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Act. 444 RESPUESTAREQUE Folios: 25

Clarke, Modet & C^o
COLOMBIA

ESCOBAR - URIBE ASOCIADOS
Intellectual Property

2552/P

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D.

Re: Expediente No. **03-039.889**
Solicitud de patente a nombre de **Novartis AG**

Yo, JIMENA ESCOBAR URIBE, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 A No. 86-53, piso 6, obrando como apoderada de la sociedad **Novartis AG**, domiciliada en Basilea, Suiza, solicitante de la patente de la referencia, en respuesta al oficio No. 13566 notificado por fijación en lista el 26 de octubre de 2007, relacionado con el concepto técnico de fondo No. 1862, me permito informar lo siguiente:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio en donde se realizaron las siguientes modificaciones:

1.1 Se cancelaron las antiguas reivindicaciones 6, 7, 8, 9 y 10 que hacían mención a un estuche para la prevención, demora de progreso o tratamiento y uso de la combinación del inhibidor de reina de la fórmula (I).

1.2 Se modificaron las antiguas reivindicaciones 1 a 5 a fin de especificar los componentes establecidos desde la página 2 hasta la página 12 de la memoria descriptiva de la solicitud en estudio.

Para este efecto, me permito acompañar el recibo No. 08-5,797 del 22 de enero de 2008, con el cual queda cubierta la tasa correspondiente a la modificación, la cual presento junto con este memorial.

2. Claridad de la solicitud

Su despacho afirma que se carece de claridad y concisión para describir la materia a proteger dentro de la descripción. Sobre este particular, me permito manifestar que el aporte hecho por la presente invención radica en la administración combinada del inhibidor de renina de la fórmula (I) o una sal del mismo, con un agente terapéutico seleccionado a partir del grupo que consiste en (i) a (ix), lo cual da como resultado tanto un efecto terapéutico benéfico, en especial sinérgico, como también beneficios adicionales que resultan del tratamiento combinado, cuando se compara con monoterapias que aplican solamente uno de los compuestos farmacéuticamente activos utilizados en las combinaciones dadas a conocer en la presente.

De hecho, las combinaciones de la invención en cuestión muestran sinergismo entre el inhibidor de renina y uno de los componentes mostrados en la solicitud cuando se aplican para contrarrestar enfermedades cardiovasculares.

En este orden de ideas, la presente invención a través de su descripción señala la necesidad de combinaciones sinérgicas del inhibidor de renina junto con otro componente para su participación en la prevención y tratamiento de infarto de miocardio, como así se muestra a través del diseño de estudio consignado en la página 19 línea 16 hasta la página 28, línea 22 de la descripción.

En consecuencia, sí resulta claro que la invención en su descripción, señala de manera suficiente tanto la necesidad en la técnica por combinaciones sinérgicas como la solución dada mediante el inhibidor de renina junto con un compuesto como un antagonista del receptor AT1 y un inhibidor HMG-Co-A reductasa, entre otros con un efecto sinérgico y beneficios adicionales. ✓ ✓

De otra parte, respecto al término “una sal farmacéuticamente aceptable”, es necesario señalar que dicha expresión resulta clara para una persona versada en la materia, toda vez que entenderá que los componentes de la combinación pueden existir como por ejemplo en la forma de clorhidratos. En este sentido, dirijo la atención del examinador a la memoria descriptiva, en especial a la página 9, línea 17 donde se ilustra que para el inhibidor de aldosterona-sintasa no esterooidal, se prefiere el enantiómero (+) del clorhidrato del fadrozol. Por lo cual, una persona medianamente versada en la materia entenderá que la presente invención también puede llevarse a cabo con sales aceptables de manera farmacéutica tal como clorhidratos.

En cuanto a que se encuentran demasiados grupos farmacológicos y demasiadas variantes de principios activos, me permito decir que como así se expresa en la reivindicación 1, la combinación es entre el compuesto de fórmula (I) y al menos un componente de la lista de nueve opciones que mostrará entonces una característica de efecto de sinergismo en su combinación. Por lo tanto, no se considera realizar una limitación adicional a esta reivindicación.

Aún más, la reivindicación 2 y 3 son limitaciones de la reivindicación 1, toda vez que señalan cuales son los compuestos específicos para cada uno de los compuestos enlistados en la reivindicación principal. Así las cosas, no se están reivindicando "demasiados" grupos farmacológicos como lo señala ese despacho sino que se enseña de manera expresa cuales son las combinaciones para las cuales se presenta el efecto de sinergia.

3. Unidad de invención

Respecto a la antigua reivindicación 7 que hacía referencia al estuche, me permito decir que gracias a la cancelación de la misma del pliego de reivindicaciones, se subsana la objeción de unidad de invención expuesta por su despacho.

4. Novedad

Ese despacho afirma que la presente invención no posee novedad en vista de los documentos D1 (WO91/17771) y D2 (WO92/10097).

En este sentido, me permito señalar que el artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina establece que la invención se considera como nueva cuando no está comprendida en el estado de la técnica. Si de acuerdo con el espíritu de la norma legal indicada, la invención no se considera como nueva si está "comprendida" en el estado de la técnica, dicha norma se está refiriendo, naturalmente, a la totalidad del invento y no a alguno o a algunos de los componentes.

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

Esto se conoce como el concepto de **NOVEDAD ABSOLUTA**, de conformidad con lo cual para desvirtuar el requisito de novedad de una invención será necesario que lo reivindicado por la invención bajo estudio corresponda de forma total con lo revelado en un documento o

anterioridad señalada, conclusión a la que se llega después de un análisis exhaustivo mediante una comparación entre el capítulo reivindicatorio de la invención en estudio y la divulgación por separado de cada una de las referencias D1 y D2.

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

Por lo tanto, de lo anterior se entiende que como mínimo la novedad debe contemplar los siguientes aspectos:

-Que la invención en estudio esté contenida de forma **TOTAL y ABSOLUTA** en el documento que se menciona como anterioridad o de forma independiente en los documentos que se mencionan, si son varios.

-Que la invención en estudio puede partir de conocimientos conocidos, caso en el cual habrá que hacer la comparación no de lo conocido sino de lo inédito y novedoso, teniendo que analizarse el **valor agregado** que aporta la invención con sus nuevas características al campo técnico al cual pertenece la invención en estudio.

En el caso en concreto, para que las razones que aduce ese despacho para desvirtuar la novedad fuesen ciertas, sería forzoso que se demostrara que la invención en cuestión esté comprendida en el estado de la técnica de forma **TOTAL**, lo cual no sucede como ahora se demostrará.

4.1 Novedad de la solicitud en estudio sobre lo divulgado en el documento WO 91/17771 (D1).

Como bien lo aduce su despacho, D1 revela composiciones y métodos para la mejora del efecto terapéutico de la presión arterial y la insuficiencia cardíaca. De hecho, D1 señala que puede haber composiciones sinérgicas comprendidas por cantidades de por lo menos dos agentes activos seleccionados de un inhibidor de renina, un inhibidor de la enzima convertidora de angiotensina I y un antagonista de angiotensina II.

De hecho, en la tabla que aparece en el folio 188 del concepto técnico, se establece que D1 únicamente está mostrando un inhibidor de renina y un

antagonista del receptor AT1 y un inhibidor de enzima convertidora de angiotensina, pero sin embargo las otras características de la invención en cuestión no las nombra. Por lo tanto, D1 no tendría porqué afectar de manera negativa la novedad de la presente invención, pues no contiene ni revela la totalidad de la materia reivindicada en la solicitud en cuestión.

4.2 Novedad de la solicitud en estudio sobre lo divulgado en el documento WO 92/10097 (D2).

Por su parte, D2 se encuentra dirigida a composiciones que se encuentran constituidas por un antagonista del receptor de angiotensina II en un vehículo junto con diuréticos y bloqueadores del canal de calcio entre otros. No obstante, teniendo en cuenta nuevamente lo mencionado por el señor examinador en la tabla del folio 118, donde D2 únicamente enseña dos aspectos comunes con la presente invención, se puede afirmar que el examinador se encuentra consiente que no todos los aspectos de la invención en estudio son revelados por este documento. Por lo cual, debería entonces reconocer que la solicitud estudiada honesta en su totalidad en la técnica anterior y así pues debe entonces considerar su posición en cuanto a la evaluación de la novedad del presente invento.

En este sentido, resulta pertinente señalar que según los lineamientos de la Comunidad Andina esbozados en el *"Manual para el examen de Solicitudes de Patentes de Invención en las Oficinas de Propiedad Industrial de los Países de la Comunidad Andina"* del 2004, y como se manifestó anteriormente, una solicitud no es nueva cuando se encuentran explícitamente todas sus características dentro del estado del arte.

En efecto, el citado manual en su numeral 9.4 respecto a la novedad manifiesta que: *"Para determinar la novedad de la invención, se debe comprobar si existen anticipaciones del estado de la técnica que contengan explícitamente todas las características técnicas esenciales de la invención. El examen de novedad se efectúa comparando elemento por elemento de la invención tal como está definido por las reivindicaciones con los del estado de la técnica, "(El subrayado es mío).*

Así las cosas, si bien el señor examinador realizó una comparación de elemento por elemento de la invención en estudio con lo revelado en D1 y D2, también es cierto que como él mismo establece en la tabla del folio 118, ninguno de los dos documentos comprende explícitamente todas las características de la presente solicitud.

En ese orden de ideas, el Manual también muestra como se hace necesario el hecho de que un solo documento sea el que contenga todas las características de la invención en estudio, cuando dice que: *"Se deberá comparar las reivindicaciones independientes de la solicitud con el contenido de cada antecedente del estado de la técnica, uno a*

uno, a fin de determinar si un antecedente por sí solo describe las características técnicas contenidas en dichas reivindicaciones" (El subrayado es mío).

