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|---------------------------------------------------|--------------------------------------------|
| SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO          |                                            |
| RAD: 13-238680- -7                                | FECHA: 2014-10-29 08:55:49                 |
| DEP: 2020 DIRECCION DE NUEVAS CREACIONES          | EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I |
| TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II | FOLIOS: 9                                  |
| ACT: 519 PRESENTACION                             |                                            |

DIRECCIÓN DE NUEVAS CREACIONES

2020  
Bogotá, D.C.,  
  
Abogado(a)/Señor(a)  
SILVANA MARIA LOPEZ DIAZ

Asunto:      Radicación:      13-238680- -7  
                 Trámite:            11  
                 Evento:            378  
                 Actuación:           519  
                 Oficio:                    13621  
                 Folios:                    9

|                                     |                   |            |   |                               |  |
|-------------------------------------|-------------------|------------|---|-------------------------------|--|
| Patente de Invención                | X                 |            |   | Patente de Modelo de Utilidad |  |
| Vía Nacional                        |                   |            |   |                               |  |
| Vía PCT (Fase Nacional)             | X                 | Capítulo I | X | Capítulo II                   |  |
| No. Solicitud Internacional         | PCT/KR2011/005754 |            |   |                               |  |
| Fecha de Presentación Internacional | 08/08/2011        |            |   |                               |  |

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

|                            |                                   |                            |
|----------------------------|-----------------------------------|----------------------------|
| Descripción:               | Radicado No. 13-238680-00000-0000 | 08/Octubre/2013            |
| Reivindicaciones:          | Radicado No. 13-238680-00000-0000 | 08/Octubre/2013            |
| Oposiciones:               | Radicado No. 13-238680-00004-0000 | Lafrancol<br>28/Marzo/2014 |
| Respuesta del solicitante: | Radicado No. 13-238680-00006-0000 | 07/Julio/2014              |
| Prioridad:                 | KR 10-2011-0033856                | 12/Abril/2011              |

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: “Composición farmacéutica antihipertensiva que contiene Fimasartan o una sal o solvato o hidrato de la misma y Amlodipina, un isómero o sal o soltrato o hidrato de la misma” (Modicado en el radicado No. Radicado No. 13-238680-00002-0000).

El problema planteado en esta solicitud consiste en la necesidad de suministrar métodos terapéuticos, preparaciones de combinación y composiciones farmacéuticas contra la hipertensión.

Para resolver este problema técnico la solicitud en estudio proporciona una composición farmacéutica antihipertensiva, caracterizada porque comprende: Fimasartan, una sal farmacéuticamente aceptable del mismo, un solvato del mismo o un hidrato del mismo como un bloqueador del receptor de angiotensina II; y Amlodipina, un isómero de la misma, una sal farmacéuticamente aceptable de la misma, un solvato de la misma o un hidrato de la misma como un bloqueador del canal de calcio.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 31/41, A61K 31/44, A61K 31/505, A61P 9/12.

#### **4. De la Solicitud de Patente**

##### **Título**

El título de la solicitud comprende la palabra soltrato, en lugar de solvato, cuando se refiere a los solvatos de amlodipina, se sugiere hacer la corrección necesaria.

##### **Reivindicaciones (Art 30 D 486, Art 22 PCT)**

La reivindicación no es concisa, ni se encuentra totalmente soportada en la descripción, porque reclama una composición farmacéutica sin establecer que tipo de composición es, y la descripción solo soporta composiciones en tableta. Adicionalmente, se recuerda al solicitante que una composición farmacéutica se debe caracterizar por sus componentes y las proporciones de éstos. Se sugiere modificar la reivindicación de tal forma que se encuentre totalmente soportada en la descripción.

La reivindicación 2 no se encuentra totalmente soportada en la descripción porque la descripción solo presenta ensayos y composiciones con trihidrato potásico de fimasartan. Se sugiere restringir la reivindicación a la materia que efectivamente cuenta con soporte descriptivo.

La reivindicación 5 no se encuentra totalmente soportada en la descripción porque la descripción solo presenta ensayos y composiciones con besilato de amlodipina. Se sugiere restringir la reivindicación a la materia que efectivamente cuenta con soporte descriptivo.

La reivindicación 7 no es concisa porque el rango de cantidad de cada uno de los principios activos es demasiado amplio. Se sugiere restringir la materia reclamada a aquella que cuenta con soporte descriptivo, e incluir la materia reclamada en la reivindicación independiente.

La reivindicación 14 no es clara porque a pesar que se refiere a una composición farmacéutica, hace referencia al uso de la misma para la prevención, alivio o tratamiento de diversas patologías, lo cual no es materia patentable. Adicionalmente, la reivindicación 14 tiene el mismo alcance de la reivindicación 1, por lo cual no se tomará en cuenta para el examen de patentabilidad. Se sugiere eliminarla del capítulo reivindicatorio.

#### **5. Análisis de la Oposición**

##### **Argumentaciones de Lafranco**

1. Afirma el oponente que las composiciones y combinaciones farmacéuticas que comprenden Fimasartán, o una sal o solvato, o hidrato del mismo, con un

- bloqueador del receptor de angiotensina II y amlodipino, un isómero del mismo, una sal, solvato o hidrato del mismo como un bloqueador del canal de calcio, son prácticas y eventos totalmente conocidos en la industria farmacéutica, y que no existe ninguna novedad sobre estos preceptos y mucho menos nivel inventivo. El oponente afirma que la solicitante no puede pretender acceder a un beneficio patentario sobre composiciones y combinaciones farmacéuticas que comprenden moléculas que se encuentran descubiertas y plenamente identificadas en el sector químico farmacéutico, para lo cual menciona el libro "The Merck Index" y señala que dicho libro indica que el amlodipino ya había sido patentado por medio de las patentes EP89167 y US4572909 y que por lo tanto no puede ser otorgado un beneficio de patente a composiciones y combinaciones farmacéuticas que comprenden moléculas identificadas y utilizadas.
2. Afirma el oponente que la solicitud de la referencia pretende proteger mediante el derecho de patente objetos que no son considerados invenciones porque reivindica combinaciones, las cuales no se consideran invenciones., y que la mezcla de productos (moléculas) conocidos y su variación, bien sea en uso, forma, dimensión o de materia, no se consideran invenciones porque no son susceptibles de aplicación industrial, nivel inventivo y novedad.
  3. El oponente señala que un efecto sinérgico nuevo no se considera inventivo como tal, dado que puede ser obvio para una persona versada en la material. El oponente referencia al Doctor Carlos Correa y el libro pautas para el examen de patentes farmacéuticas, quien señala que: *"El efecto sinérgico entre dos o más drogas se puede considerar un "descubrimiento" y no una invención", dado que la sinergia ocurre en el cuerpo y se descubre a través de ensayos clínicos. Asimismo, cabe destacar que, en algunos casos, las reivindicaciones de combinaciones pueden equivaler, en términos prácticos, a reivindicaciones sobre tratamientos médicos (cuya patentabilidad está excluida en la mayoría de los países), cuando sólo brindan un método para administrar una combinación de drogas conocidas. Asimismo, la combinación de drogas para evitar la resistencia particular a una de ellas es una práctica normal en el desarrollo farmacéutico y, por lo general, es evidente para el hombre del oficio de nivel medio"*.
  4. Finalmente establece el oponente que la solicitud en curso carece de los requisitos fundamentales para otorgársele el privilegio de patente de invención, particularmente en materia de novedad y/o nivel inventivo si se tiene en cuenta la materia revelada en los documentos: "Pharmacokinetic interaction of Fimasartan, a new angiotensin II receptor antagonist, with Amlodipine in healthy volunteers", "Role of combination therapy in the treatment of hypertension: focus on valsartan plus amlodipine", "Pharmacokinetics of repeated oral doses of amlodipine and amlodipine plus telmisartan in healthy volunteers" y "The combination of olmesartan Medoxomil and Amlodipine Besylate in controlling High Blood Pressure: COACH, a randomized, Double-Blind, Placebo-Controlled, 8-Week factorial Efficacy and Safety Study".

### Respuestas del Solicitante

El solicitante señala que el artículo de los autores Yi, So Jeong et al., corresponde a una publicación hecha por el mismo inventor y presenta una fecha de publicación que resulta ser solo un mes antes de la fecha de prioridad de la presente solicitud, siendo éstas 9 de marzo de 2011 y 12 de abril de 2011, respectivamente, por tanto y de acuerdo a lo establecido por el artículo 17 de la Dec. 486, dicha anterioridad no es válida como documento a citar del estado previo de la técnica.

