAPCC records report dose certainty as 'observed', 'evidenced', 'suspected', 'possible' or 'unknown', dose was first examined for all cases and controls and then for the subset of animals having doses assessed as 'observed' or 'evidenced'. Dose was also evaluated in the subset of animals who received no intervention in order to eliminate possible confounding or interactive effects of intervention on a dose–response relationship.

An absorbed dose of ibuprofen was calculated for cases and controls, where both the dose ingested and the time to intervention were recorded. Since intervention strategies included the induction of emesis and the administration of activated charcoal, only a fraction of the ingested dose was actually absorbed when intervention occurred within 2 h post-exposure (the time required for maximum absorption of ibuprofen). The dose absorbed was determined by extrapolation from a time-vs.-absorption curve for ibupro-

fen in the dog (Adams, 1969). For interventions which occurred beyond 2 h post-exposure, the entire dose was assumed to have been absorbed.

Time to intervention was examined in three ways: as a dichotomous variable noting whether or not intervention occurred; as the hours from ingestion to intervention; and as the logarithm of the hours to intervention. Biologically, the difference of 1 h early in the course of intervention was expected to have a larger impact than the difference of 1 h later in the course of intervention. The coding giving the best model (described below) was used. Since data on intervention was missing for many dogs, a variable was created to denote missing intervention times. This 'intervention missing or known' variable was coded as '0' if the time to intervention was missing, and '1' if the time to intervention was known. This allowed dogs to be included in the assessment of other variables even if their time to intervention variable was missing.

Table 1
Distribution of all variables across cases and controls

Variable	GIU cases	GIU controls	ARF cases	ARF controls
Age				
≤ 12 months	35	30	26	14
13–84 months	47	26	27	30
> 84 months	11	7	4	3
Breed				
Cocker	4	2	4	2
Dachshund	3	4	3	3
German Shepherd[c]	7	1	8	0
Golden Retriever	2	3	3	3
Siberian Husky	3	4	3	4
Labrador Retriever[a]	10	16	7	7
Mixed	18	21	9	17
Dose ≥ 200 mg/kg	52	38	38	33
Dose < 200 mg/kg	33	31	14	13
Sex				
Male	20	26	11	13
Male castrated	23	8	11	9
Female	28	24	19	18
Female spayed	29	20	27	10
Time to treat[a,b]				
> 2 h	17	12	9	11
≤ 2 h	8	46	6	27
Weight[a,b] *(kg)*				
0 to < 11	37	27	15	19
11 to < 22	27	26	16	18
22 to < 45	42	37	35	19
≥ 45	4	2	3	1

Advanced to backward elimination model: [a]GIU and [b]ARF.

Effects of breed were examined for the seven 'breeds' having a sum of cases and controls greater than or equal to an arbitrary cut-point of 5. The seven breeds were Cocker Spaniel, Dachshund, German Shepherd, Golden Retriever, Labrador Retriever, Siberian Husky and Mixed breed. Mixed breed dogs were compared against all other groups. Dogs were divided into four size groups based on their reported body weights: small dogs weighed 0 to < 11 kg, medium dogs weighed 11 to < 22 kg, large dogs weighed 22 to < 45 kg, and giant dogs weighed > 45 kg. Age was evaluated using three groups based on reported ages: young dogs (0–12 months), middle aged dogs (13–84 months), and old dogs (> 84 months). Middle-aged dogs were used as the comparison group. The risk was predicted to be high for the very young and the very old, and moderate for the middle aged dogs. Four sex groups were compared: intact males, castrated males, intact females, and spayed females. Intact males were used as the comparison group in the univariable screening. Orthogonal contrasts for sex were developed for logistic multiple regression. These contrasts compared males to females (mavfe = 0.5 for sex = male and for sex = male castrate, mavfe = −0.5 for sex = female and for sex = female spayed), castrated males to intact males (mavmc = 1 for sex = male, mavmc = −1 for sex = male castrate and mavmc = 0 where sex = female or sex = female spayed), and spayed females to intact females (fevfs = 0 where sex = male or sex = male castrate, fevfs = 1 where sex = female and fevfs = −1 where sex = female spayed) (Table 1).

2.4. Statistical analysis

The seven main effects variables of interest yielded fifteen variables for modeling. Interaction was initially evaluated with the use of Cochran–Mantel–Haenzel stratification (Afifi and Clark, 1984). Variable combinations with a Breslow–Day chi-square $p < 0.25$ were tested for interaction in logistic multiple regression. Two-way interaction terms were created by using the products of the main-effects terms.

GIU and ARF were modeled separately. All possible variables were screened using a univariable approach. Categorical risk factors such as breed were included in multivariable analysis if the p-values from the univariable analyses (breed vs. mixed) were ≤ 0.25 (Hosmer and Lemeshow, 1989). For continuous and contrast-coded variables, each factor was first tested separately in a simple regression model. Factors yielding a p-value for the Wald chi-square statistic of ≤ 0.25 were offered to the multivariable analysis. The variables for age and the orthogonal contrast variables for sex were assessed as groups and remained in the model if any one of the members yielded a Wald chi-square with a $p \leq 0.25$. Variables were individually deleted from models in a backwards stepwise method to evaluate the effects of each variable on the coefficients of other variables. Variables were deleted one-at-a-time, beginning with interaction terms and then the variable having the least significant Wald chi-square p-value (Afifi and Clark, 1984). Covariate patterns were examined to determine how well each model represented the entire data set. An alpha of 0.10 was used as the initial criterion for elimination. Choice of optimal models was based on comparison of likelihood ratios and Hosmer–Lemeshow Goodness of Fit statistics. Individual variables with higher alpha levels were included if this led to a large improvement in fit (change in Hosmer–

Lemeshow statistic or Beta coefficient of greater than 20%) (Hosmer and Lemeshow, 1989). SAS for Windows (SAS Institute, 1995) was used for logistic regression analyses.

Statistical significance was identified using an alpha level of 0.05. Two-tailed tests were used in all evaluations.

3. Results

3.1. Case sampling

There were 1502 calls regarding ibuprofen to the NAPCC from 1992–1996. Of the 1502 calls, 116 met the criteria for GI case definition, while 93 met the criteria for GI control definition.

There were 109 dogs which exhibited clinical signs subsequent to ibuprofen exposure but did not meet the inclusion criteria for the case definition. These dogs showed very mild clinical signs such as vomiting or diarrhea, with no direct evidence of gastrointestinal ulceration. It was impossible to determine whether this vomiting was due to an initial mild irritation of the gastric mucosa by ibuprofen, or whether it was an indicator of the presence of a gastric ulcer. Since these intermediates may or may not have been indicative of the disease of interest, they were not included as cases or controls in the data analysis. Eighty calls met renal case criteria and 64 calls met renal control criteria. The most frequent reason for exclusion from the study was that the call had never been followed up. The second most frequent reason for exclusion was that the call involved the ingestion of other products in addition to ibuprofen.

3.2. Multivariable analysis

For the GIU model, variables remaining significant in the final model were logarithm of time to intervention, time to intervention missing or known, Labrador breed, and

Table 2
Results of multiple logistic regression analysis of risk factors for gastrointestinal ulceration in dogs ingesting ibuprofen

Variable	β	SE (β)	p	Adjusted OR	95% CI(OR)
Log time to intervention (h)	0.97	0.26	0.0002	2.63	1.59–4.37
Intervention time (missing/known)	−2.12	0.36	0.0001	0.12	0.06–0.24
Labrador breed	−1.54	0.54	0.004	0.22	0.08–0.62
German Shepherd breed	1.74	1.17	0.14	5.67	0.57–56.65

Likelihood ratio $\chi^2 = 73.56$, $p = 0.0001$.
Hosmer–Lemeshow statistic = 1.76, $p = 0.88$.
Constant term = 0.60.

Table 3
Results of multiple logistic regression analysis of risk factors for renal disease in dogs ingesting ibuprofen

Variable	β	SE(β)	p	Adjusted OR	95% CI
Log time intervention	0.77	0.31	0.01	2.16	1.16–4.00
Intervention time (missing/known)	−1.89	0.42	0.0001	0.15	0.07–0.34

Likelihood ratio $\chi^2 = 30.37$, $p = 0.0001$.
Hosmer–Lemeshow statistic = 0.80, $p = 0.94$.
Constant term = 0.27.