Por lo tanto, sería forzoso que se demostrara que un solo documento de D1 ó D2 enseñara todas las características de la invención en estudio. Así las cosas, se solicita de manera atenta se reconsideren las objeciones de novedad y se acepte dicho requisito para la solicitud en cuestión.

5. NIVEL INVENTIVO

Según lo estipulado en el Artículo 18 de la Decisión 486 *"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."*

Respecto a las combinaciones del compuesto de la fórmula (I) con por lo menos uno de los componentes enlistados, estas no habrían sido evidentes para un persona medianamente versada en la técnica, toda vez que nada en los documentos mencionados como anterioridades sugiere que combinaciones de este estilo puedan presentar todas, un efecto de sinergia cuando se mezclan.

En este sentido, es deseo del solicitante poner en consideración del señor examinador el artículo: "Aliskiren, a novel, orally effective renin inhibitor, lowers blood pressure in marmosets and spontaneously hypertensive rats" publicado en Journal of Hipertensión 2005, 23: 417-426, el cual se debe tener en cuenta como una evidencia de que la presente invención de hecho se encuentra basada en un concepto inventivo.

En efecto, la publicación en referencia claramente demuestra que una combinación de aliskiren (compuesto de la fórmula I) de la reivindicación independiente 1, junto con el benazepril inhibidor de ACE (reivindicación 3) y el antagonista del receptor de angiotensina II valsartan (reivindicación 5) muestra un efecto de sinergismo en el tratamiento de la hipertensión en títis y ratas espontáneamente hipertensos. En este sentido, es importante señalar que el benazepril es un representante de la clase de inhibidores de ACE, en tanto que el valsartan es un representante de la clase de antagonistas del receptor de angiotensina II.

Ambos modelos animales resultan ser relevantes desde el punto de vista clínico ya que se ha demostrado en ensayos clínicos con monoterapias con aliskiren, como así se establece en la página 425 columna derecha segundo párrafo de la publicación en mención. En este artículo se puede evidenciar el efecto de sinergia en las figuras 6 y 7 más específicamente en las gráficas a y c de ambas figuras donde se muestra que la combinación (cuadrados sin relleno) muestran

una reducción en el cambio del promedio de la presión arterial en milímetros de mercurio (mmHg). Esto también se puede constatar en el último párrafo de la página 423 y que continúa en la página 424.

Por lo tanto, este artículo resulta ser un indicio más sobre la solución al problema de nuevas combinaciones del inhibidor de renina con otros compuestos que mostrarán efectos sinérgicos en tratamiento de enfermedades cardiovasculares.

Aún más, en esta publicación las combinaciones de acuerdo con la presente invención muestran además una característica sorprendente en términos de prolongación del efecto, toda vez que en las gráficas a y c de las figuras 6 y 7 se puede ver que dicho efecto se mantiene desde el día 2 hasta el día 15 a 16 de la terapia. En este sentido, de las gráficas se puede concluir que por ejemplo en el día 15 del tratamiento, el cambio en el promedio de la tensión arterial (MAP) para el aliskiren ó el benazepril o el valsartan, respectivamente se encuentra cerca del control, en tanto que las correspondientes combinaciones aún muestran a este mismo tiempo, una reducción significativa de la MAP.

Por lo tanto, no cabe duda que efectos correspondientes también pueden ser alcanzados con los otros representantes de los inhibidores de ACE o antagonistas del receptor de angiotensina II, entre otros.

En este sentido, otra publicación que brinda soporte inventivo a las combinaciones sinérgicas de la invención en estudio, es el póster "*The novel oral renin inhibitor aliskiren decreases plasma renin activity (PRA) and neutralizes hydrochlorothiazine-induced RAAS activation in hypertensive patients*" que es un estudiopresentado ante la American Society of Hipertensión (ASH) en la conferencia del 16 al 20 de mayo de 2006 en Nueva York, Estados unidos.

En dicho póster, se ilustran los efectos de la combinación del inhibidor de aliskiren de la fórmula (I) y un diurético, principalmente el llamado hidroclorotiazida sobre la actividad de la renina en plasma y la concentración de renina en pacientes hipertensos. Ambos son biomarcadores para los efectos antihipertensivos como se explica en la introducción del póster. El examinador lo puede constatar en el aspecto segundo y cuarto de la introducción mostrada en dicho póster.

En la parte de conclusiones, se muestra cómo resulta sorprendente que la combinación de estos componentes no sólo reduce la actividad de renina en plasma sino que también eleva la concentración de renina (RI) de manera sinérgica. Esto claramente indica un efecto sinérgico sobre la estimulación de la liberación de renina, lo cual se puede apreciar en más detalle en las figuras 2 y 3, respectivamente donde estos efectos son ilustrados.

En conclusión se puede decir que en la reducción en la actividad de la renina en plasma (PRA) y el incremento drástico en la concentración de renina (RI), el compuesto de la fórmula (I) complementa de manera muy favorable la terapia del diurético y conlleva a un bloqueo más potente del sistema de RAAS en pacientes hipertensos. Estos efectos positivos resultan ser totalmente inesperados y no habrían podido ser de ninguna manera anticipados a partir del arte previo enseñado antes de estos estudios.

Resumiendo, ese despacho puede constatar que ciertamente en ninguna parte de los documentos citados como anterioridades relevantes D1 y D2 se sugiere o se halla un indicio que llevara de manera evidente a la persona versada en la materia a las novedosas combinaciones mostradas en la invención en estudio.

Así las cosas, me permito solicitar a ese despacho aceptar las modificaciones realizadas y retirar las objeciones de novedad y nivel inventivo presentadas en su concepto técnico no. 1862 y conceder el privilegio de patente a la solicitud en estudio.

Señor Superintendente,

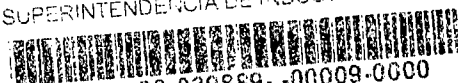

JIMENA ESCOBAR URIBE
C.C.39.779.452 de Usaquén
T.P. 68228
Cód. 5376

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 5,797
FECHA : ENERO 22 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Act 444 RESPUESTAREQUE Folios: 25

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CLARKE MODET Y CO. COLOMB	CONSIGNACION	BANCO DE BOGOTA	062754387	83221	22/01/2008	38,815,000.00

***** CONCEPTO *****

CONCEPTO	TOTAL CONCEPTO
1. RENTISTICO	
1 50005-01-03 CAMBIO DE MODALIDAD Y MODIFICA	
12 MARCAS Y LEMAS COMERCIALES	103,000.00
TOTAL :	\$ 103,000.00

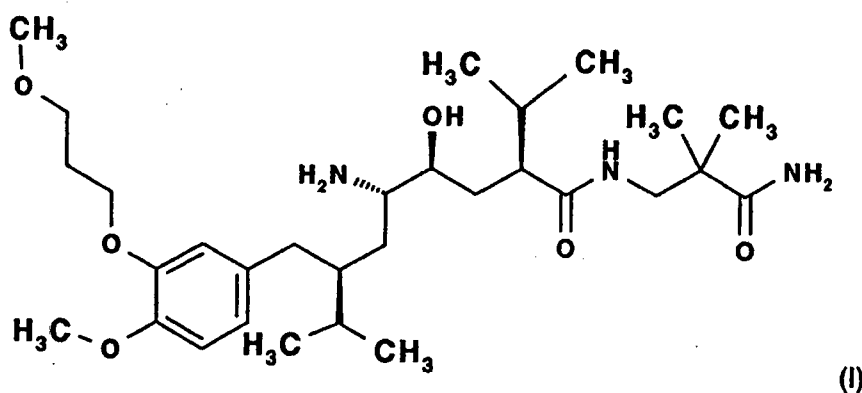
SON: CIENTO TRES MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

REIVINDICACIONES

1. Una composición farmacéutica que comprende el inhibidor de renina de la fórmula (I):



o una sal farmacéuticamente aceptable del mismo, y al menos un agente terapéutico seleccionado a partir del grupo que consiste de:

(i) un antagonista del receptor AT₁ o una sal farmacéuticamente aceptable del mismo,

(ii) un inhibidor de HMG-Co-A reductasa o una sal farmacéuticamente aceptable del mismo,

(iii) un inhibidor de la enzima convertidora de angiotensina (ACE) o una sal farmacéuticamente aceptable del mismo,

(iv) un bloqueador del canal de calcio una sal farmacéuticamente aceptable del mismo,

(v) un inhibidor de aldosterona-sintasa o una sal farmacéuticamente aceptable del mismo,

(vi) un antagonista de aldosterona o una sal farmacéuticamente aceptable del mismo,

(vii) un inhibidor doble de la enzima convertidora de angiotensina/endopeptidasa neutra (ACE/NEP) o una sal farmacéuticamente aceptable del mismo,

(viii) un antagonista de endotelina o una sal farmacéuticamente aceptable del mismo, y

(ix) un diurético o una sal farmacéuticamente aceptable del mismo;

y un portador.

2. Una composición de acuerdo con la reivindicación 1, en donde el inhibidor de renina está presente en la forma del hemi-fumarato.