En lo que respecta al resto de referencias citadas, el solicitante señala que todas ellas hacen mención a composiciones farmacéuticas que comprenden ya sea el fimasartan o el amlodipina, pero nunca, que la composición tenga ambos compuestos, tal y como si se reclama en la presente solicitud y concluye, que el opositor no ha comprendido aun la presente invención y por tanto afirma erróneamente, que por el hecho de que alguno de los compuestos reivindicados haya sido empleado previamente en diferentes

formulaciones, la presente formulación por ende, carece de novedad. El solicitante señala que para que un documento del estado de la técnica afecte la novedad de una nueva creación, éste debe comprender TODAS las características técnicas esenciales que se estén reivindicando, de lo contrario, no existe afectación por novedad.

Respecto al nivel inventivo, el solicitante establece que la solicitud presenta nivel inventivo por cuanto la combinación de la composición reclamada presenta efecto sinérgico para lo cual hace referencia a las tablas 1 y 2 de la presente solicitud, cita al Doctor Carlos Correa, presenta un ensayo de la combinación de ramipril y telmisartán que demuestra que entre dichos agentes no se presenta un efecto sinérgico y un documento donde se analizan los resultados de dicho estudio, donde se señala que "la acción combinada de esos dos medicamentos es una fuente de tensión en los riñones" y "por tanto la combinación de esos medicamentos no genera un efecto benéfico adicional y por lo contrario, cada uno de ellos está asociado a efectos secundarios, por lo cual en el momento de combinarlos, lo que se está haciendo es aumentar el efecto de dichos efectos secundarios".

### **Respuesta del Examinador Técnico**

Este despacho considera que los documentos EP89167 y US4572909 no afectan la patentabilidad de la solicitud ya que dichos documentos se refieren a la molécula amlodipino y las composiciones que lo contienen, pero no divulgan, ni sugieren una composición que comprenda amlodipino en combinación con un antagonista de angiotensina II como Fimasartan.

Por otro lado, la materia reclamada en la presente solicitud no es considerada como materia no patentable o como no invención, ya que no las composiciones farmacéuticas no se encuentran dentro de las excepciones señaladas en los Artículos 15 y 20 de la Decisión 486 del 2000 y la materia objeto de la presente solicitud no corresponde a un método de tratamiento.

Finalmente, respecto a los documentos citados por el solicitante y que fueron citados en el reporte de búsqueda internacional se encuentra que:

El documento "Pharmacokinetic interaction of Fimasartan, a new angiotensin II receptor antagonist, with Amlodipine in healthy volunteers" no puede ser tenido en cuenta durante el examen de patentabilidad debido a que su publicación fue realizada por los mismos inventores de la presente solicitud dentro del año anterior a la fecha de prioridad, y en consecuencia, se encuentra dentro de lo establecido en el Art. 17 de Decisión 486.

El documento "Pharmacokinetics of repeated oral doses of amlodipine and amlodipine plus telmisartan in healthy volunteers" se refiere a un estudio realizado en humanos, en el cual se concluye que no hay variación clínicamente significativa en los parámetros farmacocinéticos de amlodipino en presencia de telmisartán, y la seguridad de la combinación es comparable a la de amlodipino solo. El documento no afecta la patentabilidad de la presente solicitud debido a que no se refiere a una composición que comprenda amlodipino y fimasartan, y por el contrario desalentaría a la persona de nivel medio versada en la materia a combinar un ARA II y un antagonista de canales de calcio.

El documento "The combination of Olmesartan Medoxomil and Amlodipine Besylate in controlling high blood pressure" divulga el efecto mejorado de la terapia de combinación de olmesartan medoxomilo y amlodipino besilato frente al uso de cada uno de estos compuestos en monoterapia. Adicionalmente, el documento no especifica que haya un efecto sinérgico entre dichos compuestos. La anterioridad en cuestión no afecta la novedad de la materia reclamada porque no presenta una composición que comprenda fimasartán y amlodipina, tampoco puede afectar el nivel inventivo de la presente solicitud si se usa de forma independiente y el oponente no señala como

podría ser combinado con otro documento del estado de la técnica para afectar el mencionado requisito de patentabilidad.

Finalmente, respecto al documento “Role of combination therapy in the treatment of hypertension: focus on valsartan plus amlodipine”, dicho documento señala que existe un efecto sinérgico de la combinación del ARA II valsartán y el antagonista de canales de calcio amlodipina. La mencionada anterioridad no puede afectar el requisito de novedad porque no se refiere a una composición que comprenda fimasartán y amlodipina, y tampoco puede afectar el requisito de nivel inventivo si se usa de forma independiente. El oponente no señala como se puede combinar el mencionado documento con otra anterioridad para anular el nivel inventivo de la presenta solicitud.

Los documentos presentados en la oposición no desvirtúan por si solos la novedad o el nivel inventivo del objeto de esta solicitud. Sin embargo, el documento “Role of combination therapy in the treatment of hypertension: focus on valsartan plus amlodipine” será usado durante el examen de patentabilidad.

## 6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: Abril 12 de 2011 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

| Ítem | Documento    | Título                                                                                                              | Fecha de Publicación* |
|------|--------------|---------------------------------------------------------------------------------------------------------------------|-----------------------|
| D1   | WO2007001066 | Pharmaceutical preparation containing an angiotensin ii receptor antagonist and a calcium channel blocker           | 06/Enero/2005         |
| D2   | WO9955681    | Pyrimidinone compounds, pharmaceutical compositions containing the compounds and the process for preparing the same | 04/Noviembre/1999     |
| D3   | WO03024956   | Method for preparing pyrimidinone compound and pharmaceutically acceptable salts thereof                            | 27/Marzo/2003         |

D1 [http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en\\_EP&FT=D&date=20070104&CC=WO&NR=2007001066A1&KC=A1](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20070104&CC=WO&NR=2007001066A1&KC=A1) Divulga composiciones farmacéuticas antihipertensivas que comprenden un antagonista del receptor de angiotensina II (ARA II) (losartán, candesarán, valsartán, telmisartán, prazosartán, olmesartán, o irbesartan) (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1, 5, 6 y 7), en combinación con un bloqueador de canales de calcio tal como amlodipino besilato (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1 y 11).

D2 [http://worldwide.espacenet.com/publicationDetails/biblio?DB=worldwide.espacenet.com&II=0&ND=3&adjacent=true&locale=en\\_EP&FT=D&date=19991104&CC=WO&NR=9955681A1&KC=A1](http://worldwide.espacenet.com/publicationDetails/biblio?DB=worldwide.espacenet.com&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19991104&CC=WO&NR=9955681A1&KC=A1) Divulga composiciones en tableta o cápsulas que comprenden el compuesto 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one (compuesto 2) y excipientes farmacéuticamente aceptables como lactosa, almidón de maíz, celulosa microcristalina y estearato de magnesio (Pág. 20, Ejs. 15 – 42, Reiv. 9; Pág. 10, lín. 7 – 9, Ej. 2).

D3 [http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en\\_EP&FT=D&date=20030327&CC=WO&NR=03024956A1&KC=A1](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20030327&CC=WO&NR=03024956A1&KC=A1) Divulga el compuesto 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one y su sal potásica, así como su forma

trihidratada (Pág. 10, Ej. 3; Pág. 8, primer párr. completo; reivs. 1, 9 y 10). El documento D3 señala que los compuestos allí revelados son 50 veces más activos que los compuestos revelados en el arte previo (Pág. 2, Arte previo; Pág. 3, primer párrafo completo).

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en una composición farmacéutica antihipertensiva, caracterizada porque comprende:

Fimasartan, una sal farmacéuticamente aceptable del mismo, un solvato del mismo o un hidrato del mismo como un bloqueador del receptor de angiotensina II; y amlodipina, un isómero de la misma, una sal farmacéuticamente aceptable de la misma, un solvato de la misma o un hidrato de la misma como un bloqueador del canal de calcio.

Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

| Características esenciales                                                                                                                                                   | D1                                                                                                                                                                                     | D2                                                                                                                                               | D3                                                                                                                                                                                                                                                             |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Una composición farmacéutica antihipertensiva, caracterizada porque comprende:                                                                                               | Una composición farmacéutica antihipertensiva, caracterizada porque comprende:                                                                                                         | Una composición farmacéutica antihipertensiva en cápsula o tableta, caracterizada porque comprende (Pág. 20, Ejs. 15 – 42, Reiv. 9).             | Divulga el compuesto:                                                                                                                                                                                                                                          |
| Fimasartan, una sal farmacéuticamente aceptable del mismo, un solvato del mismo o un hidrato del mismo como un bloqueador del receptor de angiotensina II; y                 | Un antagonista del receptor de angiotensina II (losartán, candesarán, valsartán, telmisartán, prazosartán, olmesartán, o irbesartan) (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1, 5, 6 y 7). | 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one (Pág. 10, lín. 7 – 9, Ej. 2). | 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one, así como la preparación de su sal potásica, incluyendo el compuesto en forma trihidrato (Pág. 8, primer párr. completo; reivs. 1, 9 y 10). |
| Amlodipina, un isómero de la misma, una sal farmacéuticamente aceptable de la misma, un solvato de la misma o un hidrato de la misma como un bloqueador del canal de calcio. | Un bloqueador de canales de calcio tal como amlodipino besilato (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1 y 11).                                                                           | No lo menciona.                                                                                                                                  | No lo menciona.                                                                                                                                                                                                                                                |

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

El documento D1 divulga composiciones farmacéuticas antihipertensivas que comprenden un antagonista del receptor de angiotensina II (ARA II) (losartán, candesarán, valsartán, telmisartán, prazosartán, olmesartán, o irbesartan) (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1, 5, 6 y 7), en combinación con un bloqueador de canales de calcio tal como amlodipino besilato (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1 y 11).