German Shepherd breed (Table 2). Only logarithm of time to intervention and whether or not time was known were significant risk factors in the final renal model (Table 3).

4. Discussion

For both outcomes, intervention was an important protective measure regardless of the dose ingested. The more rapidly intervention occurred, the greater the likelihood of a favorable outcome. The improved fit with the logarithmic transformation of time highlighted the greater consequence of even brief delays in treatment shortly after exposure as compared to decreasing effects of treatment later in the course of the syndrome. Early interventions (< 2 h post-ingestion) would help remove the NSAID before complete absorption occurred. This finding should encourage veterinarians to intervene early and aggressively (induction of emesis, administration of activated charcoal with a cathartic, or both). It is difficult to say whether increased risk for dogs with unknown intervention time is real or surrogate. It may reflect unsupervised ingestions, where the dog was left alone for a large portion of the day and ingestion time could not be estimated with any reasonable margin of error.

Appropriate intervention was more effective for gastrointestinal disease than for renal disease. The odds ratios were 2.63 for GIU and 2.16 for ARF for each unit change in the log of the time to intervention. This would correspond to an increase in risk of 80% for GIU and 60% or ARF if intervention was made in 20 min rather than 5, 60 min rather than 15 or 4 h rather than 1 h. The slightly slower rate of increase in risk for ARF may have been a reflection of a higher minimum dose required to produce this syndrome. It may also have been influenced by lower precision in recall of time to intervention. ARF generally occurred ≥ 36 h after exposure while signs of GIU occurred within a few hours. Exact recall of time to intervention would be more difficult when the call was further from the event.

Neither the univariable nor multivariable analyses identified the expected dose–response relationship. We had anticipated that dose would be significant. Previous reports have suggested a minimum toxic dose for ibuprofen of between 50 and 100 mg/kg. An exposure dose of 300 mg/kg is required experimentally to produce renal failure in the dog (Kore, 1989, 1990; Spyridakis, 1986). In our study, seven cases of GIU had reported exposure doses below 50 mg/kg and 32 cases of ARF had exposure doses below 300 mg/kg.

While controls with high-dose exposures might have been explained by rapid and aggressive intervention, there is no similar explanation for the large number of cases which occurred at low doses. The most probable explanation is reporting error although limiting analyses to records with the best dose-assurance levels also did not demonstrate a dose–response relationship. There appeared to be at least three components of reported dose: the actual dose ingested, the dose that the owner observed, and the dose that the owner reported. The amount observed may not have been equal to the amount ingested simply because people may not have known how many pills were in the bottle before the dog began to chew. Some dog owners were likely better able to seek out and quantify the remaining pills to aid in dose estimation than were other owners. Estimating doses for ibuprofen exposures may be more difficult than for other medications because of the small tablet size and large number of tablets that can fit into relatively small bottles. A bottle of 100 acetaminophen tablets is more than twice as large as a bottle of 100 ibuprofen tablets. Estimates may, therefore, be lower than the actual or observed amount. People may not realize how rapidly a dog can ingest these small ibuprofen tablets. These findings regarding dose should serve as a reminder for veterinarians that a dose reported by owners must be interpreted with caution. Both GIU and ARF were seen in dogs at levels of ibuprofen previously reported as 'no effect' levels (Kore, 1989; Conlon, 1991). Veterinarians should be prepared to aggressively decontaminate the majority of dogs exposed to ibuprofen.

Minor modification of the NAPCC form for data collection might improve the accuracy of the reported doses. Addition of a line to record the number of pills in the original container, the number of pills remaining in the container after the ingestion was discovered, the number of pills that the owner was taking per day and the number of days that the owner had been taking the medication would allow for improved assessment of dose accuracy. This would be especially useful in flagging improbable owner estimates and would remind NAPCC veterinarians to ask these questions. This information is often requested verbally, but not recorded in a specific location on the case record.

Another explanation for the cases which occurred at low doses could be the presence of a confounding or interacting risk factor such as a particularly sensitive segment of the canine population. For many intoxicants (such as anticoagulant rodenticides), the very old and the very young are more susceptible (Buck et al., 1987). Breed differences in sensitivity may occur with certain compounds. Collies and mixed-breed collies appear to be about ten times as sensitive to ivermectin as non-Collie breeds (Lovell, 1990). We did not have sufficient numbers of exposures to detect such interaction.

We did find that the German Shepherd breed was at increased risk for GIU. This breed was not retained in the multivariable model of ARF due to the small sample size. In fact, seven out of eight exposed German Shepherds developed GIU and eight of eight developed ARF. The Labrador breed was at decreased risk for GIU. These differences could be related to the types of behaviors selected for in each breed. Perhaps Labradors pick up a bottle of pills and 'retrieve' them without swallowing the pills, while German Shepherds chew and ingest more pills. There could also be differences in the sensitivity of the gastric mucosa or other metabolic differences. Further study of the German Shepherd breed is needed to explore this finding.

No significant differences were found across various weight groups of dogs. One potential bias was that our size group categories were rather broad in order to achieve adequate numbers in each category. Behavioral differences between a 2-kg Yorkshire Terrier and a 10-kg mixed breed working dog, for example, would not have been detectable. Some of this potential bias was accounted for through the evaluation of breed as a risk factor. Although some dog owners may have mistakenly classified the breed of their dog (especially if it was a mixed breed mistaken for a purebred), most owners would be fairly reliable in this estimation. The breed categories, then, were more homogeneous, barring wide variations for age within the breed categories.

There were no significant differences across the rather broadly defined age groups tested for either GIU or ARF. A 4-month old puppy was categorized with a 4-week old puppy, despite the fact that there may be large differences in how these dogs metabolize xenobiotics (Casarett and Doull, 1991). There were not enough exposures to separate dogs with these subtle age differences. Treating age as a continuous variable would not have overcome these differences as the very young and the very old were hypothesized to be at higher risk, yielding a nonlinear relationship.

Reporting biases could have meant a greater likelihood of owners and veterinarians reporting high dose exposures and animals showing clinical signs. In our data, seven of 86 total GIU cases and one of 52 ARF cases where the dose was reported, were below 50 mg/kg. Nine of 67 GIU controls and three of 46 ARF controls where the dose was reported, were below 50 mg/kg. Thus, while lower doses were less likely to be reported than larger doses, the bias was similar among cases and controls. The case:control ratio was about 5:4 for both GIU and ARF. This bias should also have had only minimal effects on the results of this study, since the tendency to call for 'sick' dogs should have extended uniformly across demographic factors.

Another potential bias was the relatively large number of dogs which did not receive a follow-up and thus could not be included in the study. The NAPCC attempts to follow-up on as many calls as possible but is limited by economic and staffing constraints. One would think that the more critically ill dogs would be more likely to receive follow-up calls but the data shows a case:control ratio of 5:4. In reality, the single biggest factor determining whether or not a follow-up call is made is the availability of student labor.

In conclusion, the most important recommendation to veterinarians from this study is to initiate early decontamination of dogs ingesting ibuprofen. The time at which the intervention occurs may be more important than the reported dose ingested. For people who live a considerable distance from a veterinary clinic, the induction of emesis at home may be preferable to delaying emesis until arrival at a veterinary facility. This does not mean that intervention at greater than 2 h post-exposure is ineffective, but intervention must occur as early as possible. Ingestions involving the German Shepherd breed should be managed more aggressively than ingestions involving other common breeds. In light of evidence reported in this study, it would be prudent to add a GI protectant such as sulcralfate, misoprostil and/or cimetidine to the treatment regimen of all German Shepherds who have ingested ibuprofen. Data are needed to evaluate and compare specific treatment modalities for canine ibuprofen intoxications, so that the most efficacious and cost-effective management can be employed.