3. Una composición de acuerdo con las reivindicaciones 1 ó 2, en donde, independientemente unos de otros,

- el antagonista del receptor AT_1 es valsartan o una sal farmacéuticamente aceptable del mismo;

- el inhibidor de HMG-Co-A reductasa es fluvastatina, pitavastatina ó atorvastatina o una sal farmacéuticamente aceptable de las mismas;

- el inhibidor de la enzima convertidora de angiotensina (ACE) es benazepril o enalapril, o una sal farmacéuticamente aceptable de los mismos;

- el bloqueador del canal de calcio es amlodipina o verapamil ó una sal farmacéuticamente aceptable de la misma;

- el inhibidor de aldosterona-sintasa es fadrozol, o una sal farmacéuticamente aceptable del mismo;
 - el antagonista de aldosterona es eplerenona;
 - el inhibidor doble de enzima convertidora de angiotensina/endoropeptidasa neutra (ACE/NEP) es omapatrilato, fasidotril o fasidotrilato o Z13752A o una sal farmacéuticamente aceptable del mismo;
 - el antagonista del endotelina es bosentan, enrasentan, atrasentan o una sal farmacéuticamente aceptable del mismo; y
 - el diurético es clorothiazida, hidroclorotiazida, methylchlorotiazida y clorotadilon o una sal farmacéuticamente aceptable del mismo;
- y un portador.

4. Una composición de acuerdo con cualquiera de las reivindicaciones 1 a 3, caracterizada porque comprende el compuesto de la fórmula (I) y valsartan o una sal farmacéuticamente aceptable de los mismos.

5. Una composición de acuerdo con la reivindicación 4, caracterizada porque el compuesto de la fórmula (I) está en la forma de la sal de hemi-fumarato y junto con valsartan.

ANEXO 1

“Aliskiren, a novel, orally effective renin inhibitor, lowers blood pressure in marmosets and spontaneously hypertensive rats” publicado en Journal of Hipertensión 2005, 23: 417-426

Aliskiren, a novel, orally effective renin inhibitor, lowers blood pressure in marmosets and spontaneously hypertensive rats

Jeanette M. Wood^a, Christian R. Schnell^a, Frederic Cumin^a, Joël Menard^b and Randy L. Webb^c

Objectives Aliskiren is a new renin inhibitor of a novel structural class that has recently been shown to be efficacious in hypertensive patients after once-daily oral dosing. We report the results of animal experiments performed in marmosets and rats in order to characterize aliskiren before its recent investigation in humans.

Methods The effects of aliskiren were investigated in sodium-depleted marmosets (oral dosing) and in spontaneously hypertensive rats (dosing via subcutaneous osmotic minipumps). Blood pressure (BP) and heart rate were measured by radiotelemetry.

Results In sodium-depleted marmosets, single oral doses of aliskiren (1–30 mg/kg) dose-dependently lowered BP. At a dose of 3 mg/kg, peak effects were observed 1 h after dosing (-30 ± 4 mmHg, $n = 6$) and the response persisted for more than 12 h. A single oral dose of 3 mg/kg aliskiren was more effective than the same dose of either remikiren or zankiren, two orally active renin inhibitors previously tested in humans. Aliskiren (10 mg/kg) was at least as effective as equal doses of the AT₁-receptor blocker valsartan or the angiotensin-converting enzyme inhibitor benazepril. In spontaneously hypertensive rats, aliskiren dose-dependently (10–100 mg/kg per day) decreased BP. Aliskiren also potentiated the antihypertensive effects

of low doses of valsartan or benazepril (1 or 3 mg/kg per day).

Conclusions Aliskiren is an orally effective, long-lasting renin inhibitor that shows antihypertensive efficacy in animals superior to previous renin inhibitors and at least equivalent to angiotensin-converting enzyme inhibitors and AT₁-receptor blockers. Aliskiren may therefore represent an effective, novel approach to the treatment of hypertension and related disorders, alone or in combination with other antihypertensive agents. *J Hypertens* 23:417–426 © 2005 Lippincott Williams & Wilkins.

Journal of Hypertension 2005, 23:417–426

Keywords: renin, marmoset, blood pressure, hypertension, telemetry

^aNovartis Institute for BioMedical Research, Basel, Switzerland, ^bINSERM U367, Paris, France and ^cNovartis Institutes for BioMedical Research, East Hanover, New Jersey, USA.

Sponsorship: This study was supported by Novartis Pharma AG.

Potential conflicts of interest: J.M.W., C.R.S., F.C. and R.L.W. are employees of Novartis Pharma AG.

Correspondence and requests for reprints to Randy L. Webb, Ph.D., Executive Director, Cardiovascular Research, Novartis Institutes for BioMedical Research, One Health Plaza, East Hanover, NJ 07936, USA. Tel: +1 862 778 0451; fax: +1 973 781 2713; e-mail: randy.webb@pharma.novartis.com

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Introduction

The renin–angiotensin system (RAS) is a key regulator of blood pressure (BP) and body fluid volume, acting primarily via the effects of the octapeptide hormone, angiotensin (Ang) II. The formation of Ang II involves two main steps: the aspartyl protease renin cleaves angiotensinogen to form the decapeptide Ang I, and Ang I is then converted by angiotensin-converting enzyme (ACE) into Ang II. *In vitro* conversion of angiotensinogen to Ang I is also mediated by other enzymes, such as cathepsin D, although at a rate several orders of magnitude slower than renin [1]. RAS activity is a major underlying determinant of many pathological states, because Ang II increases BP and aldosterone levels and has direct growth-promoting

effects on cardiac, vascular and renal tissue that contribute to end-organ damage [2,3]. Indeed, RAS inhibitors such as ACE inhibitors and AT₁-receptor blockers have proven to be highly successful treatments for disorders such as hypertension, heart failure and left ventricular dysfunction after myocardial infarction [4–6] and renal conditions [7,8].

Renin inhibitors would similarly be expected to lower BP and provide organ protection benefits in humans because they target the first step of the RAS, and thereby block the pathway at its source [9,10]. Moreover, the fact that renin catalyses the initial rate-limiting step of the RAS, and that angiotensinogen is its only naturally occurring substrate [11], means that renin inhibitors offer the potential for highly specific RAS blockade with a low likelihood of side effects [12].

Previous presentation: Some of the work in this article has been presented previously as an abstract.

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A combination of molecular modelling techniques with crystal structure elucidation has recently led to the development of the alkane carboxamide renin inhibitor, aliskiren [2(S),4(S),5(S),7(S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)-phenyl]-octanamide], a potent inhibitor of human renin activity *in vitro* [13]. Species differences in the amino acid sequence of renin and angiotensinogen make human renin inhibitors relatively weak inhibitors of renin isoforms from non-primate species [9,10]. Aliskiren inhibits human, marmoset and rat plasma renin with IC_{50} values of 0.6, 2 and 80 nmol/l, respectively [14]. These characteristics have allowed its biological properties to be evaluated in two different species, sodium-depleted marmosets (oral administration), and spontaneously hypertensive rats (SHR) (subcutaneous administration by osmotic mini-pumps).

The aim of the preclinical development of aliskiren was to investigate its ability to lower BP in these two animal models and to compare its effects on BP and heart rate (HR) — measured by telemetry — with two renin inhibitors, remikiren [15] and zankiren [16], the clinical development programs for which were discontinued for reasons of insufficient efficacy during repeated oral administration. The effects of aliskiren were also compared with an ACE inhibitor, benazepril, and an AT_1 -receptor blocker, valsartan. The antihypertensive effects of combinations of aliskiren with an ACE inhibitor and an AT_1 -receptor blocker were also investigated in SHR.

Methods

Marmoset studies

Marmosets (*Callithrix jacchus*) of both sexes weighing approximately 350 g were used (Ciba-Geigy, Sisseln, Switzerland) [17]. Animals were kept in a room ventilated with air maintained at a temperature of 24–27°C, a humidity of 50–80% and a 12-h light/dark cycle. BP and HR were measured by telemetry in conscious marmosets moving freely in their home cages, as described previously [18]. Briefly, pressure transmitters (AM Unit, model TA11PA-C40; Data Sciences Inc., St Paul, Minnesota, USA) were implanted into the peritoneal cavity under aseptic conditions and light anaesthesia (combination of 10 mg/kg alfaxalone, 0.15 mg/kg atropine, and 0.75 mg/kg diazepam, intramuscularly). The sensor catheter was placed in the aorta below the renal artery pointing upstream. Only male marmosets were implanted with the telemetry transmitters, and were allowed to recover for at least 4 weeks before any experiment was started. The marmosets were kept in single male/female pairs.

Marmosets were maintained on a low-sodium diet (pellets with 9 mmol/kg Na^+ and 128 mmol/kg K^+ ; NAFAG, Gossau, Switzerland) supplemented with fruit and with free access to water for 1 week before and during a BP

experiment. Marmosets received the diuretic furosemide in their drinking water (6 mg/kg per day) 2 days before starting an experiment, and a bolus dose of furosemide (9 mg/kg, intramuscularly) was administered 20 h before drug administration.

Aliskiren (hydrochloride salt) was given to sodium-depleted marmosets orally by gavage as a single dose of 0.3, 1, 3 or 10 mg/kg. Vehicle (distilled water) was given as a control. Two renin inhibitors, remikiren and zankiren, were administered as single 3 mg/kg doses, whereas the ACE inhibitor benazepril and the AT_1 -receptor blocker valsartan were administered as single 10 mg/kg doses. Measurements were made for 48 h before, and up to 24 h after, drug administration. No recordings were made for 30 min after dosing in order to avoid interference from the stress of handling.

Plasma renin activity (PRA) and plasma concentrations of aliskiren were measured in the female marmosets. Distilled water vehicle or aliskiren in doses of 0.3, 1, 3 and 10 mg/kg were given orally by gavage. Blood samples were collected using ethylenediamine tetraacetic acid as an anticoagulant by direct puncture of a femoral vein before dosing, and at 1.5, 3, 6 and 24 h after dosing. PRA was measured by the antibody trapping method [19], and the percentage inhibition of renin activity at each time point was calculated using the pretreatment value for PRA as the baseline. Plasma concentrations of aliskiren were measured using an enzymatic assay [20].