La diferencia entre la invención, definida en la reivindicación 1 y la composición farmacéutica divulgada en el documento D1 consiste en que las composiciones del documento D1 no comprenden fimasartán como antagonista del receptor de angiotensina II.

El efecto que logra el incluir esta característica es que las composiciones de la presente solicitud presentan un efecto antihipertensivo sinérgico.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar las composiciones reveladas en el documento D1 para obtener una composición antihipertensiva que presente un efecto sinérgico superior”.

Sin embargo, el documento D2 divulga composiciones en tableta o cápsulas que comprenden el compuesto 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one (compuesto 2) y excipientes farmacéuticamente aceptables como lactosa, almidón de maíz, celulosa microcristalina y estearato de magnesio (Pág. 20, Ejs. 15 – 42, Reiv. 9; Pág. 10, lín. 7 – 9, Ej. 2). El documento D2 señala que los compuestos allí revelados son superiores en actividad a los compuestos ARA II revelados en el arte previo (Pág. 2, lín. 29 – 34 y Pág. 3, lín. 1 – 3).

En consecuencia, la persona del oficio normalmente versada en la materia, que conoce por el documento D2 que el compuesto 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one (fimasartán) presenta una actividad superior a la de compuestos como el olmesartán, se vería motivada a incluirlo en una composición en combinación con amlodipina como se enseña en el documento D1, porque es del conocimiento común de una persona medianamente versada en la materia que la combinación de antagonistas del receptor de angiotensina II (ARA II) y antagonistas de canales de calcio presenta efectos antihipertensivos sinérgicos<sup>1 o 2 o 3</sup>, por lo cual tendría la expectativa razonable de encontrar un efecto sinérgico superior debido a que el fimasartán presenta mejores resultados que los presentados por los otros ARA II conocidos con anterioridad. Por lo cual se considera que la reivindicación 1 es obvia.

### Examen en base a los documento D1 y D3

Los documentos D1 y D3 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

El documento D1 divulga composiciones farmacéuticas antihipertensivas que comprenden un antagonista del receptor de angiotensina II (losartán, candesarán, valsartán, telmisartán, prazosartán, olmesartán, o irbesartan) (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1, 5, 6 y 7), en combinación con un bloqueador de canales de calcio tal como amlodipino besilato (Ejs. 1 – 10, Págs. 19 – 23; Reivs. 1 y 11).

La diferencia entre la invención, definida en la reivindicación 1 y la composición farmacéutica divulgada en el documento D1 consiste en que las composiciones del documento D1 no comprenden fimasartán como antagonista del receptor de angiotensina II.

El efecto que logra el incluir esta característica es que las composiciones de la presente solicitud presentan un efecto antihipertensivo sinérgico.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar las composiciones reveladas en el documento D1

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1 Synergism of Telmisartan and Amlodipine on Blood Pressure Reduction and Cardiorenal Protection in Hypertensive Rats. J Cardiovasc Pharmacol. Volume 57, Number 3, March 2011.

2 Beneficial Effects of the Combination of Amlodipine and Losartan for Lowering Blood Pressure in Spontaneously Hypertensive Rats. Arch Pharm Res Vol 32, No 3, 353-358, 2009.

3 Role of Combination Therapy in the Treatment of Hypertension: Focus on Valsartan plus Amlodipine. Adv Ther. 2008;25(4):300–320.

para obtener una composición antihipertensiva que presente un efecto sinérgico superior”.

Sin embargo, el documento D3 divulga el compuesto 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one y su sal potásica, así como su forma trihidratada (Pág. 10, Ej. 3; Pág. 8, primer párr. completo; reivs. 1, 9 y 10). El documento D3 señala que los compuestos allí revelados son 50 veces más activos que los compuestos revelados en el arte previo (Pág. 2, Arte previo; Pág. 3, primer párrafo completo).

En consecuencia, la persona del oficio normalmente versada en la materia, que conoce por el documento D3 que el compuesto 2-n-butil-5-dimetilaminotiocarbonilmetil-6-metil-3-[[2'-(1H-tetrazol-5-il)bifenil-4-il]metil]-pirimidina-4(3H)-one (fimasartán) presenta una actividad superior a la de otros compuestos ARA II revelados previamente, se vería motivada a incluirlo en una composición en combinación con amlodipina como se enseña en el documento D1, porque es del conocimiento común de una persona medianamente versada en la materia que la combinación de antagonistas del receptor de angiotensina II (ARA II) y antagonistas de canales de calcio presenta efectos antihipertensivos sinérgicos<sup>4 o 5 o 6</sup>, por lo cual tendría la expectativa razonable de encontrar un efecto sinérgico superior debido a que el fimasartán presenta mejores resultados que los presentados por los otros ARA II conocidos con anterioridad. Por lo cual se considera que la reivindicación 1 es obvia.

#### Conclusión:

La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18), de la misma manera que sus reivindicaciones dependientes 2-13.

### 8. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

| Ítem | Documento    | Reivindicaciones afectadas | Requisito que afecta |
|------|--------------|----------------------------|----------------------|
| D1   | WO2007001066 | 1 - 14                     | Nivel Inventivo      |
| D2   | WO9955681    | 1 – 14                     | Nivel Inventivo      |
| D3   | WO03024956   | 1 - 14                     | Nivel Inventivo      |

4 Synergism of Telmisartan and Amlodipine on Blood Pressure Reduction and Cardiorenal Protection in Hypertensive Rats. J Cardiovasc Pharmacol. Volume 57, Number 3, March 2011.

5 Beneficial Effects of the Combination of Amlodipine and Losartan for Lowering Blood Pressure in Spontaneously Hypertensive Rats. Arch Pharm Res Vol 32, No 3, 353-358, 2009.

6 Role of Combination Therapy in the Treatment of Hypertension: Focus on Valsartan plus Amlodipine. Adv Ther. 2008;25(4):300–320.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



**JOSÉ LUIS SALAZAR LÓPEZ**  
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-11-04

Elaboró: Juanita Catalina Rico Valbuena  
Revisó: Gloria Jacqueline Alfonso Roza

# Synergism of Telmisartan and Amlodipine on Blood Pressure Reduction and Cardiorenal Protection in Hypertensive Rats

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**Aim:** This study was designed to investigate the effects of telmisartan and amlodipine on reduction of blood pressure (BP), myocardial hypertrophy, and renal injury in hypertensive rats.

**Method:** In acute experiments, the BP was measured in conscious freely moving rats. Spontaneously hypertensive rats were treated with intragastric administration of amlodipine (1, 2, 4 mg/kg), telmisartan (4, 8, 12, 16, 20 mg/kg), and their different combinations (4 + 4, 2 + 4, 4 + 8, 4 + 12, 1 + 4, 2 + 8, 4 + 16, 2 + 12, 1 + 8, 2 + 16, 2 + 20, 1 + 12, 1 + 16, 1 + 20 mg/kg). The probability sum test (q test) was used to evaluate the synergistic action on BP reduction. In two-kidney, one-clip rats, the effects of amlodipine (1 mg/kg), telmisartan (6 mg/kg) and their combination on BP reduction were observed. In the chronic study, spontaneously hypertensive rats were treated with amlodipine (1 mg/kg), telmisartan (6 mg/kg), and their combination for 4 months. Histopathologic examinations were performed after the determination of BP and BP variability.

**Results:** There is a synergistic interaction between amlodipine and telmisartan on BP reduction. The optimal dose ratio was found at 1:6. The synergistic effect of this dose ratio (1:6) was also seen in two-kidney, one-clip rats. Long-term treatment with this combination results in a beneficial effect on the reduction of BP and BP variability. The end-organ damage, including myocardial hypertrophy, glomerular atrophy, and fibrosis, was significantly attenuated by this combination.

**Conclusion:** The optimal dose ratio of amlodipine and telmisartan on BP was 1:6. This combination is beneficial for the BP and BP variability reduction and end-organ damage prevention.