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RIMADYL®
(carprofen)

Sterile Injectable Solution

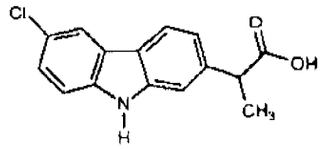
50 mg/mL

Non-steroidal anti-inflammatory drug

For subcutaneous use in dogs only

CAUTION: Federal law restricts this drug to use by or on the order of a licensed veterinarian

DESCRIPTION: Rimadyl Injectable is a sterile solution containing carprofen, a non-steroidal anti-inflammatory drug (NSAID) of the propionic acid class that includes ibuprofen, naproxen, and ketoprofen. Carprofen is the non-proprietary designation for a substituted carbazole, 6-chloro-α-methyl-9H-carbazole-2-acetic acid. The empirical formula is $C_{15}H_{12}ClNO_2$ and molecular weight 273.72. The chemical structure of carprofen is



Each mL of Rimadyl Injectable contains 50.0 mg carprofen, 30.0 mg arginine, 88.5 mg glycocholic acid, 189.0 mg lecithin, 10.0 mg benzyl alcohol, 6.17 mg sodium hydroxide, with additional sodium hydroxide and hydrochloric acid as needed to adjust pH, and water for injection.

CLINICAL PHARMACOLOGY: Carprofen is a non-narcotic, non-steroidal anti-inflammatory agent with characteristic analgesic and antipyretic activity approximately equipotent to indomethacin in animal models.[1]

The mechanism of action of carprofen, like that of other NSAIDs, is believed to be associated with the inhibition of cyclooxygenase activity. Two unique cyclooxygenases have been described in mammals.[2] The constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal and renal function. The inducible cyclooxygenase, COX-2, generates prostaglandins involved in inflammation. Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity. The specificity of a particular NSAID for COX-2 versus COX-1 may vary from species to species.[3] In an *in vitro* study using canine cell cultures, carprofen demonstrated selective inhibition of COX-2 versus COX-1.[4] Clinical relevance of these data has not been shown. Carprofen has also been shown to inhibit the release of several prostaglandins in two inflammatory cell systems: rat polymorphonuclear leukocytes (PMN) and human rheumatoid synovial cells, indicating inhibition of acute (PMN system) and chronic (synovial cell system) inflammatory reactions.[1]

Several studies have demonstrated that carprofen has modulatory effects on both humoral and cellular immune responses.[5-9] Data also indicate that carprofen inhibits the production of osteoclast-activating factor (OAF), PGE_1, and PGE_2 by its inhibitory effects on prostaglandin biosynthesis.[1]

Based upon comparison with data obtained from intravenous administration, carprofen is rapidly and nearly completely absorbed (more than 90% bioavailable) when administered orally.[10] Peak blood plasma concentrations are achieved in 1–3 hours after oral administration of 1, 5, and 25 mg/kg to dogs. The mean terminal half-life of carprofen is approximately 8 hours (range 4.5–9.8 hours) after single oral doses varying from 1–35 mg/kg of body weight. After a 100 mg single intravenous bolus dose, the mean elimination half-life was approximately 11.7 hours in the dog. Rimadyl is more than 99% bound to plasma protein and exhibits a very small volume of distribution.

Comparison of a single 25 mg dose in Beagle dogs after subcutaneous and oral administration demonstrated that the dorsoscapular subcutaneous administration results in a slower rate of drug input (as reflected by mean peak observed concentrations) but comparable total drug absorption within a 12 hour dosing interval (as reflected by area under the curve from hours zero to 12 postdose).

Carprofen is eliminated in the dog primarily by biotransformation in the liver followed by rapid excretion of the resulting metabolites (the ester glucuronide of carprofen and the ether glucuronides of 2 phenolic metabolites, 7-hydroxy carprofen and 8-hydroxy carprofen) in the feces (70–80%) and urine (10–20%). Some enterohepatic circulation of the drug is observed.

INDICATIONS: Rimadyl is indicated for the relief of pain and inflammation associated with osteoarthritis and for the control of postoperative pain associated with soft tissue and orthopedic surgeries in dogs.

DOSAGE AND ADMINISTRATION: The recommended dosage for subcutaneous administration to dogs is 2 mg/lb (4.4 mg/kg) of body weight daily. The total daily dose may be administered as either 2 mg/lb of body weight once daily or divided and administered as 1 mg/lb (2.2 mg/kg) twice daily. For control of postoperative pain, administer approximately 2 hours before the procedure.

EFFECTIVENESS: Confirmation of the effectiveness of Rimadyl for the relief of pain and inflammation associated with osteoarthritis, and for the control of postoperative pain associated with soft tissue and orthopedic surgeries was demonstrated in 7 placebo-controlled, masked studies examining the anti-inflammatory and analgesic effectiveness of Rimadyl caplets and injectable in various breeds of dogs.

Separate placebo-controlled, masked, multicenter field studies confirmed the anti-inflammatory and analgesic effectiveness of Rimadyl caplets when dosed at 2 mg/lb once daily or when divided and administered at 1 mg/lb twice daily. In these two field studies, dogs diagnosed with osteoarthritis showed statistically significant overall improvement based on lameness evaluations by the veterinarian and owner observations when administered Rimadyl at labeled doses.

Based upon the blood level comparison between subcutaneous and oral administration, Rimadyl effectiveness for osteoarthritis after dorsoscapular subcutaneous and oral administration should be similar, although there may be a slight delay in the onset of relief after subcutaneous injection.

Separate placebo-controlled, masked, multicenter field studies confirmed the effectiveness of Rimadyl injectable for the control of postoperative pain when dosed at 2 mg/lb once daily in various breeds of dogs. In these studies, dogs presented for ovariohysterectomy, cruciate repair and aural surgeries were administered Rimadyl preoperatively and for a maximum of 3 days (soft tissue) or 4 days (orthopedic) postoperatively. In general, dogs administered Rimadyl showed statistically significant improvement in pain scores compared to controls.

ANIMAL SAFETY: Laboratory studies in unanesthetized dogs and clinical field studies have demonstrated that Rimadyl is well tolerated in dogs after oral and subcutaneous administration.

In target animal safety studies, Rimadyl was administered orally to healthy Beagle dogs at 1, 3, and 5 mg/lb twice daily (1, 3 and 5 times the recommended total daily dose) for 42 consecutive days with no significant adverse reactions. Serum albumin for a single female dog receiving 5 mg/lb twice daily decreased to 2.1 g/dL after 2 weeks of treatment, returned to the pre-treatment value (2.6 g/dL) after 4 weeks of treatment, and was 2.3 g/dL at the final 6-week evaluation. Over the 6-week treatment period, black or bloody stools were observed in 1 dog (1 incident) treated with 1 mg/lb twice daily and in 1 dog (2 incidents) treated with 3 mg/lb twice daily. Redness of the colonic mucosa was observed in 1 male that received 3 mg/lb twice daily.

Two of 8 dogs receiving 10 mg/lb orally twice daily (10 times the recommended total daily dose) for 14 days exhibited hypoalbuminemia. The mean albumin level in the dogs receiving this dose was lower (2.38 g/dL) than each of 2 placebo control groups (2.88 and 2.93 g/dL, respectively). Three incidents of black or bloody stool were observed in 1 dog. Five of 8 dogs exhibited reddened areas of duodenal mucosa on gross pathologic examination. Histologic examination of these areas revealed no evidence of ulceration, but did show minimal congestion of the lamina propria in 2 of the 5 dogs.

In separate safety studies lasting 13 and 52 weeks, respectively, dogs were administered orally up to 11.4 mg/lb/day (5.7 times the recommended total daily dose of 2 mg/lb) of carprofen. In both studies, the drug was well tolerated clinically by all of the animals. No gross or histologic changes were seen in any of the treated animals. In both studies, dogs receiving the highest doses had average increases in serum L-alanine aminotransferase (ALT) of approximately 20 IU.

In the 52 week study, minor dermatologic changes occurred in dogs in each of the treatment groups but not in the control dogs. The changes were described as slight redness or rash and were diagnosed as non-specific dermatitis. The possibility exists that these mild lesions were treatment related, but no dose relationship was observed.