SHR studies

Male adult SHR aged 20–30 weeks (Tac:N(SHR)1B; Taconic Farms, Germantown, New York, USA) were used. SHR were housed in individual cages, maintained on a 12-h light/dark cycle and had access to standard laboratory rat chow and water *ad libitum*. All experiments were performed in accordance with the guidelines set forth by the Novartis Animal Care Committee.

Rats were anaesthetized with sodium amytal (80 mg/kg sodium amobarbital, intraperitoneally) and a fluid-filled telemetric catheter was introduced into the lower abdominal aorta. Rats were allowed to recover for 2 weeks prior to the start of experimentation [21]. Receivers (RA1010; Data Sciences Inc.) allowed measurement of BP and HR, and all data were collected using a computerized data acquisition system (Dataquest IV; Data Sciences Inc.).

Drugs or saline vehicle control were infused subcutaneously via osmotic minipumps (2 ML21; Alza Corporation, Palo Alto, California, USA) for a period of 2 weeks. Two minipumps were used in each animal. Aliskiren (hydrochloride salt) was infused through one minipump at concentrations of 10, 30 and 100 mg/kg per day. Continuous infusion of higher doses of aliskiren was required in the SHR due to the lower potency of aliskiren and less

favourable pharmacokinetics of aliskiren in the rat, compared with the marmoset [14]. Benazeprilat or valsartan were infused at concentrations of 1 or 3 mg/kg per day through the second minipump.

Pharmacokinetic studies

The pharmacokinetics of aliskiren (hydrochloride salt) were studied following intravenous and oral administration in marmosets and rats. Aliskiren was administered to fasting marmosets (weight 300–400 g) either intravenously (at a dose of 3 mg/kg via the femoral vein), or orally (at a dose of 10 mg/kg) by gavage in a 5% dextrose solution. In the rat studies, aliskiren was administered to conscious, fed, male Sprague–Dawley rats (Tif: RA25, weight 250–300 g; BRL, Füllinsdorf, Switzerland) either intravenously (at a dose of 3 mg/kg via the femoral vein), or orally (at a dose of 30 mg/kg) by gavage in a 5% dextrose solution. In both marmoset and rat studies, blood samples were taken from the femoral vein and plasma concentrations of aliskiren were measured using an enzymatic assay [20]. Pharmacokinetic parameters, including the area under the curve ($AUC_{0-\infty}$), elimination half-life ($t_{1/2}$), maximum plasma concentration (C_{max}), plasma clearance (Cl) and steady-state volume of distribution (V_{ss}) were estimated by standard methods after fitting the plasma concentration data to a bi-exponential equation using an iterative least-squares method.

Data analysis and statistics

In the marmoset studies, the mean values of mean arterial BP and HR were calculated over 1-h periods. Delta values of BP and HR were measured using each animal as its own control; values for each time point in the post-administration diurnal profile were subtracted from the corresponding time point in the pre-administration diurnal profile. In SHR experiments, waveforms of BP and HR for each animal were sampled for 10 s at 10-min intervals, and 24-h averages were later derived from these values. Baseline mean arterial pressure (MAP) and HR consisting of the mean of three consecutive 24-h averages, were obtained immediately preceding the 2-week drug infusion. Cardiovascular parameters were measured during infusion of drugs, and for one additional week following completion of drug treatment. Results are expressed as the mean $\pm$ standard error of the mean.

For studies in both species, comparison of peak changes in BP from baseline data were carried out. In the SHR studies, changes in BP from baseline data were also analysed by comparison of the areas over the curve (AOC) as calculated by the trapezoidal method (mmHg $\times$ days). In order to identify possible beyond-additive effects of combination therapy, the AOC values for aliskiren in combination with either valsartan or benazeprilat were also compared with the sums of the AOC values for the respective monotherapies as described previously [22]. Changes in HR were analysed

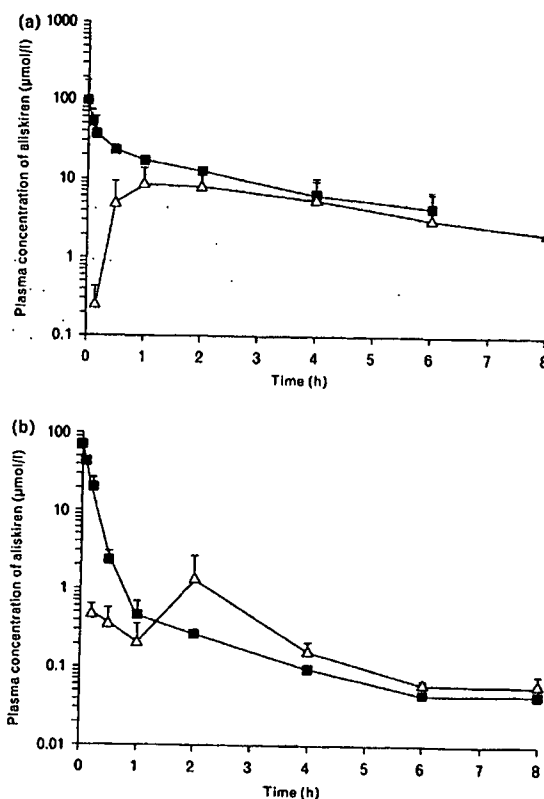
by comparison of the AUCs, calculated according to the trapezoidal method (beats/min $\times$ days). Statistical analysis was performed by a one-way analysis of variance followed by Bonferroni's *post-hoc* test. $P < 0.05$ was considered statistically significant.

Results

Pharmacokinetics of aliskiren in marmosets and rats

Elimination of aliskiren following intravenous administration of a single 3 mg/kg dose to marmosets followed a two-compartment or three-compartment model (Fig. 1a). The $AUC_{0-\infty}$ was $90.2 \pm 16.2 \mu\text{mol/l} \times \text{h}$ (mean $\pm$ standard deviation), and the mean $t_{1/2}$ was $2.1 \pm 0.6 \text{ h}$. Plasma clearance was $0.058 \pm 0.009 \text{ l/h per kg}$ and the volume of distribution was $0.16 \pm 0.04 \text{ l/kg}$. Following

Fig. 1



Plasma concentration–time data following administration of a single intravenous (filled squares) or oral dose (open triangles) of aliskiren in (a) marmosets or (b) rats. The oral dose of aliskiren was (a) 10 mg/kg in marmosets and (b) 30 mg/kg in rats; the intravenous dose was 3 mg/kg in both marmosets and rats. Plasma concentrations of aliskiren were determined by an enzymatic assay, as described in Methods. Data presented as mean $\pm$ standard deviation; note that plasma concentration data are plotted on a logarithmic scale. Estimated pharmacokinetic parameters for aliskiren in marmosets and rats are presented in Results.

oral administration of a single 10 mg/kg dose, aliskiren was slowly absorbed from the gastrointestinal tract, reaching a maximum 1–2 h after administration (Fig. 1a). The $AUC_{0-\infty}$ was $49.0 \pm 30.7 \mu\text{mol/l} \times \text{h}$ and the mean $t_{1/2}$ was $2.3 \pm 1.0 \text{ h}$. The calculated bioavailability was 16.3%.

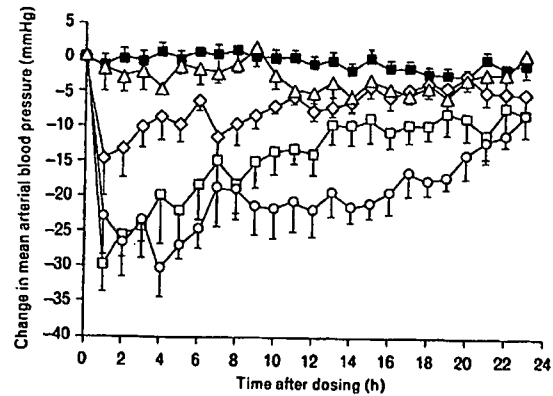
Following intravenous administration of a bolus dose of 3 mg/kg aliskiren to rats, elimination of aliskiren also followed a two-compartment or three-compartment model of elimination (Fig. 1b). The $AUC_{0-\infty}$ was $12.7 \pm 2.6 \mu\text{mol/l} \times \text{h}$, and the mean $t_{1/2}$ was $2.24 \pm 0.47 \text{ h}$. Plasma clearance was $0.41 \pm 0.08 \text{ l/h per kg}$ and the volume of distribution was $0.19 \pm 0.05 \text{ l/kg}$. Following oral administration of a single 30 mg/kg dose, aliskiren was rapidly absorbed from the gastrointestinal tract ($< 25 \text{ min}$), with a second peak of absorption observed 2 h after administration (Fig. 1b). The $AUC_{0-\infty}$ was $3.06 \pm 1.8 \mu\text{mol/l} \times \text{h}$ and the mean $t_{1/2}$ was $1.9 \pm 0.4 \text{ h}$. The calculated bioavailability was 2.4%.

Dose-response of aliskiren in sodium-depleted marmosets

Oral administration of single doses of aliskiren (0.3–10 mg/kg) caused dose-dependent reductions in mean arterial BP in sodium-depleted marmosets (Fig. 2). No significant change in mean arterial BP was observed after administration of either vehicle or aliskiren, 0.3 mg/kg. The threshold dose required to lower mean arterial BP significantly ($P < 0.05$) was between 0.3 and 1 mg/kg, and 1 mg/kg aliskiren lowered BP by a maximum of $14 \pm 5 \text{ mmHg}$ ($n = 6$). The maximum fall in BP of $30 \pm 3 \text{ mmHg}$ ($P < 0.001$ compared with vehicle control) was achieved with 3 mg/kg aliskiren ($n = 6$), and BP was lowered by $> 20 \text{ mmHg}$ for approximately 6 h. With a higher dose of aliskiren, 10 mg/kg, the magnitude of the BP response did not increase, but the duration of the response increased such that BP was lowered by $> 20 \text{ mmHg}$ for approximately 16 h ($n = 6$) compared with the pre-administration diurnal BP profile.