**Key Words:** amlodipine, telmisartan, spontaneously hypertensive rat, two-kidney, one clip, end-organ damage

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## INTRODUCTION

Hypertensive complications such as stroke, heart failure, renal failure, and myocardial infarction are often lethal. End-organ damage (EOD) is the early phase of these complications in hypertension. It is well accepted that reduction of blood pressure (BP) can effectively reduce or reverse EOD and consequently prevent the possible complications in hypertension.

Randomized controlled trials have shown that single-drug treatment is usually not adequate to achieve BP goal in most hypertensive patients.<sup>1</sup> Initiating therapy with more than one agent offers the potential advantages of achieving BP control more rapidly and avoiding dose-related adverse effects of individual drugs by producing greater BP reduction at lower doses of the component agents.<sup>2</sup>

Amlodipine, a calcium channel blocker, and telmisartan, an AT(1) receptor blocker, are two widely used drugs in the treatment of hypertension and the prevention of hypertensive complications.<sup>3–7</sup> They belong to two different classes of antihypertensives and the mechanisms of action are quite different for these two drugs, so they might have a synergistic effect on BP reduction. In fact, the beneficial effects of the combination of amlodipine and AT(1) receptor blocker on endothelial function have been found to be greater than the effect of either drug alone.<sup>8</sup> However, it remains to be established whether the combination of amlodipine and telmisartan would have a synergistic effect on BP reduction, what is the optimal dose ratio for the combination of these two drugs, and what are their effects on hypertensive organ damage. Therefore, the present work was designed to investigate the optimal dose ratio for this combination and to define the preventive effect of the combination on the myocardial hypertrophy and renal injury in hypertensive rats.

## METHODS

### Animals and Chemicals

Amlodipine was purchased from Zhejiang Pharmaceutical Co Ltd (Zhejiang, China) and telmisartan was purchased from Jiangsu Pharmaceutical Co Ltd (Jiangsu, China). Male Sprague-Dawley rats (used for preparation of hypertensive models) were purchased from the Sino-British SIPPR/BK Lab Animal Ltd (Shanghai, China). Male spontaneously hypertensive rats (SHR) were provided by the animal center of our university. The rats were housed with controlled temperature (23–25°C) and lighting (8:00 AM to 8:00 PM light, 8:00 PM to

8:00 AM dark) and with free access to food and tap water. All the animals used in this work received humane care in compliance with institutional animal care guidelines.

### Blood Pressure Measurements

Systolic BP (SBP), diastolic BP (DBP) and heart period (HP) were continuously recorded described in previous studies.<sup>9,10</sup> Rats were anesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). A floating polyethylene catheter was inserted into the lower abdominal aorta through the left femoral artery for BP measurement. In Experiments 1 and 2, the other catheter was placed into the stomach through a midabdominal incision for the drug's administration. The catheters were exteriorized through the interscapular skin. After operation, each animal was treated intramuscularly with a dose of penicillin ( $8 \times 10^4$  IU) and housed individually with controlled temperature (23–25°C) and with free access to food and tap water. After 1-day recovery, the animals were placed in individual cylindrical cages containing food and water for BP recording. The aortic catheter was connected to a BP transducer through a rotating swivel that allowed the animals to move freely in the cage. After approximately 4-hour habituation (from 8:00 AM to 12:00 PM), the BP signal was digitized by a microcomputer beat-to-beat. SBP, DBP, and HP values from every heartbeat were determined online. The mean values of these parameters during 6 hours before administration (from 12:00 PM to 6:00 PM) and 6 hours after administration (from 6:00 PM to 12:00 AM) for each rat were calculated by the BP recording system (MPA-HBBS; Shanghai Alcott Biotech Co, Ltd, Shanghai, China). The standard deviation of the mean during 6 hours before and 6 hours after administration for each rat were calculated and defined as the quantitative parameter of variability; that is SBP variability, DBP variability, and HP variability. In Experiment 3, after approximately 4 hours habituation (from 8:00 AM to 12:00 PM), SBP, DBP, and HP values were recorded during 6 hours (from 12:00 PM to 6:00 PM).

### Preparation of Two-Kidney, One-Clip Hypertensive Rats

Male Sprague-Dawley rats weighing 180 to 200 g were anaesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). The left renal artery of each animal was isolated through a flank incision, and a silver clip (0.25-mm internal gap) was placed on the left renal artery.<sup>11,12</sup> All animals were fed standard rat chow and tap water ad libitum. On Day 30 after operation, BP was measured using the previously mentioned methods. The rats with SBP greater than 140 mmHg were used for this study and received the treatment with different drugs.

### Morphologic Examination

In Experiment 3, after determination of BP, the animals were euthanized and the right kidney, thoracic aorta, and heart were dissected quickly. Morphologic examination was performed using the previously mentioned methods. Ratios of heart weight to body weight, left ventricular weight to body weight (LVW/BW), and aortic weight to the length of aorta

(AW/length) were calculated.<sup>13,14</sup> Histopathologic observation was also carried out with our conventional method.<sup>15</sup>

### Glomerulosclerosis Score

For the semiquantitative evaluation of glomerular damage, the glomerulosclerosis score (GSS) was defined as previously described.<sup>16</sup> On the light microscopic specimens, approximately 50 glomeruli from the outer cortex and the same number of glomeruli from the inner cortex for each kidney were graded according to the degree of sclerosis: 0, no mesangial expansion; 1, mild mesangial expansion (less than 30% of a glomerular area); 2, moderate mesangial expansion (30–60% of a glomerular area); 3, marked mesangial expansion (greater than 60% of a glomerular area); and 4, sclerosis was global. This was performed by one observer in a blind fashion using coded slides. A weighted composite sclerosis score was then calculated for each kidney according to the following formula: glomerulosclerosis score  $[1 \times (\text{number of grade 1 glomeruli}) + 2 \times (\text{number of Grade 2 glomeruli}) + 3 \times (\text{number of Grade 3 glomeruli}) + 4 \times (\text{number of Grade 4 glomeruli})] \times 100/(\text{number of glomeruli observed})$ .

### Experimental Protocols

#### Experiment 1: Effect of a Single Dose of Different Combinations of Amlodipine and Telmisartan on the Blood Pressure in Spontaneously Hypertensive Rats

Two hundred fifty SHR aged 40 weeks were used in this study. They were divided into 23 groups ( $n = 10$ – $11$  per group) and treated by the following drugs: amlodipine (1 mg, 2 mg, 4 mg/kg), telmisartan (4, 8, 12, 16, 20 mg/kg), and the combination of amlodipine and telmisartan (4 + 4, 2 + 4, 4 + 8, 4 + 12, 1 + 4, 2 + 8, 4 + 16, 2 + 12, 1 + 8, 2 + 16, 2 + 20, 1 + 12, 1 + 16, 1 + 20 mg/kg). After approximately 4 hours training, the drugs were dissolved in the 0.8% carboxymethylcellulose sodium and were given through a catheter inserted into the stomach. The BP and HP were recorded during 12 hours. Before this experiment, six SHR were given a dose of vehicle (0.8% carboxymethylcellulose sodium). No significant changes in BP and HP were observed in these control rats.

#### Experiment 2: Effect of the Single Dose of the Combination of Amlodipine and Telmisartan (1:6) on the Blood Pressure in Two-Kidney One-Clip Rats

The rats with SBP greater than 140 mm Hg after two-kidney one-clip operation were used for this study. Animals were divided into six groups ( $n = 10$ – $11$  per group). Amlodipine (1 mg/kg), telmisartan (6 mg/kg), and their combinations (1:6: 0.5 + 3, 1 + 6, 2 + 12 mg/kg) were dissolved in the 0.8% carboxymethylcellulose sodium. Drugs were administered by catheter of gastric fistula implanted 1 day before the experiment. After approximately 4 hours habituation (from 8:00 AM to 12:00 PM), the BP and HP were recorded during 12 hours (from 12:00 PM to 12:00 AM during 6 hours before administration and 6 hours after administration) in conscious freely moving rats.

### Experiment 3: Effects of Long-Term Treatment at Optimal Dose Ratios of Amlodipine and Telmisartan on the Blood Pressure and End-Organ Damage in Spontaneously Hypertensive Rats

Seventy-five SHR aged 40 weeks were divided into six groups ( $n = 12$ – $13$  per group). Amlodipine and telmisartan were mixed in the rat chow. The consumption of rat chow containing drugs was determined previously.<sup>17–19</sup> The content of drugs in the rat chow was calculated according to the chow consumption. The daily ingested dose of amlodipine, telmisartan, and their combinations (1:6) were approximately 1, 6 3.5, 7, and 14 mg/kg per day, respectively. The control group received the same diet without any drugs. After 4 months of drug administration, the BP was recorded during 6 hours (from 12:00 PM to 6:00 AM after training for 4 hours); then the BP variability (BPV) was calculated in conscious freely moving rats. Histopathologic examinations were performed after BP recording with mentioned methods.