Clinical field studies were conducted with 549 dogs of different breeds at the recommended oral doses for 14 days (297 dogs were included in a study evaluating 1 mg/lb twice daily and 252 dogs were included in a separate study evaluating 2 mg/lb once daily). In both studies the drug was clinically well tolerated and the incidence of clinical adverse reactions for Rimadyl-treated animals was no higher than placebo-treated animals (placebo contained inactive ingredients found in Rimadyl). For animals receiving 1 mg/lb twice daily, the mean post-treatment serum ALT values were 11 IU greater and 9 IU less than pre-treatment values for dogs receiving Rimadyl and placebo, respectively. Differences were not statistically significant. For animals receiving 2 mg/lb once daily, the mean post-treatment serum ALT values were 4.5 IU greater and 0.9 IU less than pre-treatment values for dogs receiving Rimadyl and placebo, respectively. In the latter study, 3 Rimadyl-treated dogs developed a 3-fold or greater increase in (ALT) and/or (AST) during the course of therapy. One placebo-treated dog had a greater than 2-fold increase in ALT. None of these animals showed clinical signs associated with the laboratory value changes. Changes in clinical laboratory values (hematology and clinical chemistry) were not considered clinically significant. The 1 mg/lb twice daily course of therapy was repeated as needed at 2-week intervals in 244 dogs, some for as long as 5 years.

Clinical field studies were conducted on 331 dogs undergoing orthopedic or soft tissue surgery. Dogs were administered 2 mg/lb of Rimadyl subcutaneously two hours prior to surgery and once daily thereafter, as needed, for 2 days (soft tissue surgery) or 3 days (orthopedic surgery). Rimadyl was well tolerated when used in conjunction with a variety of anesthetic-related drugs. The type and severity of abnormal health observations in Rimadyl- and placebo-treated animals were approximately equal and few in number (see Adverse Reactions). The most frequent abnormal health observation was vomiting and was observed at approximately the same frequency in Rimadyl- and placebo-treated animals. Changes in clinicopathologic indices of hematopoietic, renal, hepatic, and clotting function were not clinically significant. The mean post-treatment serum ALT values were 8.4 IU and 7.0 IU less than pre-treatment values for dogs receiving Rimadyl and placebo, respectively. The mean post-treatment AST values were 1.5 IU and 0.7 IU greater for dogs receiving Rimadyl and placebo, respectively.

Swelling and warmth were associated with the injection site after subcutaneous administration of Rimadyl injectable. These findings were not clinically significant. Long term use of the injectable has not been studied.

CONTRAINDICATIONS: Rimadyl should not be used in dogs exhibiting previous hypersensitivity to carprofen.

PRECAUTIONS: As a class, cyclooxygenase inhibitory NSAIDs may be associated with gastrointestinal, renal and hepatic toxicity. Effects may result from decreased prostaglandin production and inhibition of the enzyme cyclooxygenase which is responsible for the formation of prostaglandins from arachidonic acid.[11-14] When NSAIDs inhibit prostaglandins that cause inflammation they may also inhibit those prostaglandins which maintain normal homeostatic function. These anti-prostaglandin effects may result in clinically significant disease in patients with underlying or pre-existing disease more

often than in healthy patients.[12,14] NSAID therapy could unmask occult disease which has previously been undiagnosed due to the absence of apparent clinical signs. Patients with underlying renal disease for example, may experience exacerbation or decompensation of their renal disease while on NSAID therapy.[11–14] The use of parenteral fluids during surgery should be considered to reduce the potential risk of renal complications when using NSAIDs perioperatively.

Carprofen is an NSAID, and as with others in that class, adverse reactions may occur with its use. The most frequently reported effects have been *gastrointestinal signs. Events involving suspected renal, hematologic, neurologic,* dermatologic, and hepatic effects have also been reported. Patients at greatest risk for renal toxicity are those that are dehydrated, on concomitant diuretic therapy, or those with renal, cardiovascular, and/or hepatic dysfunction. Concurrent administration of potentially nephrotoxic drugs should be approached cautiously, with appropriate monitoring. Since many NSAIDs possess the potential to induce gastrointestinal ulceration, concomitant use of Rimadyl with other anti-inflammatory drugs, such as corticosteroids and NSAIDs, should be avoided or very closely monitored. Sensitivity to drug-associated adverse reactions varies with the individual patient. For example, Rimadyl treatment was not associated with renal toxicity or gastrointestinal ulceration in well-controlled safety studies of up to ten times the dose in dogs. As with any parenterally injected product, good hygienic procedures should be used when administering Rimadyl Injectable. It is suggested to use different sites for additional injections.

Rimadyl is not recommended for use in dogs with bleeding disorders (e.g., Von Willebrand's disease), as safety has not been established in dogs with these disorders. The safe use of Rimadyl in animals less than 6 weeks of age, pregnant dogs, dogs used for breeding purposes, or in lactating bitches has not been established. Safety has not been established for IV or IM administration. Studies to determine the activity of Rimadyl when administered concomitantly with other protein-bound or similarly metabolized drugs have not been conducted. Drug compatibility should be monitored closely in patients requiring additional therapy. Such drugs commonly used include cardiac, anticonvulsant and behavioral medications. It has been suggested that treatment with carprofen may reduce the level of inhalant anesthetics needed.[15] If additional pain medication is warranted after administration of the total daily dose of Rimadyl, alternative analgesia should be considered. The use of another NSAID is not recommended. Consider appropriate washout times when switching from one NSAID to another or when switching from corticosteroid use to NSAID use.

INFORMATION FOR DOG OWNERS:
Rimadyl, like other drugs of its class, is not free from adverse reactions. Owners should be advised of the potential for adverse reactions and be informed of the clinical signs associated with drug intolerance. Adverse reactions may include decreased appetite, vomiting, diarrhea, dark or tarry stools, increased water consumption, increased urination, pale gums due to anemia, yellowing of gums, skin or white of the eye due to jaundice, lethargy, incoordination, seizure, or behavioral changes. **Serious adverse reactions associated with this drug class can occur without warning and in rare situations result in death (see Adverse Reactions). Owners should be advised to discontinue Rimadyl therapy and contact their veterinarian immediately if signs of intolerance are observed.** The vast majority of patients with drug related adverse reactions have recovered when the signs are recognized, the drug is withdrawn, and veterinary care, if appropriate, is initiated. Owners should be advised of the importance of periodic follow up for all dogs during administration of any NSAID.

WARNINGS: Keep out of reach of children. Not for human use. Consult a physician in cases of accidental human exposure. **For use in dogs only.** Do not use in cats.

All dogs should undergo a thorough history and physical examination before initiation of NSAID therapy. Appropriate laboratory tests to establish hematological and serum biochemical baseline data prior to, and periodically during, administration of any NSAID should be considered. **Owners should be advised to observe for signs of potential drug toxicity (see Adverse Reactions, Animal Safety and Post-Approval Experience).**

ADVERSE REACTIONS: During investigational studies for the caplet formulation, no clinically significant adverse reactions were reported. Some clinical signs were observed during field studies (n=297) which were similar for carprofen- and placebo-treated dogs. Incidences of the following were observed in both groups: vomiting (4%), diarrhea (4%), changes in appetite (3%), lethargy (1.4%), behavioral changes (1%), and constipation (0.3%). The product vehicle served as control.

There were no serious adverse events reported during clinical field studies with once daily oral administration of 2 mg/lb. The following categories of abnormal health observations were reported. The product vehicle served as control.

Percentage of Dogs with Abnormal Health Observations Reported in Clinical Field Study (2 mg/lb once daily)

Observation	Rimadyl (n=129)	Placebo (n=132)
Inappetance	1.6	7.5
Vomiting	3.1	3.8
Diarrhea/Soft stool	3.1	4.5
Behavior change	0.8	0.8
Dermatitis	0.8	0.8
PU/PD	0.8	
SAP increase	7.8	8.3
ALT increase	5.4	4.5
AST increase	2.3	0.8
BUN increase	3.1	1.5
Bilirubinuria	16.3	12.1
Ketonuria	14.7	9.1

Clinical pathology parameters listed represent reports of increases from pretreatment values; the use of clinical judgement is necessary to determine clinical relevance (refers also to table below).

There were no serious adverse events reported during clinical field studies for the injectable formulation. The following categories of abnormal health observations were reported. Saline served as placebo control.

Percentage of Dogs with Abnormal Health Observations Reported in Clinical Field Studies with the Injectable

Observation*	Rimadyl (n=168)	Placebo (n=163)
Vomiting	10.1	9.2
Diarrhea/soft stool	2.4	3.7
Dermatitis	0.6	1.2
Dysrhythmia	0.6	0.6
Swelling	0	1.2
Dehiscence	1.2	0
WBC increase	13.7	6.7

* A single dog may have experienced more than one occurrence of an event.

Post-Approval Experience
Although not all adverse reactions are reported, the following adverse reactions are based on voluntary post-approval adverse drug experience reporting. The categories of adverse reactions are listed by body system.