Plasma concentrations of aliskiren increased dose-dependently following oral administration of aliskiren (Table 1). PRA was completely inhibited 1.5 and 3 h after oral administration of 0.3 mg/kg aliskiren ($n = 3$). It was still

Fig. 2



Changes in mean arterial blood pressure (BP) following administration of a single oral dose of aliskiren, 0.3 mg/kg (open triangles), 1 mg/kg (open diamonds), 3 mg/kg (open squares) or 10 mg/kg (open circles), or vehicle control (filled squares) to sodium-depleted marmosets. BP was continuously measured by telemetry in conscious, freely-moving marmosets as described in Methods. Data presented as mean $\pm$ standard error of the mean. Mean baseline arterial BP and heart rate (HR) values in each group as follows: vehicle (BP, $80 \pm 2 \text{ mmHg}$; HR, $219 \pm 5 \text{ beats/min}$); 0.3 mg/kg aliskiren (BP, $78 \pm 4 \text{ mmHg}$; HR, $212 \pm 3 \text{ beats/min}$); 1 mg/kg aliskiren (BP, $80 \pm 4 \text{ mmHg}$; HR, $208 \pm 12 \text{ beats/min}$); 3 mg/kg aliskiren (BP, $81 \pm 6 \text{ mmHg}$; HR, $210 \pm 12 \text{ beats/min}$); and 10 mg/kg aliskiren (BP, $73 \pm 5 \text{ mmHg}$; HR, $208 \pm 8 \text{ beats/min}$).

inhibited by 87% after 6 h, and had returned to above baseline levels after 24 h (Table 1). Aliskiren at 1 mg/kg caused complete inhibition of renin activity for at least 6 h, and renin activity was inhibited by 78% after 24 h ($n = 4$). Higher oral doses gave even longer inhibition, with renin activity still being completely inhibited 24 h after administration of 3 or 10 mg/kg aliskiren ($n = 4$).

Comparison of aliskiren with remikiren and zankiren in sodium-depleted marmosets

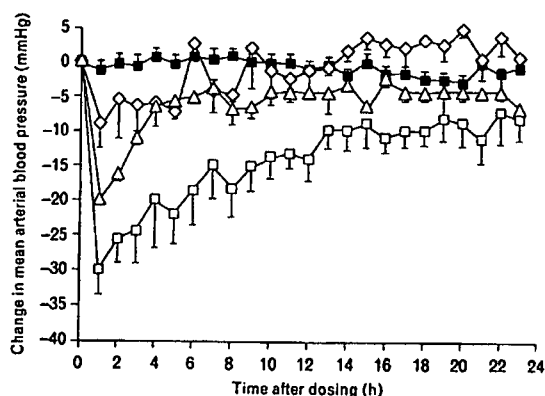
The BP-lowering effects of a single 3 mg/kg oral dose of aliskiren in sodium-depleted marmosets were compared with the same dose of Ro 42,5892 (remikiren) and A-72517 (zankiren). The maximum reduction in MAP achieved with aliskiren was significantly greater ($P < 0.001$) than

Table 1 Plasma concentrations of aliskiren and inhibition of plasma renin activity (PRA) following administration of a single oral dose of aliskiren, 0.3–3 mg/kg, to sodium-depleted marmosets

Dose (mg/kg)	Plasma parameter	Time after administration				n
		1.5 h	3 h	6 h	24 h	
0.3	Aliskiren ($\mu\text{mol/l}$)	0.12 ± 0.07	0.11 ± 0.07	0.04 ± 0.02	0.003 ± 0.003	4
	PRA inhibition (%)	100 $\pm$ 1	99 $\pm$ 1	87 $\pm$ 12	–23 $\pm$ 62	3
1	Aliskiren ($\mu\text{mol/l}$)	0.68 ± 0.3	0.44 ± 0.04	0.35 ± 0.36	0.028 ± 0.023	4
	PRA inhibition (%)	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 1	78 $\pm$ 19	4
3	Aliskiren ($\mu\text{mol/l}$)	1.42 ± 1.29	0.90 ± 0.93	0.24 ± 0.17	0.06 ± 0.04	4
	PRA inhibition (%)	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	4
10	Aliskiren ($\mu\text{mol/l}$)	5.48 ± 2.22	3.13 ± 2.34	1.73 ± 0.92	0.33 ± 0.21	4
	PRA inhibition (%)	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	4

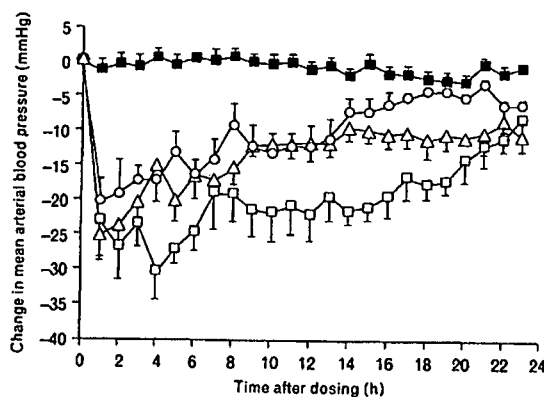
Values presented as mean $\pm$ standard deviation.

Fig. 3



Changes in mean arterial blood pressure (BP) following administration of a single oral 3 mg/kg dose of aliskiren (open squares), or renin inhibitors Ro 42,5892 (remikiren; open diamonds) or A-72517 (zankiren; open triangles), or vehicle control (filled squares) to sodium-depleted marmosets. BP was continuously measured by telemetry in conscious, freely-moving marmosets as described in Methods. Data presented as mean $\pm$ standard error of the mean. Mean baseline arterial BP and heart rate (HR) values were as follows: 3 mg/kg remikiren (BP, 81 ± 4 mmHg; HR, 218 ± 3 beats/min); and 3 mg/kg zankiren (BP, 84 ± 5 mmHg; HR, 206 ± 5 beats/min). Data for the effects of the vehicle control and 3 mg/kg aliskiren are reproduced from Fig. 2 for clarity.

Fig. 4



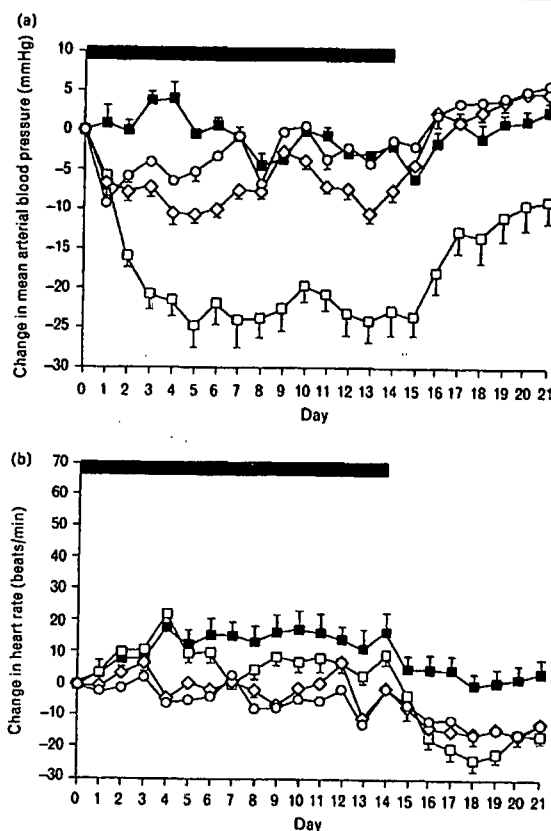
Changes in mean arterial blood pressure (BP) following administration of a single oral 10 mg/kg dose of aliskiren (open squares), the angiotensin-converting enzyme inhibitor benazepril (open triangles), the AT_1 -receptor blocker valsartan (open circles), or vehicle control (filled squares) to sodium-depleted marmosets. BP was continuously measured by telemetry in conscious, freely-moving marmosets as described in Methods. Data presented as mean $\pm$ standard error of the mean. Mean baseline mean arterial BP and heart rate (HR) values were as follows: 10 mg/kg benazepril (BP, 83 ± 4 mmHg; HR, 212 ± 12 beats/min); and 10 mg/kg valsartan (BP, 80 ± 2 mmHg; HR, 206 ± 5 beats/min). Data for the effects of the vehicle control and 10 mg/kg are aliskiren reproduced from Fig. 2 for clarity.

either the maximum reduction of 9 ± 4 mmHg observed with remikiren ($n = 4$) or the reduction of 19 ± 1 mmHg ($n = 4$) achieved with zankiren (Fig. 3).

Comparison of aliskiren with benazepril and valsartan in sodium-depleted marmosets

The effects of a single 10 mg/kg dose of aliskiren on MAP in sodium-depleted marmosets were compared with the actions of the ACE inhibitor benazepril and the AT_1 -receptor blocker valsartan (Fig. 4). Aliskiren

Fig. 5



Changes in (a) mean arterial blood pressure (BP) and (b) heart rate (HR) in spontaneously hypertensive rats (SHR) following administration of aliskiren, 10 mg/kg per day (open circles), 30 mg/kg per day (open diamonds) or 100 mg/kg per day (open squares), or vehicle control (filled squares). Drugs were administered by subcutaneous infusion via osmotic minipumps for 14 days (solid horizontal bar indicates the period of infusion). BP and HR were continuously measured by telemetry in SHR as described in Methods. Data presented as mean $\pm$ standard error of the mean. Mean baseline BP and HR values in each group were as follows: vehicle (BP, 142 ± 2 mmHg; HR, 314 ± 4 beats/min); 10 mg/kg per day aliskiren (BP, 142 ± 1 mmHg; HR, 320 ± 3 beats/min); 30 mg/kg per day aliskiren (BP, 142 ± 1 mmHg; HR, 332 ± 3 beats/min); and 100 mg/kg per day aliskiren (BP, 145 ± 3 mmHg; HR, 340 ± 7 beats/min).