#### Probability Sum Test

To determine if the drugs were acting synergistically, we used the probability sum test ( $q$  test).<sup>20,21</sup> Compared with the mean values in the control group of rats, treated rats with a decrease in BP greater than 15 mm Hg were defined as responders. Data with a decrease in blood pressure ranging from 12 to 15 mm Hg were discarded and less than 12 mm Hg were defined as nonresponders. The formula used is as follows:  $q = P_{A+B} / (P_A + P_B - P_A \times P_B)$ . Here, A and B indicate Drug A and Drug B, respectively; P is the percentage of responders in each group.  $P_{A+B}$  is the real percentage of responders and  $(P_A + P_B - P_A \times P_B)$  is the expected response rate.  $(P_A + P_B)$  is the sum of the probabilities when Drug A and Drug B are used alone.  $P_A \times P_B$  is the probability of rates responding to both drugs when they were used alone. When  $q$  was less than 0.85, the combination was thought to be antagonistic; when  $q$  greater than 1.15, the combination was thought to be synergistic; and when  $q$  was between 0.85 and 1.15, the combination was thought to be additive.

#### Statistical Analysis

Data are expressed as mean  $\pm$  standard deviation. The comparisons between before and after drug administration were made by paired  $t$  tests and between two groups by unpaired  $t$  tests. The comparisons between more than two groups were made by one-way analysis of variance followed by Dunnett  $t$  test.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Experiment 1: Effects of a Single Dose of Different Combinations of Amlodipine and Telmisartan on the Blood Pressure in Spontaneously Hypertensive Rats

#### Blood Pressure Reduction

As shown in Table 1 and Figure 1, compared with before administration, the SBP values were significantly reduced in three amlodipine alone treatment groups by

approximately 8, 12, and 18 mm Hg ( $P < 0.05$ ) and in four telmisartan alone treatment groups by approximately 6, 10, 11, 16, and 17 mm Hg ( $P < 0.05$ ). The different combinations of these two drugs were also found to significantly reduce SBP in SHR ( $P < 0.05$ ). In amlodipine (4 mg/kg) combination groups, adding different doses of telmisartan (0–16 mg/kg) produced a similar effect on BP reduction. Similar results were also found in telmisartan (20 mg/kg) combination groups. The SBP only in the 1 + 4 and 2 + 12 groups was reduced significantly more than in the amlodipine and telmisartan alone groups (shown in Table 1).

#### Effect on the Heart Period

Tachycardia (shortened HP) was seen in rats treated with large-dose telmisartan alone or its combinations but was minor and inconsistent.

#### Synergistic Effect in Blood Pressure Reduction

Table 2 presents the result of the probability sum test in data from SHR treated with amlodipine, telmisartan, and their combinations. It was found that not all the  $q$  values were larger than 1.15. The  $q$  value in 2 + 12 group ( $q = 3.00$ ) was larger than any other group, which implied this combination (1:6) might be the optimal dose ratio.

### Experiment 2: Effects of the Single Dose of the Combination of Amlodipine and Telmisartan (1:6) on the Blood Pressure in Two Kidney One Clip Rats

#### Blood Pressure Reduction

As shown in Figure 2, compared with former administration, the SBP did no change in the control group but showed a significant decrease in amlodipine (1:  $149 \pm 9.72$  versus  $162 \pm 12.0$  mmHg,  $P < 0.01$ ), telmisartan (6:  $155 \pm 22.5$  versus  $170 \pm 28.9$  mm Hg,  $P < 0.01$ ), and their combination groups (0.5 + 3:  $152 \pm 17.3$  versus  $166 \pm 17.9$  mm Hg,  $P < 0.01$ ; 1 + 6:  $148 \pm 26.4$  versus  $171 \pm 27.0$  mm Hg,  $P < 0.01$ ; 2 + 12:  $139 \pm 11.9$  versus  $176 \pm 16.7$  mm Hg,  $P < 0.01$ ). The SBP reduction was dose-dependent in the combination treatment groups and the small dose group (0.5

**TABLE 1.** Effects of a Single Dose of Amlodipine (Aml), Telmisartan (Tel), and Their Combinations on the Change of Mean Blood Pressure (BP) Over 6 Hours in Spontaneously Hypertensive Rats

| Aml (mg/kg) | 0  | 4    | 8   | Tel (mg/kg) | 12  | 16  | 20 |
|-------------|----|------|-----|-------------|-----|-----|----|
| 0           | 2  | 6    | 10  | 11          | 16  | 17  |    |
| 1           | 8  | 13*† | 12* | 14*         | 14* | 15* |    |
| 2           | 12 | 16†  | 14  | 17*†        | 17* | 19* |    |
| 4           | 18 | 18†  | 19† | 20†         | 21  | —   |    |

The change of mean BP (mm Hg) over 6 hours;  $n = 10$ – $11$  per group.

\* $P < 0.05$  the combination versus Aml alone.

† $P < 0.05$  the combination versus Tel alone.

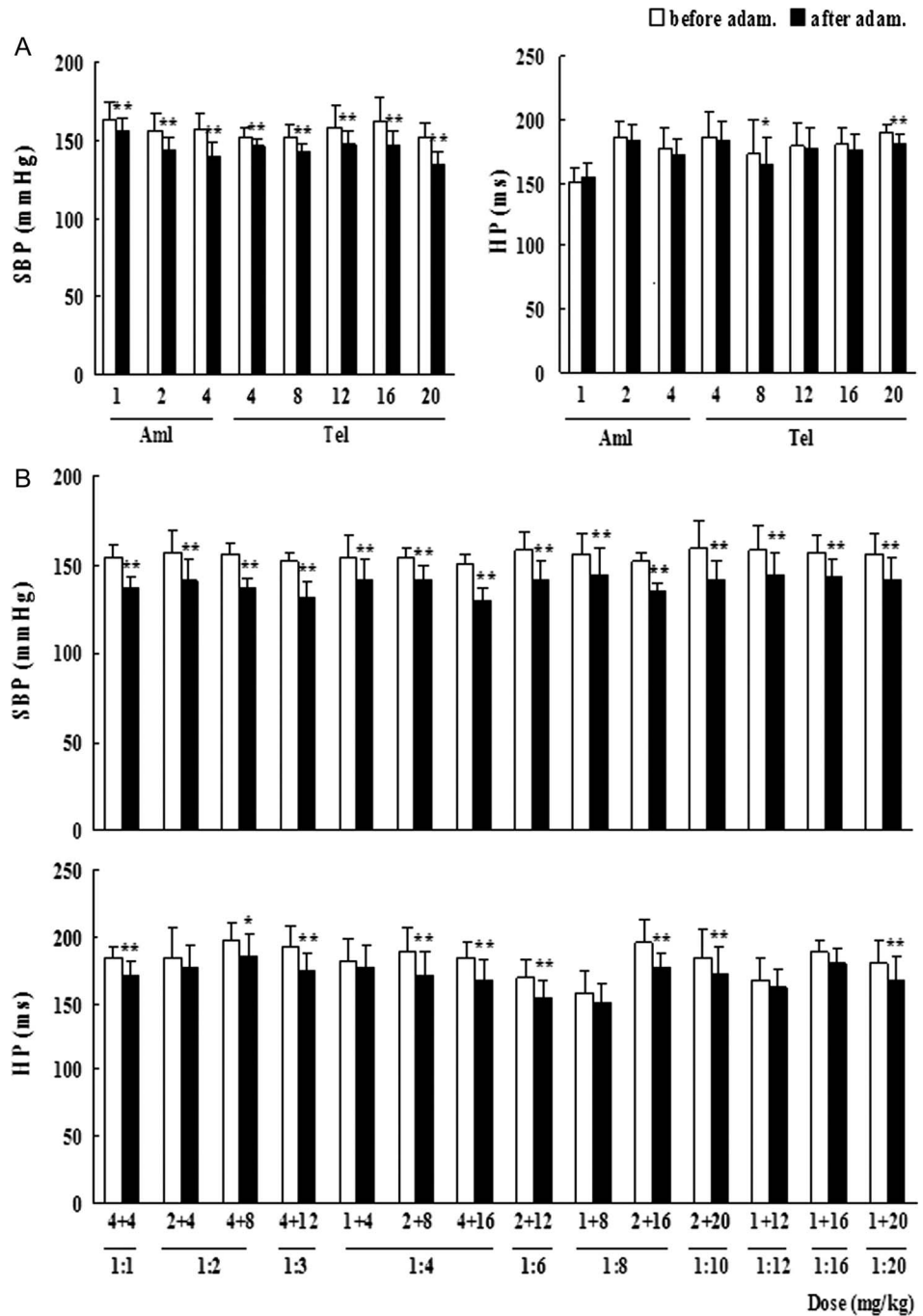


**FIGURE 1.** Effects of a single dose of amlodipine and telmisartan alone (A) and in combination (B) on the blood pressure and heart period in conscious spontaneously hypertensive rats. SBP, systolic blood pressure; DBP, diastolic blood pressure; HP, heart period. N = 10–11 in each group. \**P* < 0.05, \*\**P* < 0.01 versus before administration.