Gastrointestinal: *Vomiting, diarrhea, constipation, inappetence, melena, hematemesis, gastrointestinal ulceration, gastrointestinal bleeding, pancreatitis.*

Hepatic: *Inappetence, vomiting, jaundice, acute hepatic toxicity, hepatic enzyme elevation, abnormal liver function test(s), hyperbilirubinemia, bilirubinuria, hypoalbuminemia. Approximately one-fourth of hepatic reports were in Labrador Retrievers.*

Neurologic: *Ataxia, paresis, paralysis, seizures, vestibular signs, disorientation.*

Urinary: *Hematuria, polyuria, polydipsia, urinary incontinence, urinary tract infection, azotemia, acute renal failure, tubular abnormalities including acute tubular necrosis, renal tubular acidosis, glucosuria.*

Behavioral: *Sedation, lethargy, hyperactivity, restlessness, aggressiveness.*

Hematologic: *Immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, blood loss anemia, epistaxis.*

Dermatologic: *Pruritus, increased shedding, alopecia, pyotraumatic moist dermatitis (hot spots), necrotizing panniculitis/vasculitis, ventral ecchymosis. In rare situations, injection site reactions including necrosis, abscess and seroma formation, and granulomas have been reported with the injectable formulation.*

Immunologic or hypersensitivity: *Facial swelling, hives, erythema.*

In rare situations, death has been associated with some of the adverse reactions listed above.

To report a suspected adverse reaction, call 1-800-366-5288.

STORAGE: Store under refrigeration 2° - 8°C (36°–46°F). Once broached, product may be stored at temperatures up to 25°C (77°F) for 28 days.

HOW SUPPLIED: Rimadyl Injectable is supplied in 20-mL, amber, glass, sterile, multi-dose vials.

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For a copy of the Material Safety Data Sheet (MSDS) or to report adverse reactions call Pfizer Animal Health at 1-800-366-5288.

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DIVISIÓN DE NUEVAS CREACIONES
Respuesta al Recurso de reposición

Radicación N° 04-109926

Clasificación Internacional Patentes: A61K 47/10

Estudiada nuevamente la presente solicitud, ahora junto con el memorial del recurso de reposición se conceptúa:

Documentos estudiados:

✓ Capitulo descriptivo folios 76-89;
✓ Reivindicaciones 90 a 93.
✓Examen de patentabilidad, folios 119-127.
✓Respuesta al examen de patentabilidad, folios 128- 136.
✓Capitulo reivindicatorio modificado, folios 135 y 136.
✓2° examen de patentabilidad, folios 138-140.
✓Respuesta al 2° examen de patentabilidad, folios 141-151.
✓Capitulo reivindicatorio modificado nuevamente, folios 150 y 151
✓Examen de fondo definitivo, 154 a 155.
✓Memorial del recurso, folios 166-209, 179 a 209 anexos que introducen materia por lo tanto no se tendrán en cuenta.

ARGUMENTO DEL SEÑOR MEMORIALISTA:

"Lo anterior por las siguientes razones jurídicas y técnicas sobre las cuales respetuosamente solicitamos un pronunciamiento expreso de su Despacho:

5.1 Violación de los artículos 14 y 18 de la Decisión 486 de la Decisión 486 de la Comisión de la Comunidad Andina:

De acuerdo con los artículos 14 y 18 de la Decisión 486:

"Artículo 14.- Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento,en todos los campos de la tecnología, siempre que sean" nuevas, tengan nivel inventivo inventivo y sean susceptibles de aplicación industrial."(Subrayas fuera del texto)

"Artículo 18.- Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica"

Ahora bien, el Manual para el Examen de Solicitudes de Patentes de invención en las Oficinas de Propiedad Industrial de los Países de la Comunidad Andina con respecto a la evaluación del nivel inventivo indica:

*"La pregunta a contestar es si teniendo en cuenta el estado de la técnica en su conjunto existe alguna **indicación que lleve a la persona versada en la materia a modificar o adaptar el estado de la técnica más cercano para resolver el problema técnico,** de tal forma que llegue a un resultado que estuviera incluido en el tenor de la (s) reivindicación(es) ". (Negrita fuera del texto).*

La obligatoriedad de este Manual no reside en su fuerza normativa intrínseca sino en que el mismo es mencionado por el Tribunal de Justicia en reiterada jurisprudencia cuando interpreta estas normas de la Decisión 486 y por ende, cuando establece cómo debe adelantarse el estudio de nivel inventivo de una invención.

Por lo tanto, teniendo en cuenta el carácter vinculante que tienen las interpretaciones prejudiciales del Tribunal para el Consejo de Estado y el hecho de que éste último revisa la legalidad de las decisiones adoptadas por su Despacho, consideramos que dicho Manual si resulta relevante para el estudio del nivel inventivo de la presente invención.

*Asi entonces, es posible concluir que para ser pertinentes en la la evaluación de nivel inventivo de la presente invención, los documentos citados **deberían** revelar sugerencias y enseñanzas claras para el técnico en la materia que lo lleven a obtenerla. Para este fin el Señor Examinador debe examinar si, de conformidad con dichos documentos (el estado de la técnica más próximo), una persona versada en la materia habria o no propuesto las características técnicas que distinguen la invención reivindicada y que permiten alcanzar los resultados obtenidos por dicha invención.*

Para los anteriores efectos, el Examinador al realizar el examen de nivel inventivo debe verificar las enseñanzas del arte previo sin adoptar la posición de un inventor que se preguntarla cómo solucionar el problema técnico en cuestión. Lo anterior, por cuanto si se adopta la posición del inventor y se tiene conocimiento tanto del arte previo como de la forma como efectivamente se solucionó el problema técnico, la invención en todos los casos le resultará obvia y evidente.

A este respecto, consideramos pertinente poner de presente la cita del tratadista GÓMEZ SEGADE, mencionada por el Tribunal de Justicia del Acuerdo de Cartagena en la sentencia proferida, dentro del Proceso No. 26-IP-99, el 23 de julio de 1999, en relación con el significado que la doctrina y la jurisprudencia le han dado a la característica de nivel inventivo.

"Tal y como lo establece GÓMEZ SEGADE (obra citada) el inventor debe reunir los méritos que le permitan atribuirse una patente, sólo si la invención fruto de su investigación y desarrollo creativo constituyen "un salto cualitativo en la elaboración de la regla técnica", actividad intelectual mínima que le permitirá que su invención no sea evidente (no obvia) del estado de la técnica.".

Teniendo en cuenta el anterior planteamiento sobre lo que debe considerarse nivel inventivo, podemos concluir que una invención solamente cumplirá con este requisito cuando la misma haya resultado a través de estudio y experimentación (investigación) y constituya un adelanto tecnológico, materializado en un resultado inesperado (mejorado) y además no evidente para alguien del oficio normalmente versado en la materia.

Es asi que el Tribunal de Justicia del Acuerdo de Cartagena, ha manifestado en reiterada jurisprudencia los elementos que debe tener en cuenta el examinador de patentes para

establecer la existencia o no de NIVEL INVENTIVO en una invención:

"El nivel inventivo se configura con referencia a dos elementos que son:

a) el estado de la técnica y,

b)la persona experta en la técnica en cuestión.

El estado de la técnica es el conjunto de elementos técnicos que se han hecho públicos antes de la fecha de presentación de la patente",

"El experto en la técnica es una figura ficticia a la que se recurre con el propósito de obtener un parámetro: objetivo que permita distinguir la actividad: verdaderamente inventiva de la que no es. Se tratará de una persona normalmente versada en el ámbito tecnológico; a que se refiere el pretendido invento. Su nivel de conocimientos es más elevado en comparación con el nivel de conocimientos del público en general, pero no excede lo que puede esperarse de una persona debidamente calificada. Se busca la figura de un técnico de conocimientos medios, pero no especializados".
(Sentencia del 21 de julio de 2000, proferida en el proceso 39-IP-2000, Magistrado Ponente Rubén HERDOIZA. MERA).

Asimismo, es importante resaltar que si bien el resultado y/o los medios reivindicados en la invención objeto de análisis ya eran conocidos (estado de la técnica), la invención carecerá de nivel inventivo.