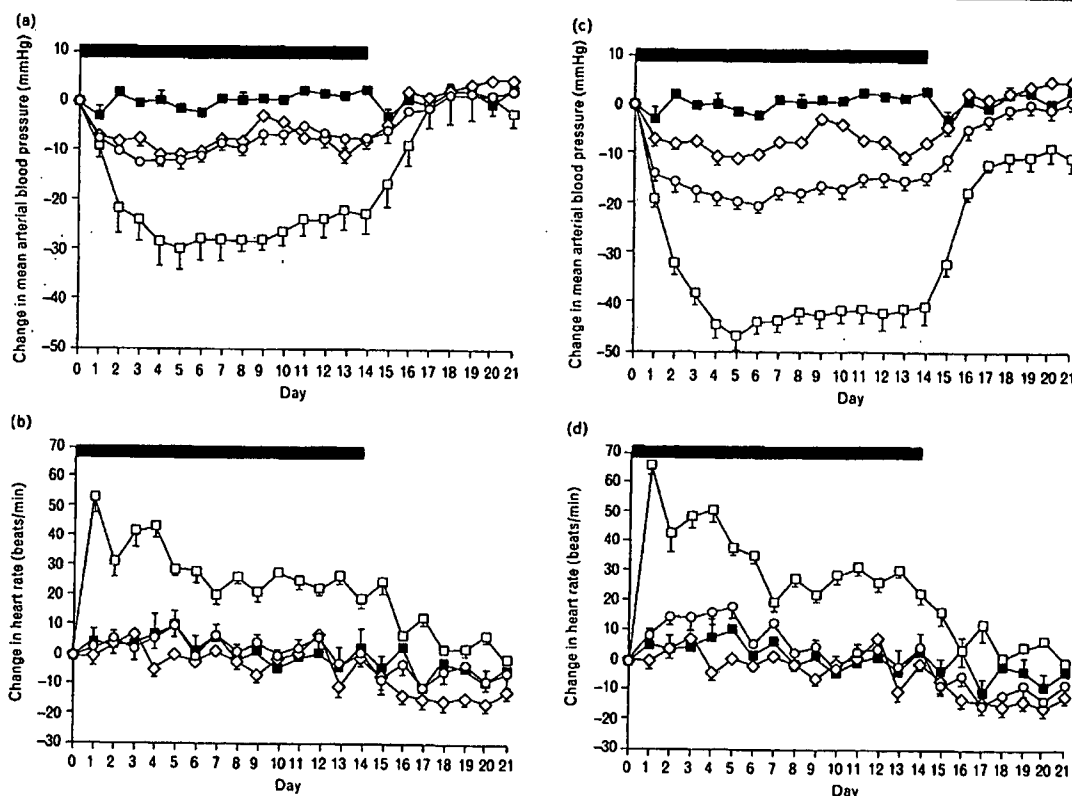
lowered MAP to a significantly greater extent ($P < 0.05$) compared with either benazepril or valsartan, which lowered MAP maximally by 25 ± 4 mmHg ($n = 13$) and 20 ± 3 mmHg ($n = 5$), respectively, at this dose.

At a dose of 3 mg/kg, no significant changes in HR were observed following administration of aliskiren, remikiren, zankiren, benazepril or valsartan. However, a reflex tachycardia could be observed during the first 2 h after administration of 10 mg/kg aliskiren or 10 mg/kg benazepril (changes in HR, $+42 \pm 17$, $+49 \pm 10$ and $+11 \pm 6$ beats/min for aliskiren, benazepril and vehicle controls, respectively).

Combination of aliskiren with benazepril or valsartan in SHR

Aliskiren at 10–100 mg/kg per day subcutaneously, elicited dose-dependent reductions in BP during the first week of administration. Thus MAP was lowered maximally by 9 ± 1 mmHg ($n = 9$), 11 ± 1 mmHg ($n = 8$) and 25 ± 3 mmHg ($n = 8$) by 10, 30 and 100 mg/kg per day aliskiren, respectively (Fig. 5a). The BP lowering achieved with 10 mg/kg per day aliskiren was not significantly greater than the vehicle control over the 2-week dosing period ($P > 0.05$ comparing AOC values from days 0–14). Aliskiren at 30 and 100 mg/kg per day caused a prolonged antihypertensive effect throughout

Fig. 6



Effects of aliskiren and valsartan alone or in combination, on blood pressure (BP) and heart rate (HR) in spontaneously hypertensive rats (SHR). Changes in (a) and (c) mean arterial BP, and (b) and (d) HR in SHR following administration of vehicle control (filled squares), 30 mg/kg per day aliskiren (open diamonds), or valsartan alone (open circles) or in combination with aliskiren (open squares). Doses of valsartan were (a) and (b) 1 mg/kg per day, or (c) and (d) 3 mg/kg per day. Drugs were administered by subcutaneous infusion via osmotic minipumps for 14 days (solid horizontal bar indicates the period of infusion). BP was continuously measured by telemetry in SHR as described in Methods. Data presented as mean $\pm$ standard error of the mean. Mean baseline BP and HR values in each group were as follows: vehicle (BP, 144 ± 3 mmHg; HR, 308 ± 4 beats/min); 1 mg/kg per day valsartan (BP, 142 ± 2 mmHg; HR, 310 ± 2 beats/min); 30 mg/kg per day aliskiren plus 1 mg/kg per day valsartan (BP, 143 ± 2 mmHg; HR, 298 ± 2 beats/min); 3 mg/kg per day valsartan (BP, 144 ± 2 mmHg; HR, 310 ± 4 beats/min); 30 mg/kg per day aliskiren plus 3 mg/kg per day valsartan (BP, 145 ± 6 mmHg; HR, 301 ± 4 beats/min). Data for the effects of 30 mg/kg per day aliskiren alone are reproduced from Fig. 5a for clarity.

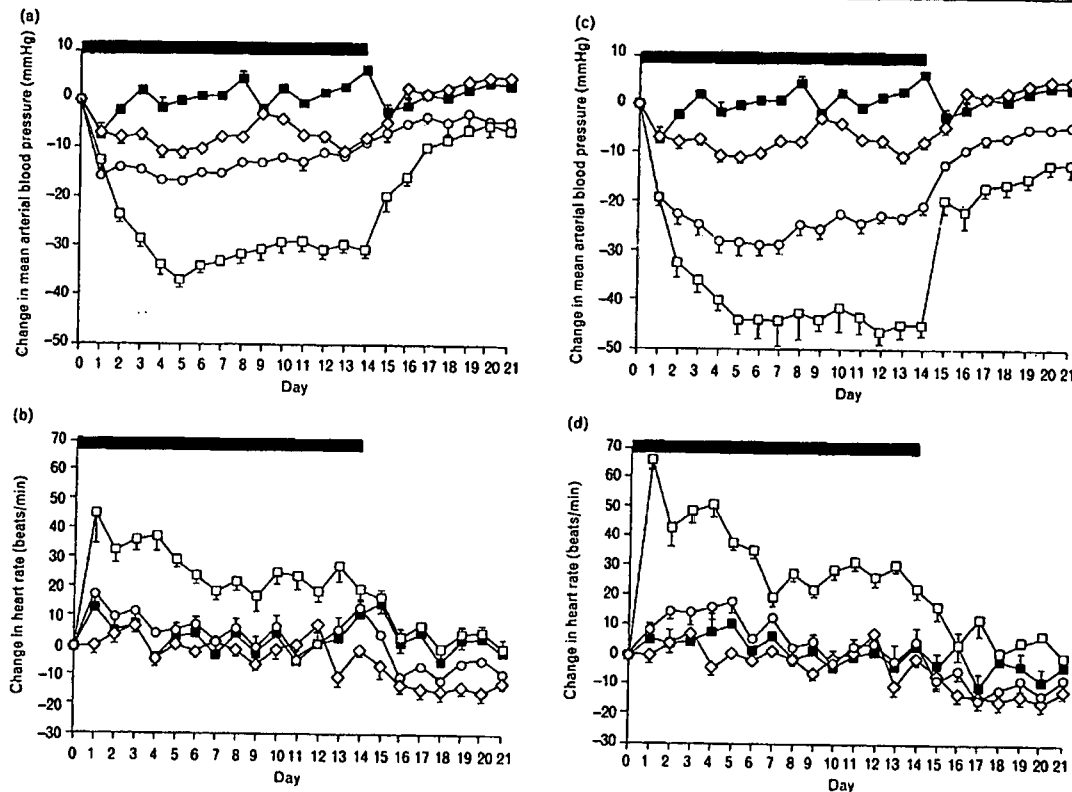
the dosing period ($P < 0.001$ compared with vehicle control), with no significant changes in HR (Fig. 5b).

The AT_1 -receptor blocker valsartan exerted dose-dependent antihypertensive effects in the SHR. Thus, 1 and 3 mg/kg per day valsartan lowered MAP by a maximum of 12 ± 1 mmHg ($n = 7$) and 20 ± 1 mmHg ($n = 7$), respectively (Fig. 6a, c). Neither dose of valsartan significantly altered the HR (Fig. 6b, d). Benazeprilat, the active form of the ACE inhibitor benazepril, lowered MAP by a maximum of 22 ± 3 mmHg ($n = 7$) and 29 ± 2 mmHg ($n = 7$) at doses of 1 and 3 mg/kg per day, respectively (Fig. 7a, c). Although the 1 mg/kg per day dose did not significantly affect HR, 3 mg/kg per day

benazeprilat caused a significant increase ($P < 0.001$) (Fig. 7b, d).