+ 3) caused a BP reduction similar to that of amlodipine (1) and telmisartan (6) alone groups. The decrease in DBP was similar to SBP. The HP remained unchanged in all groups except for the low-dose combinations.

**Synergistic Effect in Blood Pressure Reduction**

The q value in 1 + 6 group (q = 1.38) was larger than 1.15, which implied this combination (1:6) has a synergistic effect on BP reduction.

**Experiment 3: Effects of Long-Term Treatment at Optimal Dose Ratios of Amlodipine and Telmisartan on the Blood Pressure and End-Organ Damage in Spontaneously Hypertensive Rats**

**Effects on Blood Pressure Reduction**

As shown in Figure 3A, long-term amlodipine (1 mg/kg per day) alone in conscious SHR slightly decreased SBP

**TABLE 2.** Results of the Probability Sum Test in Spontaneously Hypertensive Rats Treated With Amlodipine (Aml) and Telmisartan (Tel)

| Aml:Tel | P <sub>Aml</sub> | P <sub>Tel</sub> | P <sub>Aml+Tel</sub> | q Value |
|---------|------------------|------------------|----------------------|---------|
| 1: 1    | A4 = 0.667       | T4 = 0           | A4 + T4 = 0.75       | —       |
| 1: 2    | A2 = 0.125       | T4 = 0           | A2 + T4 = 0.714      | —       |
|         | A4 = 0.667       | T8 = 0.125       | A4 + T8 = 1.00       | 1.410   |
| 1: 3    | A4 = 0.667       | T12 = 0.143      | A4 + T12 = 0.80      | 1.120   |
| 1: 4    | A1 = 0.125       | T4 = 0           | A1 + T4 = 0.5        | —       |
|         | A2 = 0.125       | T8 = 0.125       | A2 + T8 = 0.571      | 2.436   |
|         | A4 = 0.667       | T16 = 0.5        | A4 + T16 = 1.00      | 1.200   |
| 1: 6    | A2 = 0.125       | T12 = 0.143      | A2 + T12 = 0.75      | 3.000   |
| 1: 8    | A1 = 0.125       | T8 = 0.125       | A1 + T8 = 0.25       | 1.067   |
|         | A2 = 0.125       | T16 = 0.5        | A2 + T16 = 0.875     | 2.100   |
| 1:10    | A2 = 0.125       | T20 = 0.556      | A2 + T20 = 0.625     | 1.022   |
| 1:12    | A1 = 0.125       | T12 = 0.143      | A1 + T12 = 0.444     | 1.775   |
| 1:16    | A1 = 0.125       | T16 = 0.5        | A1 + T16 = 0.375     | 0.900   |
| 1:20    | A1 = 0.125       | T20 = 0.556      | A1 + T20 = 0.625     | 1.022   |

P<sub>Aml</sub>, P<sub>Tel</sub>, and P<sub>Aml+Tel</sub> were the percentages of animals possessing an effective decrease in systolic blood pressure by amlodipine, telmisartan, and their combinations. q greater than 1.15 means synergism. The q value is discarded when the value of P<sub>Aml</sub> or P<sub>Tel</sub> is equal to zero.

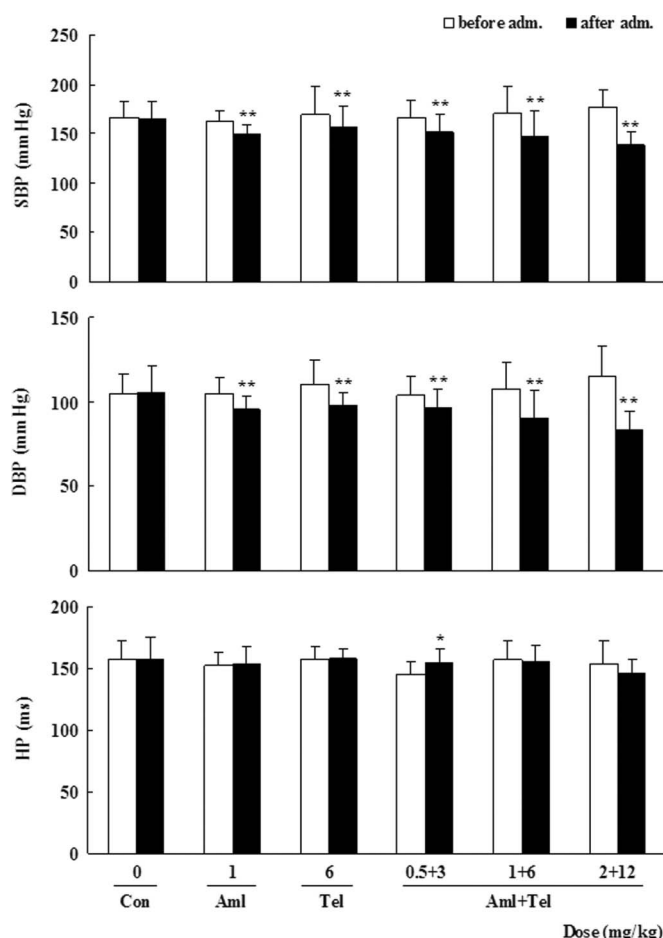


**FIGURE 2.** Effects of a single dose of amlodipine and telmisartan alone and in combination on blood pressure and heart period in two-kidney one-clip rats. SBP, systolic blood pressure; DBP, diastolic blood pressure; HP, heart period. N = 9 in each group. \* $P < 0.05$ , \*\* $P < 0.01$  versus before administration (adm).

(approximately 9 mmHg) and DBP (approximately 4 mmHg), but neither reached statistical significance. The long-term treatment with telmisartan (6 mg/kg per day) alone markedly decreased the SBP (approximately 45 mmHg) and DBP (approximately 30 mmHg). A significant reduction in both SBP and DBP was also shown in rats treated with combinations (0.5 + 3, 1 + 6, and 2 + 12 g/kg per day). These effects of combination therapy were dose-dependent.

### Effects on Heart Period

No significant change in HP was found in any group studied (data not shown).

### Effects on Systolic Blood Pressure Variability, Diastolic Blood Pressure Variability, and Heart Period Variability

As shown in Figure 3B, compared with the control group, no change in BPV was found in amlodipine alone or in the low-dose combination groups (0.5 + 3 mg/kg per day). A significant reduction in SBP variability was found in rats treated with telmisartan ( $8.37 \pm 1.11$  versus  $10.0 \pm 1.81$  mmHg,  $P < 0.05$ ) and two large doses of combination ( $7.84 \pm 0.82$ ,  $7.35 \pm 1.13$  versus  $10.0 \pm 1.81$  mmHg,  $P < 0.01$ ). A significant reduction in DBP variability was found in rats treated with the largest dose of combination (2 + 12:  $5.77 \pm 1.24$  versus  $7.10 \pm 1.45$ ,  $P < 0.05$ ). No change in HP variability was found in any group studied.