*En efecto, se pone de presente que si al momento de efectuar el examen de nivel inventivo de una invención cualquiera, los medios usados para la producción de dicho resultado son conocidos, este **solo** hecho no permitirá al examinador concluir que la invención carece de nivel inventivo y que por ende no puede ser objeto de patente de invención.*

Precisamente esto resulta plenamente concordante con la posición reciente de esa misma Corporación, quien en sentencia del 28 de enero de 2010 en el Expediente No. 2004-00401, siendo Consejero ponente el Dr. Marco Antonio Velilla Moreno, señaló:

***"[...] si el inventor para la creación en cuestión, extrajo elementos de anterioridades como componentes para su invento, no afectarla negativamente su altura inventiva, en la medida en que ésta se considere en su combinación y no en cada uno de los elementos extraídos para su estructura, tal como acertadamente lo analiza el Tribunal Andino en la interpretación solicitada e este proceso"** -se refiere a la sentencia de interpretación prejudicial del Proceso 198-IP-2006 -*

conformidad con el anterior artículo, se expone a continuación el por qué la presente invención posee nivel inventivo frente a los documentos Di y D2, toda vez que para el presente caso la decisión de la División de Nuevas Creaciones fue expedida en contravención de la Ley, toda vez que se interpretó erróneamente el artículo 18 de la Decisión 486. Veamos:

5.1.1 Con respecto a las reivindicaciones de producto:

El De problema planteado en la presente solicitud es la necesidad de composiciones acuosas inyectables de carprofeno que sean adecuadas para animales pequeños. Este problema fue el que precisamente solucionó la presente invención, toda vez que la misma proporciona composiciones inyectables que presentan efectos secundarios reducidos (estabilidad mejorada)."

§ RESPUESTA TÉCNICA

Respecto a la disertación personal del señor memorialista sobre la obligatoriedad del Manual y su carácter vinculante es necesario aclarar que el Manual ni es vinculante ni es obligatorio, lo que sí es obligatorio aplicar es la D 486 de la C.A.N. junto a las interpretaciones prejudiciales que ha emitido el Tribunal de Justicia de la Comunidad Andina y respecto a nivel inventivo, el **PROCESO 88-IP-2005** es donde interpreta lo concerniente a nivel inventivo así:

" *2. **Del nivel inventivo**:*

...se desarrolla el requisito de nivel inventivo al establecer que la invención goza de dicho nivel al no derivar de manera evidente del estado de la técnica, ni que hubiese resultado obvia para una persona entendida o versada en la materia. Este requisito ofrece al examinador la posibilidad de determinar si con los conocimientos técnicos que existían al momento de la invención, se hubiese podido llegar de manera evidente a la misma, o si el resultado hubiese sido obvio para un experto medio en la materia de que se trate, es decir, para una persona del oficio y normalmente versada en la materia técnica correspondiente.

Con respecto a lo anterior, el Tribunal de Justicia de la Comunidad Andina ha manifestado lo siguiente:

*"**Con el requisito del nivel inventivo, lo que se pretende es dotar al examinador técnico de un elemento que le permita afirmar o no si a la invención objeto de estudio no se habría podido llegar a partir de los conocimientos técnicos que existían en ese momento dentro del estado de la técnica**... En este punto conviene advertir que uno es el examen que realiza el técnico medio respecto de la novedad y otro el que se efectúa con respecto al nivel inventivo; si bien en uno y otro se utiliza como parámetro de referencia el 'estado de la técnica', en el primero, se coteja la invención con las 'anterioridades' existentes dentro de aquella, cada uno (sic) por separado, mientras que en el segundo* (nivel inventivo) **se exige que el técnico medio que realiza el examen debe partir del conocimiento general que él tiene sobre el estado de la técnica y realizar el cotejo comparativo con su apreciación de conjunto, determinando si con tales conocimientos técnicos existentes ha podido o no producirse tal invención**"... (negrilla y subrayado fuera de texto)

"En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo

más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no sea consecuencia clara y directa de dicho estado."

Ahora bien así las cosas, y con la interpretación prejudicial de lo que implica el nivel inventivo, pasaremos a demostrar que no hubo un indebido análisis para tal efecto de la solicitud, como en opinión personal del señor memorialista argumenta que ocurrió. Entonces es necesario hacer una ilustración de cómo se puede observar en la práctica real y de manera objetiva, un análisis de nivel inventivo de cualquier solicitud y de paso demostrar nuevamente porque fue negada la presente solicitud. Lo primero que se debe observar es, cual materia está definida técnicamente y que se reclama como nuevo e inventivo en la solicitud en estudio. Luego teniendo claro lo anteriormente señalado, se procede a realizar el examen, partiendo del conocimiento que debe poseer el examinador del área, junto con el conocimiento sobre el estado de la técnica y realizando el cotejo comparativo contra la materia reclamada en la solicitud, para ver sí realmente la materia reclamada es una invención con actividad inventiva o nivel inventivo, y de paso para el presente caso analizar como encajan los argumentos del señor memorialista.
Comenzamos observando de manera objetiva la materia que realmente se reclama como propiedad exclusiva, o sea, se pretende nueva e inventiva en las reivindicaciones de la solicitud:

"Una solución inyectable a temperatura ambiente para uso veterinario, que comprende 0.25 a 30% (p/v) de Carprofeno (ácido 6-cloro-α-metil-carbazol-2-ácetico) o una sal fisiológicamente aceptada de carprofeno, y desde y desde 0.5% a 20% (p/v) de un poloxámero, y agua c.s para inyectable"

Se puede observar que la materia tal como es reclamada se en cabeza como una solución inyectable, cuya patentabilidad depende de la patentabilidad de la mezcla de **Carprofeno o una de sus sales farmacéuticamente aceptables, un poloxamero y agua,** entonces buscamos esa mezcla de sustancias que son la característica técnica, del "inyectable" de Carprofeno el cual es un principio activo analgésico, las cantidades del principio activo están en un rango enorme y además la cantidad es dada por su ventana terapéutica la cual depende única y exclusivamente de la naturaleza química del Carprofeno.

De igual manera el hecho que se enuncie que dicha solución es un inyectable, no constituye una "característica técnica", **las características técnicas de la composición inyectable están establecidas por sus elementos constitutivos propios, que le permiten como primeramedida diferentes esa forma de presentación y segundo esa forma de administración**, eso no es complicado de entender. Ahora viendo la solicitud en estudio respecto a cómo aparecen los elementos constitutivos de la solución inyectable, únicamente aparece la mezcla **Carprofeno o una de sus sales farmacéuticamente aceptables, un poloxamero y agua.** Tal como debe ser sabido por experto en la materia, las composiciones inyectables. Entonces ahora veamos en las anterioridades citadas como aparecen las composiciones inyectables en D1[EP0955063], se enseña composiciones inyectables que comprenden **Un agente analgésico, un poláxamero y agua.**

• Ya teniendo establecida claramente la materia que se reclama como nueva e inventiva y la que enseña la anterioridad D1, ahora ¿cómo se puede observar objetivamente el nivel inventivo de una solicitud?

Se debe prestar atención especialmente a:

-el hecho de haber superado un prejuicio de los expertos en la materia;

-el hecho de haber superado dificultades técnicas reales;

-la originalidad de la solución, que se aparta del camino trillado y abre una vía nueva;

-Uso de equivalentes técnicos conocidos

-Intercambio de material por otro análogo conocido;

-la necesidad de combinar por lo menos tres documentos del estado de la técnica para llegar a la invención en estudio;

-el carácter inesperado del resultado;

-Extrapolación Simple y directa de hechos conocidos

-Selección entre un número de posibilidades conocidas sin ningún efecto inesperado.

Así las cosas, recordando lo anterior prestando atención a la materia realmente reclamada, podemos hacer académicamente un cuadro comparativo:

Solicitud en estudio	**D1 [EP0955063]**
Principio activo: Carprofeno Analgésico antiinflamatorio	Principio activo: Analgésico antiinflamatorio
poloxámero	poloxámero
Agua	Agua

Continuando con el análisis sobre el nivel inventivo, podemos ver que en ambos casos las composiciones comprenden principios activos analgésicos antiinflamatorios, y comprenden los mismos excipientes adecuados para inyectables. Eso nos dice diáfanamente que la anterioridad ya había presentado la solución técnica, de presentar un agente analgésico antiinflamatorio con los excipientes más adecuados fue un poloxámero y agua, afirmando claramente que los compuestos antiinflamatorios pueden ser usados ventajosamente en combinación con poloxámeros para llegar a soluciones inyectables. Entonces la solicitud en estudio presenta exactamente la misma solución técnica de D1 pero el analgésico antiinflamatorio es Carprofeno.