Administration of 30 mg/kg per day aliskiren in combination with 1 mg/kg per day valsartan lowered the MAP by a maximum of 30 ± 4 mmHg ($n = 6$), significantly ($P < 0.001$) more than either monotherapy (Fig. 6a). A similar potentiation was observed following co-administration of 3 mg/kg per day valsartan and 30 mg/kg per day aliskiren; combination therapy lowered MAP by a maximum of 47 ± 3 mmHg ($n = 6$), with sustained reductions of more than 40 mmHg throughout the 2-week dosing period (Fig. 6c). The AOC for the aliskiren/1 mg/kg per day valsartan combination (mean

Fig. 7



Effects of aliskiren and benazeprilat alone or in combination, on blood pressure (BP) and heart rate (HR) in spontaneously hypertensive rats (SHR). Changes in (a) and (c) mean arterial BP, and (b) and (d) HR in SHR following administration of vehicle control (filled squares), 30 mg/kg per day aliskiren (open diamonds), or benazeprilat alone (open circles) or in combination with aliskiren (open squares). Doses of benazeprilat were (a) and (b) 1 mg/kg per day, or (c) and (d) 3 mg/kg per day. Drugs were administered by subcutaneous infusion via osmotic minipumps for 14 days (solid horizontal bar indicates the period of infusion). BP was continuously measured by telemetry in SHR as described in Methods. Data presented as mean $\pm$ standard error of the mean. Mean baseline BP and HR values in each group were as follows: vehicle (BP, 156 ± 2 mmHg; HR, 311 ± 3 beats/min); 1 mg/kg per day benazeprilat (BP, 155 ± 3 mmHg; HR, 312 ± 4 beats/min); 30 mg/kg per day aliskiren plus 1 mg/kg per day benazeprilat (BP, 143 ± 3 mmHg; HR, 296 ± 7 beats/min); 3 mg/kg per day benazeprilat (BP, 154 ± 3 mmHg; HR, 304 ± 4 beats/min); 30 mg/kg per day aliskiren plus 3 mg/kg per day benazeprilat (BP, 144 ± 5 mmHg; HR, 294 ± 9 beats/min). Data for the effects of 30 mg/kg per day aliskiren alone are reproduced from Fig. 5a for clarity.

$\pm$ standard deviation, -331 ± 115 mmHg days) was significantly greater ($P < 0.01$) compared with the sum of AOC values for the respective monotherapies (-218 ± 42 mmHg days). Similar findings were observed for the aliskiren/3 mg/kg per day valsartan combination.

Administration of the lower dose of benazeprilat (1 mg/kg per day) in combination with 30 mg/kg per day aliskiren led to a maximum reduction in MAP of 37 ± 2 mmHg ($n = 6$). The antihypertensive effect of the combination therapy was significantly ($P < 0.001$) greater compared with the effects of 1 mg/kg per day benazeprilat alone (Fig. 7a). Co-administration of the higher dose of benazeprilat (3 mg/kg per day) with 30 mg/kg per day aliskiren elicited a maximum reduction in MAP of 47 ± 2 mmHg ($n = 5$), with reductions in MAP of greater than 40 mmHg observed throughout the 2-week dosing period (Fig. 7c). The AOC for the aliskiren/1 mg/kg per day benazeprilat combination (-398 ± 52 mmHg days) was also significantly greater ($P < 0.01$) compared with the sum of AOC values for the respective monotherapies (-286 ± 29 mmHg days). Similar findings were observed for the aliskiren/3 mg/kg per day benazeprilat combination.

Combinations of aliskiren and valsartan or aliskiren and benazeprilat caused significant ($P < 0.001$) increases in the HR compared with vehicle controls and with the respective monotherapies (Figs 6b, d and 7b, d). The combination therapy-induced tachycardia was most prominent during the first week, with elevations in the HR then progressively diminishing throughout the remainder of the 2-week infusion period.

Aliskiren treatment, either alone or in combination with valsartan or benazeprilat, was well tolerated in both marmosets and SHR, with no grossly detectable adverse effects observed in either species at any dose throughout the study.

Discussion

The results of the present study show that aliskiren is an orally effective, long-lasting renin inhibitor that lowers BP in two diverse experimental models. Inhibition of renin activity with aliskiren lowers BP at least as effectively as ACE inhibition and AT_1 -receptor blockade, and aliskiren is significantly more effective than the same dose of previously tested renin inhibitors. Moreover, the SHR studies suggest that potentiation of antihypertensive effects may occur with submaximal doses of aliskiren in combination with submaximal doses of ACE inhibitors or AT_1 -receptor blockers. Aliskiren treatment alone as well as in combination with other RAS inhibitors was well tolerated in both marmosets and SHR.

The dose-dependent BP reductions observed following oral administration of aliskiren to sodium-depleted

marmosets in the present study parallel the results of previous investigations of renin inhibitors in this animal model [23–25]. Although there was good correlation between the BP-lowering effects of aliskiren and the plasma concentration of the drug in marmosets, the observed lack of correlation between the BP-lowering and PRA inhibitory effects of aliskiren may reflect a limitation of the trapping assay used. Although the trapping assay is the best method available for direct determination of PRA, it may overestimate PRA inhibition by renin inhibitors *ex vivo*, due to complete dissociation of the inhibitor from plasma proteins under *in vitro* assay conditions [26,27]. The optimum indicator of RAS inhibition in these experiments would be measurement of Ang II levels, and studies in humans have confirmed that aliskiren effectively inhibits Ang II production [28].

Remikiren and zankiren were chosen for comparison with aliskiren in the present study because these previous generation renin inhibitors proceeded the furthest through clinical trials, with both agents having been tested in humans [15,16]. Our results clearly show that a single oral dose of aliskiren gives a significantly larger and longer-lasting reduction in BP in sodium-depleted marmosets compared with identical doses of either remikiren or zankiren. The greater effectiveness of aliskiren probably reflects increased oral bioavailability and a longer half-life, because the *in vitro* potency of aliskiren, remikiren and zankiren at inhibiting human renin is similar ($IC_{50} = 0.6, 0.8$ and 1.1 nmol/l, respectively) [13,14]. This is a notable result, as remikiren and zankiren did not succeed in hypertension clinical development programmes as a result of their insufficient oral bioavailability and/or short half-life. The 25–30 mmHg reductions in MAP achieved with aliskiren in sodium-depleted marmosets in the present study were found to be similar to the effects of identical doses of the ACE inhibitor benazepril or the AT_1 -receptor blocker valsartan, both of which are currently used widely in the treatment of hypertension, congestive heart failure and progressive renal insufficiency.

Aliskiren was effective only at higher doses in the SHR compared with the marmoset, but this is presumably due to the 100-fold lower inhibitory potency of aliskiren and less favourable pharmacokinetics of aliskiren in the rat [13,14]. However, these studies in another animal model confirm that renin inhibition with aliskiren has the potential to lower BP as effectively as other classes of RAS inhibitors. Indeed, early studies investigating the acute effects of intravenously administered renin inhibitors in humans showed that inhibition of renin activity was at least as effective as ACE inhibition in reducing BP [29,30], while the renin inhibitor enalkiren was even more effective than the ACE inhibitor enalaprilat when administered intravenously to patients with high baseline renin levels [31].

Our experiments investigating the antihypertensive effect of aliskiren in combination with either benazeprilat or valsartan in SHR were motivated by previous findings that subcutaneous co-administration of benazeprilat and valsartan had synergistic effects to decrease BP in this animal model [22]. The same observations have been made in sodium-depleted guinea pigs using the renin inhibitor CP 80-794 in combination with the ACE inhibitor captopril [32], or another renin inhibitor telakiren in combination with the AT₁-receptor blocker losartan [33]. Our results demonstrate that combination of submaximally effective doses of the renin inhibitor aliskiren (30 mg/kg per day) with low doses (1 and 3 mg/kg per day) of either benazeprilat or valsartan potentiates the antihypertensive effects of these agents. Although no endocrine investigation could be performed in these pharmacological studies in conscious rats, the observed potentiation by the combination regimen conceivably reflects the pharmacologic effects of a more complete RAS blockade [34]. Alternatively, it could ensue from a complex interplay of endocrine responses to pharmacologic interventions at multiple points along the RAS pathway. It is notable that a recent clinical study showed that high dosages of an AT₁-receptor blocker may provide comparable RAS blockade compared with an ACE inhibitor/AT₁-receptor blocker combination [35]. Further studies are therefore required in order to investigate whether combination of a renin inhibitor and an ACE inhibitor or AT₁-receptor blocker may provide additional benefits compared with high doses of individual monotherapies.

Our observation that the antihypertensive effects of the lowest dose of aliskiren (10 mg/kg per day) were not sustained beyond the first week of the dosing period in SHR is similar to previous results observed with low doses of ACE inhibitors and AT₁-receptor blockers, and is not due to changes in drug clearance or deterioration of active drug in the osmotic minipumps [22]. This finding illustrates that the antihypertensive effects of chronic RAS inhibitor monotherapy, whatever the target is within the RAS, is counteracted by the inherent reactive stimulation of the RAS that these drugs induce, an effect that can only be offset by the use of higher doses [5,36]. Combination therapy may be useful to reinforce RAS inhibition at least when submaximal doses of each inhibitor are used. For instance, addition of 50 mg losartan to the ACE inhibitor enalapril (10 mg) was found to be more effective in lowering the BP of mildly sodium-depleted normotensive men, compared with increasing the dose of the enalapril to 20 mg [34].