### Effects of Combinations on End-Organ Damage

Among EOD parameters, some representative parameters are shown in Figures 4 and 5. They are ratio of heart weight to body weight (reflecting ventricular hypertrophy), LVW/BW (reflecting left ventricular hypertrophy), AW/length (reflecting aortic hypertrophy), and GSS (reflecting glomerular damage). It was found that long-term treatment with amlodipine alone did not modify any EOD parameters except a marked reduction on AW/BW. Long-term treatment with

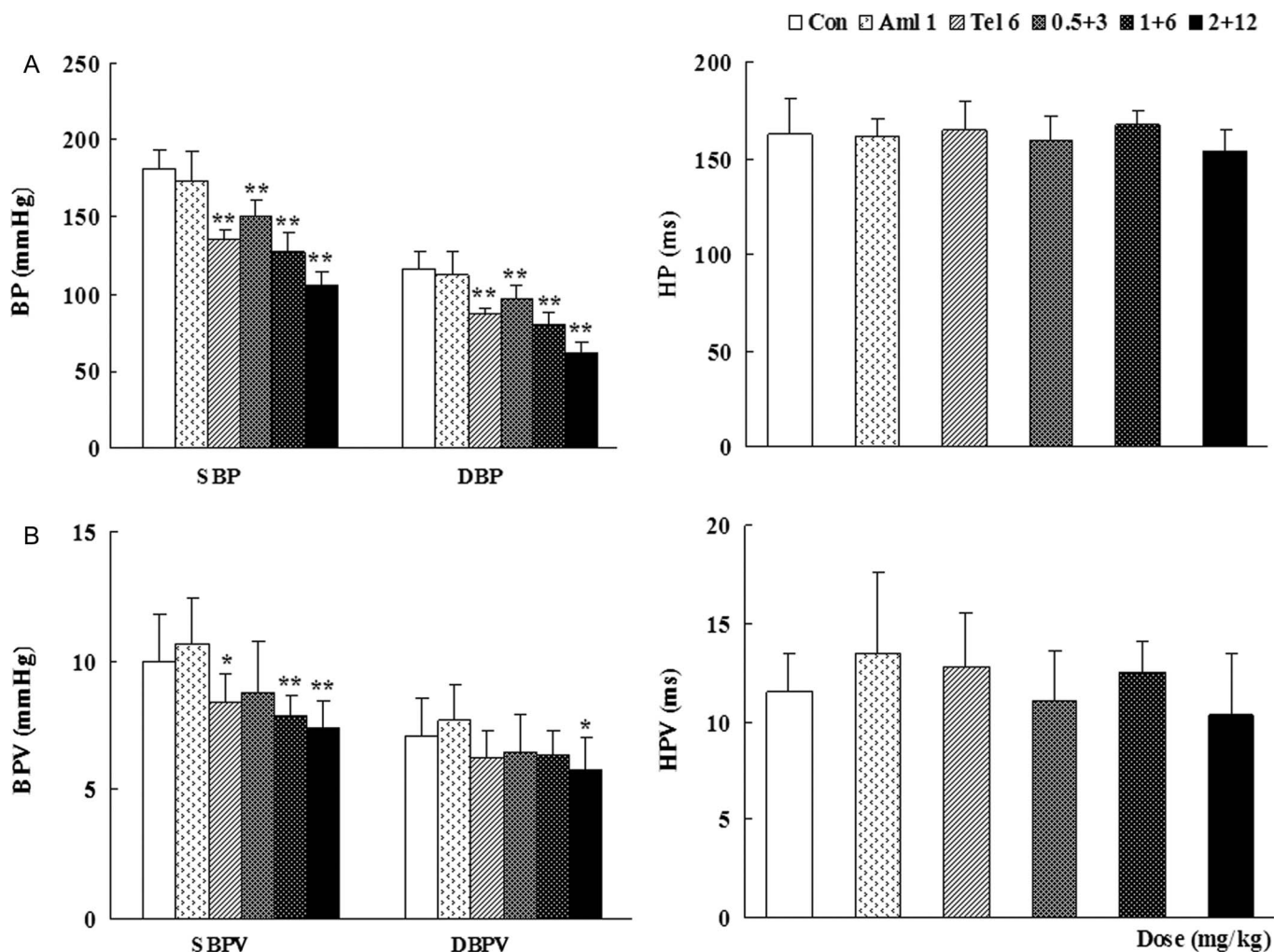


**FIGURE 3.** Effects of long-term treatment with amlodipine and telmisartan alone and in combination on blood pressure and heart period in conscious spontaneously hypertensive rats. SBP, systolic blood pressure; DBP, diastolic blood pressure; HP, heart period; SBP variability, DBP variability, and HP variability were expressed as the standard deviation of SBP, DBP, and HP, respectively. \* $P < 0.05$ , \*\* $P < 0.01$  versus before administration (adm).

telmisartan alone and three doses of combinations significantly decreased the ratio of heart weight to body weight, LVW/BW, AW/length, and GSS. These effects of combination therapy were dose-dependent. The high-dose combination of amlodipine and telmisartan (1 + 6 and 2 + 12) significantly ameliorated the heart hypertrophy and glomerulus damage ( $P < 0.05$ ). Microscopic examination of left ventricular and renal tissues from SHR demonstrated obvious pathologic changes, including cardiomyocyte hypertrophy, myocardial interstitial and perivascular fibrosis, wall thickening of intramyocardial arterioles, glomerular atrophy and fibrosis, and renal vascular sclerosis. These characteristic changes were significantly attenuated by long-term treatment with telmisartan and three doses of combinations. It should be noted that the degree of these characteristic changes were more significant in the groups of high-dose combination than in the group of telmisartan alone ( $P < 0.05$ ).

## DISCUSSION

The main findings of the present work may be summarized as follows: 1) there is a synergistic interaction between amlodipine and telmisartan on BP reduction in different hypertensive animals. The optimal dose ratio of the two drugs is 1:6; and 2) the long-term treatment with the combination of the two drugs in fixed proportion (1:6) appears effective not only with regard to reduction in BP, but also with regard to reduction in BPV and prevention of EOD.

Individualization is an important principle in the treatment of hypertension; however, identifying the best agent may take time. With combination therapy, it is obviously easier and quicker to control hypertension. We also expect that the combinations have potent synergistic effect on BP reduction. It may reduce the doses of each drug required in the treatment of hypertension and then can minimize the clinical and metabolic side effects of each individual component in larger dosage.

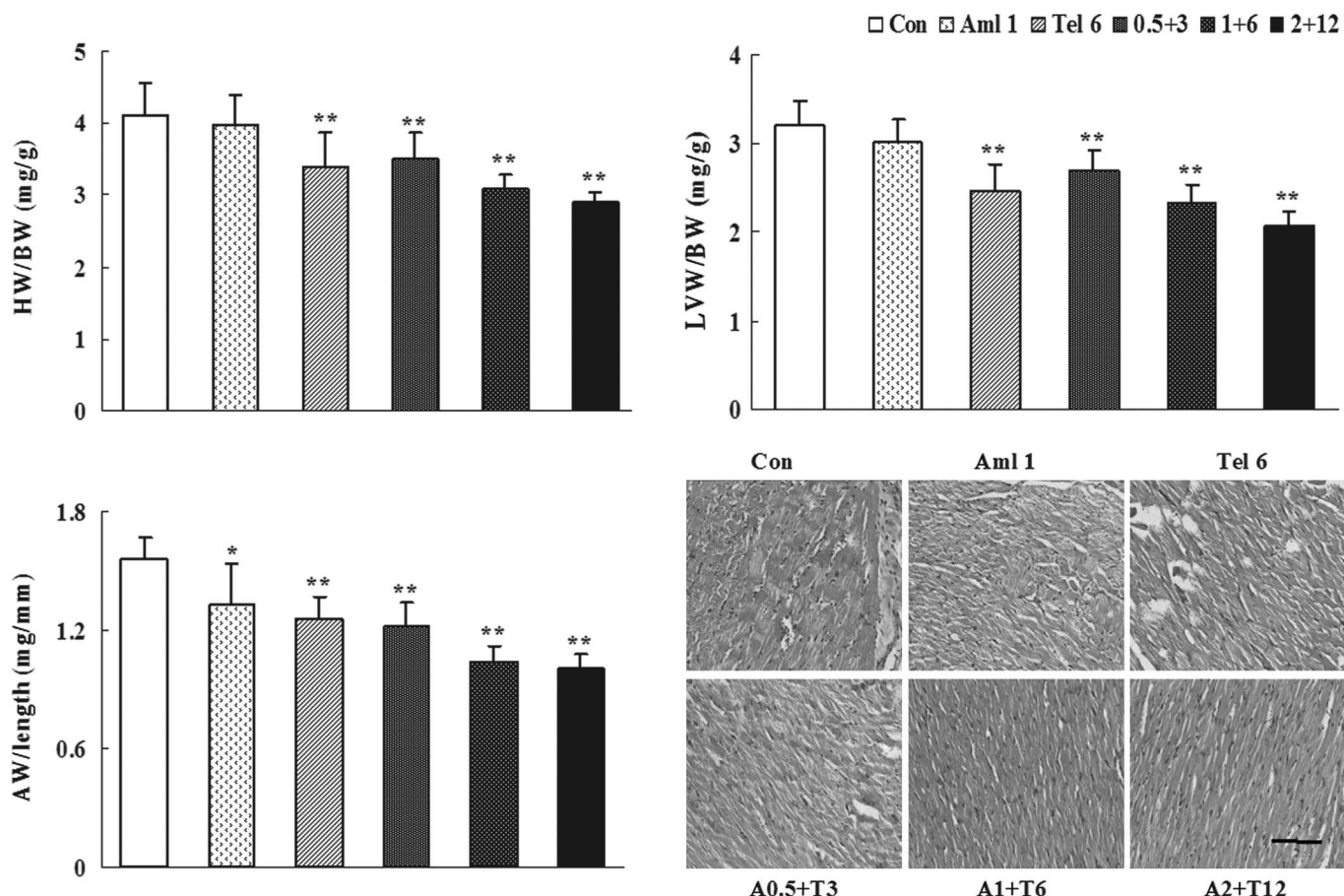


**FIGURE 4.** Effects of long-term treatment with amlodipine (Aml, 1 mg/kg per day) and telmisartan (Tel, 6 mg/kg per day) alone and in combination on end-organ damage parameters and on histopathologic changes in left ventricles in spontaneously hypertensive rats (SHR); ratio of heart weight to body weight, heart weight/body weight; LVW/BW, left ventricular weight/body weight; AW, aortic weight; Con, control untreated rats; 0.5 + 3, 1 + 6, and 2 + 12, daily doses of Aml and Can. \* $P < 0.05$ ; \*\* $P < 0.01$  versus Con. Top row: Control, Aml (1 mg/kg per day) and Tel (6 mg/kg per day); Bottom row: a low dose (0.5 + 3), a middle (1 + 6), and large dose (2 + 12). Scale bars = 500 μm.