El Carprofeno y sus sales son compuestos bien conocidos desde 1978, para una persona versada en el arte desde la publicación D2 [CH663788], y suplir el antiinflamatorio en D1 con Carprofeno, no implica haber superado un prejuicio de los expertos en la materia, no se ha superado dificultades técnicas reales, la solución técnica no es original, tampoco y abre una vía nueva técnica, es solamente el uso de equivalentes técnicos conocidos, ya que es un mero intercambio de material por otro análogo conocido, donde dicho intercambio no presenta carácter inesperado del resultado, tal como se puede ver es una extrapolación simple y directa de las enseñanzas o hechos conocidos en D1.

Entonces, así las cosas, vemos que en los estudios de patentabilidad sí se hizo un análisis técnico juicioso, ajustándose a lo exigido por la legislación actual vigente para Colombia, en relación con las solicitudes que no presentan un aporte técnico real que enriquezcan el acervo tecnológico, como el presente caso.

ARGUMENTO DEL SEÑOR MEMORIALISTA

Ahora bien, D1 no divulga una composición que es una solución inyectable estable a temperatura ambiente, como la requerida por la presente solicitud. Sin embargo, no hay evidencia en DI que demuestre que las composiciones allí reveladas sean estables a temperatura ambiente. En efecto, Di revela composiciones SOL-GEL que pueden ser administradas a temperatura ambiente en forma líquida intramuscularmente y subcutáneamente. Sin embargo, dichas composiciones a temperatura corporal producen un gel de liberación lenta (Ver párrafo 0008 de la descripción en idioma original), a partir del cual los ingredientes activos son liberados de forma controlada.

Adicionalmente, mi representada desea poner de presente que el hecho que las composiciones poloxámero-anti-inflamatorias divulgadas en DI presenten una temperatura de transición entre 20 a 40°C, no significa que dichas composiciones sean estables a temperatura ambiente en forma de solución. De hecho, la estabilidad de las composiciones necesita ser verificada a partir de estudios de estabilidad adecuados a varias temperaturas por al menos 6 a 12 meses. Sin embargo en DI, en la figura 4 simplemente se compara la estabilidad de los geles en relación con la resistencia de penetración de

los mismos, los cuales comprenden diferentes concentraciones de Lutrol F68, F127 mas o menos 0.1% Carbopol (PAA) y no se menciona nada con respecto a la estabilidad en el tiempo y a distintas temperaturas de los activos en el gel.

De otro lado, D2 enseña sales de carprofeno que son útiles para la preparación de composiciones farmacéuticas analgésicas, antiinflamatorias y antirreumáticas. Los ejemplos 1-3 divulgados en D2 se refiere a la síntesis de sales de carprofeno. El ejemplo 4 se refiere a composiciones en tableta, cápsula y/o supositorio. A diferencia de la presente solicitud, ninguna de las composiciones alli reveladas presenta un polímero con carprofeno y no son inyectables. En efecto, todas las composiciones mencionadas en dicho documento presentan formas de dosis ***sólidas****.*

Ahora bien, es importante resaltar que para una persona versada en la materia no seria obvio reemplazar el ibuprofeno de DI con carprofeno debido a que a pesar de que ambos son NSAIDs del grupo priopiónico, estos tienen diferentes propiedades.

Por ejemplo el Carprofeno es un ácido débil, con un pKa de 4.3 y un coeficiente de partición n-octanol/agua de 41 a un PH de 7.4; mientras el ibuprofeno tiene un pKa de 4.43 y un coeficiente de partición de 11.7 a un PH de 7.4. Ambas drogas poseen propiedades anti inflamatorias, analgésicas (sitios de acción periféricos y centrales) y antipiréticas.

En un contexto amplio, ambas drogas actúan disminuyendo la producción de prostaglandinas y tromboxano A2 de acido araquidónico por membranas de células dañadas.

Ahora bien, el Ibuprofeno es un NSAID no selectivo de COX. Asimismo, según estudios recientes las propiedades antiinflamatorias adicionales se deben a la modulación de la actividad de leucocito, producción reducida de citoquina, inhibición de radicales libres, transducción de señales u acción analgésica. Sin embargo, el carprofeno es un NSAID selectivo de COX-2 con una inhibición débil de las isoenzimas COX. Esta propiedad ha ayudado a obtener una licencia para el uso preoperatorio porque hay un riesgo reducido de que afecte el flujo de sangre renal durante un episodio de hipotensión en condiciones anestésicas.

Asimismo, el ibuprofeno puede ser un analgésico efectivo en caninos pero existe evidencia de que tiene un perfil de seguridad pobre en estas especies con riesgos inaceptables de ulceras gastrointestinales y fallas renales.

§ RESPUESTA TÉCNICA

Nuevamente se hace necesario tener siempre presente la materia reclamada en la solicitud en estudio para no apartarnos del objeto real que verdaderamente se reclama:

"Una solución inyectable a temperatura ambiente para uso veterinario, que comprende 0.25 a 30% (p/v) de Carprofeno (ácido 6-cloro-α-metil-carbazol-2-ácetico) o una sal fisiológicamente aceptada de carprofeno, y desde y desde 0.5% a 20% (p/v) de un poloxámero, y agua c.s para inyectable"

Ahora retomemos la materia técnica enseñada en D1, conociendo la materia técnica de solicitud, que corresponde a los elementos constitutivos de la solución inyectable, aparece la mezcla **Carprofeno o una de sus sales farmacéuticamente aceptables, un poloxamero y agua;** ahora veamos en las anterioridades citadas si aparecen las composiciones inyectables acuosas en D1[EP0955063], se enseña composiciones inyectables que comprenden **Un agente analgésico, un poláxamero y agua,** o sea, esta anterioridad ya había presentado la solución técnica, de presentar un agente analgésico antiinflamatorio con los excipientes más adecuados un poloxámero y

agua; en el que inicialmente se prueba expresamente que los compuestos antiinflamatorios pueden ser usados ventajosamente en combinación con poloxámeros para llegar a soluciones inyectables, el hecho que luego se le midan propiedades inherentes a dichas composiciones así obtenidas, como por ejemplo estabilidad a la luz, al aire, estabilidad a la "temperatura ambiente" de determinado lugar, eso no es ningún aporte tecnológico, y tal como se discutió arriba, se reitera la solicitud no presenta un aporte técnico real que enriquezcan el acervo tecnológico, lo que se aprecia es que la presente solicitud es una ejecución particular de la invención presentada en D1.

ARGUMENTOS DEL SEÑOR MEMORIALISTA

Las composiciones acuosas inyectables de la presente invención son para el suministro de analgesia en animales pequeños, especialmente animales de compañía, y en particular perros. Asi entonces, teniendo en cuenta los argumentos arriba mencionados es claro que una persona versada en la materia buscando proporcionar una nueva preparación inyectable comprendiendo carprofeno para el suministro de analgesia en animales pequeños, y en particular en perros no se verla motivada a partir de las composiciones reveladas en DI, toda vez que las mismas contienen ibuprofeno, por cuanto dicho compuesto es toxico en perros y por ende no solucionaría el problema técnico planteado en la presente solicitud.

Lo anterior, es aun más evidente si se tiene en cuenta que el ibuprofeno y el carprofeno actúan de forma muy distinta cuando son empleados como NSAID en formulaciones para perros. De tal manera que, no sería obvio para una persona versada en la materia reemplazar el ibuprofeno de DI por carprofeno para obtener la presente invención.

En efecto, es importante resaltar que al ser empleado el carprofeno como ingrediente activo(antiinflamatorio) en las composiciones reveladas en DI en lugar de iburpofeno, las composiciones obtenidas no seria adecuadas para ser administradas por inyección y , por tanto, no se obtendria la presente invención. Para demostrar lo anterior, se presentan a continuación los efectos obtenidos con composiciones de acuerdo con DI al reemplazar el ibuprofeno por carprofeno:

- Formula.
Carprofeno 5% Propilen
glicol 20% Copolimero A
22% Copolimero B 5% Agua
purificada q.s 100%.