Although chronic RAS inhibition is not generally associated with significant elevations in HR, as now demonstrated with aliskiren monotherapy, the results of the present study suggest that the magnitude and the rapidity of the blood pressure fall when aliskiren was combined with either valsartan or benazeprilat, led to marked

tachycardia in SHR. This was also observed with the highest dose of benazeprilat monotherapy, and is similar to results obtained previously with combinations of the ACE inhibitor and AT₁-receptor blocker [22]. It should be noted that the radiotelemetry procedure may reveal significant rises in HR as a result of the resting HRs in these animals being lower than those obtained for SHR with the use of indwelling arterial cannulae measurements or using a tail-cuff apparatus [21]. In addition, it has been postulated that the compensatory increase in HR following BP reduction may be activated more effectively in animals with a lower resting HR [22].

The pharmacological relevance of the animal models used and the selection of aliskiren for clinical investigation have recently been confirmed by the results of the first clinical studies in normotensive and hypertensive subjects. Sustained inhibition of plasma renin activity and Ang II production over 8 days was observed in healthy volunteers following once-daily oral doses of 40–640 mg aliskiren [36]. In patients with mild-to-moderate essential hypertension, aliskiren showed dose-dependent antihypertensive efficacy and safety similar to the AT₁-receptor blocker losartan [37]. Besides its medical usefulness as a new therapeutic approach to the treatment of hypertension, the effects of aliskiren may also aid understanding of the mechanisms of action of ACE inhibitors and AT₁-receptor blockers. By acting directly at the main, if not the exclusive, source of renin (the kidney), renin inhibitors may also exert different intra-renal effects compared with ACE inhibitors and AT₁-receptor blockers, as indicated previously by studies with remikiren in guinea pigs [38] and enalkiren in humans [39].

In summary, aliskiren is the first in a new class of potent, orally effective non-peptide renin inhibitors. The results of the present study show that oral administration of aliskiren to sodium-depleted marmosets causes greater and more prolonged BP lowering than two other human renin inhibitors, remikiren and zankiren, which failed during clinical testing. Indeed, the BP-lowering effects of aliskiren are at least equivalent to those produced by an ACE inhibitor or an AT₁-receptor blocker. Submaximal doses of aliskiren also enhance the antihypertensive effects of low doses of benazeprilat and valsartan. Aliskiren may therefore represent an effective, novel approach to the treatment of hypertension and related disorders, alone or in combination with other antihypertensive agents.

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ANEXO 2

“The novel oral renin inhibitor aliskiren decreases plasma renin activity (PRA) and neutralizes hydrochlorothiazine-induced RAAS activation in hypertensive patients”

THE NOVEL ORAL RENIN INHIBITOR ALISKIREN DECREASES PLASMA RENIN ACTIVITY (PRA) AND NEUTRALIZES HYDROCHLOROTHIAZIDE-INDUCED RAAS ACTIVATION IN HYPERTENSIVE PATIENTS

D Calhoun,¹ SG Chrysant,² A Villamil,² B Schobel,¹ H Hsu,¹ MF Prescott¹

¹Vascular Ecology and Hypertension Program, University of Alabama at Birmingham, AL; ²Oklahoma Cardiovascular and Hypertension Center and University of Oklahoma, Oklahoma City OK, USA; ³Cardiac, Los-Herns 2910-Apo 1A, Buenos Aires, Argentina; ⁴Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA

INTRODUCTION

Thiazide diuretics, such as hydrochlorothiazide (HCTZ), are commonly used in the treatment of hypertension and are recommended as first-line therapy by JNC 7 guidelines.¹ Thiazide diuretics act by blocking sodium reuptake in the distal nephron, thus reducing water retention and depleting fluid volume.

While this mechanism of action effectively reduces blood pressure (BP), volume depletion stimulates release of renin from the kidney - a compensatory mechanism to activate the renin-angiotensin-aldosterone system (renin system) and restore body fluid homeostasis. This leads to a rise in renin concentration (RC) and an increase in plasma renin activity (PRA).²

Aliskiren is a potent renin inhibitor,³ the first in a new class of agents for the management of hypertension, that has been shown to reduce PRA from baseline (unlike other agents that target the renin system, such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers).^{4,5}

Therefore, aliskiren has the potential to complement diuretic therapy and enhance its antihypertensive benefits by counteracting diuretic-induced stimulation of the renin system.

The antihypertensive efficacy and safety of aliskiren 75, 150 and 300 mg, alone and in combination with HCTZ 6.25, 12.5 and 25 mg, were investigated in patients with mild-to-moderate hypertension. Here we report an exploratory analysis of the effects of these treatments on PRA and RC.

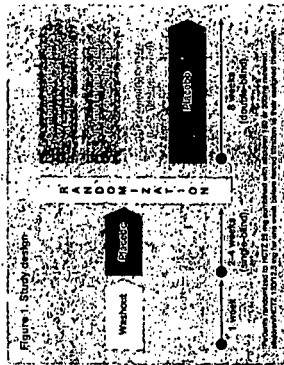
METHODS

Study population

Male and female patients aged ≥ 18 years, with mild-to-moderate hypertension (mean sitting diastolic BP (MSDBP) ≥ 95 mmHg and < 110 mmHg).

Study design

The study design is shown in Figure 1.



Assessments and variables

- Changes from baseline to study end in MSDBP and mean sitting systolic BP (MSBP) were assessed in the intent-to-treat population.
- Patients at selected study centers participated in an exploratory biomarker sub-study. PRA was measured by means of radioimmunoassay of generated angiotensin I (Diasorin kit), and RC by immunochemiluminometric assay (Nichols Direct Renin assay). In plasma samples taken at baseline and Week 8.
- Percentage changes from baseline in PRA and RC were calculated, based on geometric mean values, to provide a descriptive assessment of treatment effects on these parameters.

RESULTS

Patient characteristics

- Of the 2776 patients randomized to double-blind treatment, 611 were included in the biomarker sub-study.
- Demographics and baseline characteristics for the biomarker sub-study population are shown in Table 1.

Table 1. Patient demographics and baseline characteristics (mean $\pm$ SD, biomarker sub-study population)

Variable	Aliskiren + HCTZ (n=305)	Placebo + HCTZ (n=306)
Age, years	52.7 $\pm$ 11.9	52.7 $\pm$ 11.9
Sex, male, n (%)	210 (68.8)	210 (68.6)
Race, Caucasian, n (%)	230 (75.4)	230 (75.2)
Duration of hypertension, years	8.7 $\pm$ 8.5	8.7 $\pm$ 8.5
BP, mmHg	132.2 $\pm$ 13.4	132.2 $\pm$ 13.4
MSDBP, mmHg	100.9 $\pm$ 11.9	100.9 $\pm$ 11.9
MSBP, mmHg	131.2 $\pm$ 13.2	131.2 $\pm$ 13.2
Aliskiren, mg/day	271 (88.8)	271 (88.8)
HCTZ, mg/day	271 (88.8)	271 (88.8)
Weight, kg	87.5 $\pm$ 18.3	87.5 $\pm$ 18.3
Height, cm	175.5 $\pm$ 7.5	175.5 $\pm$ 7.5
Body mass index, kg/m ²	27.9 $\pm$ 4.5	27.9 $\pm$ 4.5

Baseline levels of PRA and RC are shown in Table 2.

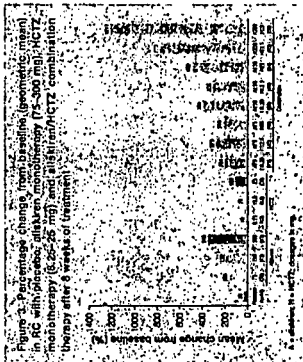
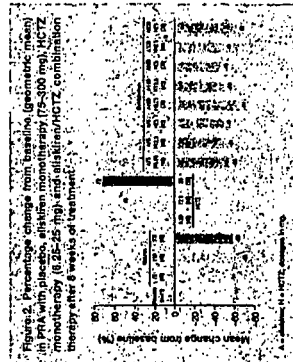
Table 2. Baseline biomarker levels (range of geometric mean values, biomarker sub-study population)

Variable	Aliskiren + HCTZ (n=305)	Placebo + HCTZ (n=306)
Baseline PRA, ng/ml/h	0.27 (0.05-1.1)	0.27 (0.05-1.1)
Baseline RC, ng/ml/h	107 (10-1000)	107 (10-1000)

Biomarkers

Changes from baseline in PRA are shown in Figure 2.

Changes from baseline in RC are shown in Figure 3.



Safety and tolerability

All active treatments were well tolerated, with the rate of adverse events (AEs) in the overall population ranging from 37.3 to 39.2% with aliskiren monotherapy, 38.7 to 42.0% with HCTZ monotherapy, and 34.6 to 45.3% with aliskiren/HCTZ combination, while 44.0% of placebo recipients experienced AEs.

CONCLUSIONS

- Aliskiren effectively blocks PRA from baseline as monotherapy, and blocks the rise in PRA seen during diuretic treatment.
- Both HCTZ and aliskiren produce dose-related increases in RC from baseline after 8 weeks of treatment, reflecting compensatory stimulation of renin release in response to volume depletion or inhibition of angiotensin II production.
- Elevation of RC from baseline with combination therapy exceeded the sum of the increases seen with each component monotherapy in most cases, suggesting a synergistic effect on stimulation of renin release.
- Aliskiren is likely to be a useful adjunct to diuretic treatment, offering an enhanced antihypertensive effect and potentially improved end-organ protection through effective suppression of the renin system.

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