En este ensayo, no se adicionó Cloruro de sodio ya que dicho compuesto tiene a aumentar la viscosidad de la solución. En todo caso, el producto ya era una pasta semi / gel a temperatura ambiente con excesiva formación de espuma lo cual lo hace inadecuado para la administración por inyección. Adicionalmente, el carprofeno no se disolvió completamente en esta formula.

De otro lado, el ejemplo 6 de lugar de DI se repitió utilizando carprofeno en ibuprofeno.
- Formula.

Carprofeno 1%
Propilen glicol 20%
Copolimero A 15%
Copolimero B 10%
Agua purificada q.s 100%.

En este caso, en la solución encontraba en composición obtenida el carprofeno no se La formulación formo espuma excesivamente incluso cuando se agito brevemente. Una formula de gel (como gel de manos) se obtuvo con burbujas de aire que dificiles de disipar. Una vez más, estas características de la formulación

la harían inadecuada para su administración por inyección.

En contraste, la presente invención cuando es administrada subcutáneamente lleva a picos de niveles de plasma de carprofeno 2.5 a 3.5 horas luego de su administración (ver tabla 1 de la solicitud) . Esto es critico debido a que el carprofeno inyectable es recomendado para el control de dolor post operatorio. Como se menciona en la descripción, productos inyectables de carprofeno conocidos en el estado del arte comercialmente son conocidos como Rimadil inyectable el cual es adecuado para administración intravenosa (ver figura 1-comparación farmacocinética). Simismo, dicho producto se formula para el alivio del dolor e inflamación asociado con osteoartritis y para el control de dolor post operatorio asociado con tejido suave o cirugías ortopédicas en perros. Para el control de dolor post operatorio, Rimadil es administrado aproximadamente 2 horas antes del procedimiento.

Asi entonces, es fundamental alcanzar los niveles circulantes de carprofeno rápidamente y no como se describe en DI a través de un gel de liberación lenta. Tales composiciones, si se administran por via subcutánea no alcanzarían los niveles terapéuticos en el tiempo requerido para una terapia eficaz. Por tanto, una persona versada no se verla motivada a reemplazar le ingrediente activo de las composiciones divulgadas en DI por carprofeno con el fin de obtener la solución inyectable acá reivindicada, toda vez que se obtendrían composiciones de carprofeno de administración paranteral para caninos para el alivio del dolor post operatorio por medio de liberación lenta.

Finalmente, en forma respetuosa considero que en el presente caso el Examinador no pudo reconocer la actividad inventiva involucrada en el desarrollo, especialmente porque entendió en forma equivocada que al reemplazar el ingrediente activo de las composiciones reveladas en DI se obtendrían la solución inyectable acá reivindicada y sus efectos ventajosos, cuando la anterioridad no se refiere al mismo tipo de composiciones que como las acá reivindicadas y no solucionan elproblema técnico planteado.

Visto lo anterior, atentamente solicito a ese Despacho tener en cuenta los argumentos arriba mencionados y también los resultados de prueba adicionales que se presentan anexo al presente memorial, en los cuales se demuestra la estabilidad mejorada de las composiciones reivindicadas en la presente solicitud, de tal forma que al' efectuar un un examen de nivel inventivo riguroso y de conformidad con las disposiciones del Manual para el Examen de Solicitudes de patente, se advierta la no-obviedad de la materia reivindicada en la presente solicitud.

Por lo expuesto anteriormente, no puede concluirse que antes de la fecha de prioridad reivindicada en la solicitud, resultara obvio para una persona versada en la materia obtener soluciones inyectables de carprofeno (de liberación rápida) estables, a partir de la combinación de las enseñanzas reveladas en los documentos DI y D2.

5.1.2 Con respecto a las reivindicaciones de método.

DI describe dos métodos para producir las composiciones:

1) "El Proceso frío" (ver párrafo 0016 Di). Este método requiere poloxámeros para ser disueltos en agua desde 0 hasta 25°C. Componentes insoluoles pueden ser disueltos en un solvente orgánico como etanol, i-propanol y propilenglicol, y luego puede ser combinado homogéneamente con la fase acuosa".

1) "El proceso caliente" (ver párrafo 0017 DI) . En este método los copolímeros son disueltos como en el proceso frío pero usando una temperatura entre 60 a 100°C, y la solución es enfriada a 25°C.

2) En ambos procesos, agentes activos que no son solubles en agua son disueltos en solventes orgánicos antes de la mezcla con agua para formar composiciones inyectables.

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1) A diferencia de lo anterior, sorprendentemente aunque el ibuprofeno y caprofeno tienen solubilidades en el agua parecidas, menor a lmg/ml, en el proceso divulgado en DI se debe disolver primero el ibuprofeno en un solvente orgánico, y posteriormente el agua es adicionada debido a que el ibuprofeno (al igual que el carprofeno) es insoluble en agua. Sin embargo, en la presente invención se ha encontrado inesperadamente que el carprofeno, polxámero y agua pueden ser adicionados simultáneamente a temperatura ambiente y ser mezclados hasta formar una solución homogénea. De modo que, dicho procedimiento se diferencia de los procedimientos usuales de preparación de soluciones, toda vez que se ha superado un prejuicio en el campo de la técnica a este respecto, lo cual es un indicio de su nivel inventivo en los términos del Manual Andino de Patentes.

§ RESPUESTA TÉCNICA

Ya se ha ilustrado lo suficientemente al señor memorialista respecto a la forma juiciosa, con argumentos técnicos y como se realizó el análisis de patentabilidad de la solicitud, entonces se debe tener claro que **las características técnicas de la composición inyectable están establecidas por sus elementos constitutivos propios, para el presente caso son Carprofeno o una de sus sales farmacéuticamente aceptables, un poloxamero y agua que le permiten como primera medida esa forma de presentación y segundo esa forma de administración, en D1 los elementos constitutivos de la solución inyectable son, analgésico antiinflamatorio, un poloxamero y agua, que de igual manera le confiere todas sus propiedades inherentes que puedan ser medibles y esos datos no son un aporte tecnológico,** respecto a los datos reportados del principio activo Carprofeno, son todos los datos normales que por regla general se deben conocer del principio activo en la preformulación, que tampoco son un aporte técnico, ya que se debe conocer todas las propiedades y todas las constantes de cualquier sustancias que se involucra en una composición farmacéutica, lo cual es una práctica normal y por demás obligatoria cuando se hace cualquier tipo de formulación, no solamente de inyectables, eso tampoco es un aporte tecnológico de una invención, lo que sí debe quedar perfectamente establecido es que D1 efectivamente presentó originalmente el aporte técnico al acervo tecnológico, donde las soluciones inyectables acuosas comprenden polaxámeros y agentes antiinflamatorios que pueden ser producidos exitosamente para proporcionar la misma solución técnica planteada en la presente solicitud, pero la presente solicitud es solamente partiendo de la solución técnica presentada en D1 y tomado un antiinflamatorio particular el Carprofeno, sin que se pueda evidenciar un aporte técnico que enriquezca el acervo tecnológico, **ya que realmente *la presente solicitud* es una ejecución particular de la invención presentada en D1**, al igual que lo son las composiciones acuosas inyectables de D1 en donde el agente antiinflamatorio particular es Ibuprofeno.

Sí erróneamente se concede el privilegio solicitado para la materia contenida en las reivindicaciones tal como está la presente solicitud, se le confiere es el derecho sobre un desarrollo tecnológico original de D1, por el mero hecho de ser una ejecución particular de este. Además la divulgación del estado de la técnica puede no haber sido hecha "*expressis verbis*" en las anterioridades, sino estar incluida en el espíritu de la invención, porque no se trata de un planteamiento "fotográfico" como si el experto en el arte no fuese capaz de interpretarla, ya que lo que *se está comparando son cuestiones puramente técnicas*, *no se está comparando material de obras literarias*.

Por lo tanto el objeto de la solicitud cumple no con los requisitos de patentabilidad y de acuerdo a lo sustentado arriba, se reitera lo expresado en el examen de fondo definitivo, donde se recomienda negar el privilegio solicitado en las reivindicaciones,
1 a 13 folios 150 y 151.

Examinador Técnico Código 202012

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