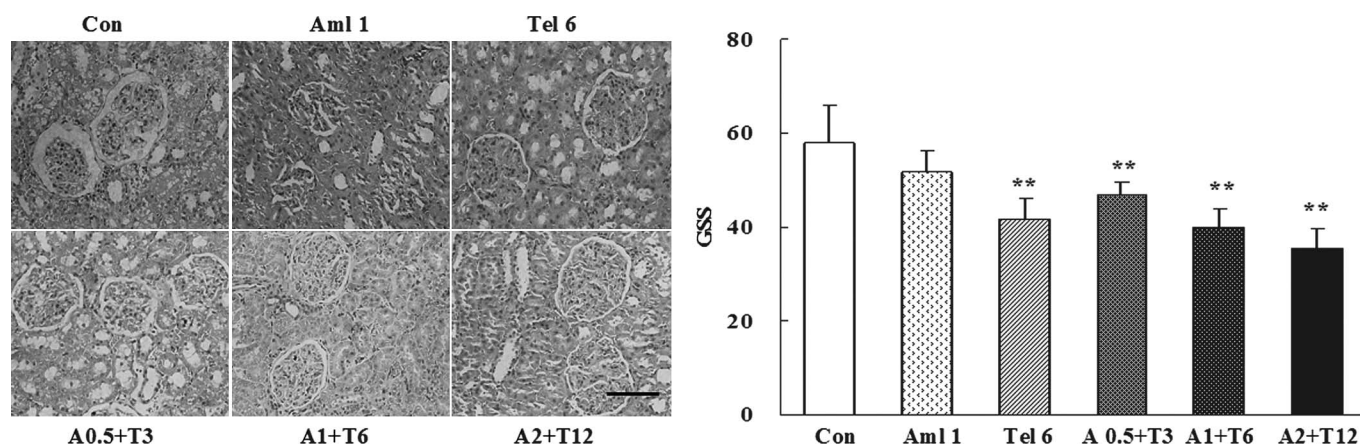


**FIGURE 5.** Effects of long-term treatment with amlodipine (Aml, 1 mg/kg per day) and telmisartan (Tel, 6 mg/kg per day) alone and in combination on histopathologic changes in the right kidney in SHR. SHR, spontaneously hypertensive rats. Top row: Control (Con), Aml (1 mg/kg per day), and Tel (6 mg/kg per day); Bottom row: a low dose (A0.5 + T3), a middle (A1 + T6), and large dose (A2 + T12). GSS, glomerulosclerosis score. \* $P < 0.05$ ; \*\* $P < 0.01$  versus Con. Scale bars = 500 μm.

when used alone.<sup>1</sup> How to combine the different antihypertensives should be carefully considered. Amlodipine and telmisartan are very common antihypertensives. They belong to calcium channel blocker and AT(1) receptor blocker classes, respectively, and have quite different mechanisms of action on the BP reduction and end-organ protection.<sup>8,22,23</sup> The mechanism responsible for the synergy should be further investigated.

The determination of synergistic interaction between drugs is not easy in practice. Recently, the probability sum test (q test) was successfully introduced to study the phenomenon of synergism of two antihypertensive drugs.<sup>20</sup> Using this test, we observed that the synergism between amlodipine and telmisartan was not significant in some proportion groups. The effect of the combination enhanced with increasing dose of medication, but the synergism decreased proportionally at higher doses. Adding different doses of telmisartan (0–16 mg/kg) to the higher dose of amlodipine group (4 mg/kg) produced a similar BP reduction, but the q values are not always larger than 1.15. In telmisartan (20 mg/kg) combination groups, the q values are smaller than 1.15 when adding different doses of amlodipine (1 and 2 mg/kg). In the proportion of 1:6, the q value ( $q = 3.00$ ) was larger than any other group, which implied this combination (1:6) might be the optimal combining proportion. When the proportion is smaller than 1:6, the synergistic effect was attenuated.

It should be noted that a large difference in q value between the combinations with the same ratio was observed. For example, the groups of 2 + 8 and 4 + 16 have the same ratio 1:4. The 4 + 16 group appears to induce a greater fall in BP, but the q value is smaller (1.200 versus 2.436). This is the result of the large doses used. Because the effectiveness of a single drug is high enough (responders = or greater than 50%), and the effectiveness of the combination reached up to the plateau (100%), the q value was attenuated. Generally, the dose of a single drug producing an effect between 10% and 40% (responders = 10–40%) might help produce a synergistic effect.

Interestingly, the synergistic effect of this proportion (1:6) was also observed in two-kidney one-clip rats. It is well accepted that SHR is the best animal model for human essential hypertension. The two-kidney one-clip model of hypertension shows high renin activity.<sup>24</sup> The totally different mechanism of action of these two drugs and the synergistic effect of the 1:6 proportion favor the blood pressure reduction in two different models of hypertension as illustrated here suggesting that this combination might be broadly suitable for treating different types of hypertensive patients.

Our results have some differences compared with another preclinical study with the  $4 \times 4$  factorial design of telmisartan/amlodipine.<sup>25</sup> Although these results show that there is no relevant amlodipine–telmisartan interaction, the combination using a ratio 1:4 or 1:8 provides a highly effective BP-lowering effect. The incidence of peripheral edema is also significantly reduced compared with telmisartan or amlodipine alone. Interestingly, the ratio 1:4 or 1:8 is near to our optimal combining proportion (1:6).

A major aim of antihypertensive therapy is to reduce cardiovascular events such as stroke, heart failure, renal failure, and acute myocardial infarction. The reduction of EOD is a very

important strategy for the prevention of these events. The parameters of EOD in this work included those reflecting left ventricular hypertrophy, aortic hypertrophy, and renal injury. LVW/BW and AW/length reflect changes in left ventricular hypertrophy and aortic hypertrophy directly. Kidney is one of the most important target organs in hypertension. Glomerulosclerosis and tubulointerstitial fibrosis lead to renal dysfunction. GSS reflect the degree of glomerulosclerosis and tubulointerstitial fibrosis.<sup>26</sup> In addition, BPV was also studied and improved considerably by the combination therapy. There is increasing evidence showing that high BPV contributes to EOD in hypertension.<sup>27–33</sup> Interestingly, the present work clearly demonstrates the benefit of this 1:6 proportion of amlodipine and telmisartan not only with regard to reduction in BP, but also with regard to reduction in BPV and protection against EOD. More importantly, in the chronic study, the combination at the low-dose ratio (0.5/3) appeared less effective than the dose ratio (2/12). Therefore, careful consideration should be given to selecting the proper doses of the combination.

In conclusion, we have demonstrated that there is a synergistic interaction between amlodipine and telmisartan on BP reduction. The greatest degree of synergism occurred when the dose ratio was 1:6 for amlodipine:telmisartan. The long-term treatment with this combination presents a beneficial effect on the reduction of BP, BPV, and the protection of end-organ with a proper dose.

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## Beneficial Effects of the Combination of Amlodipine and Losartan for Lowering Blood Pressure in Spontaneously Hypertensive Rats

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A combination of antihypertensive agents can better control blood pressure and reduce the number and severity of side effects than a monotherapy. Since both CCBs (calcium channel blockers) and ARBs (angiotensin II receptor type-1 blockers) are current and effective antihypertensive drugs, this study assessed the synergistic antihypertensive effects as well as the optimal combination ratio of these two drugs. Amlodipine (3 mg/kg) or losartan (30 mg/kg) alone or a combination of each drug at a ratio 1:10 and 1:20 was administered orally to spontaneously hypertensive rats (SHR). A four-week treatment of either 3 mg/kg amlodipine or 30 mg/kg losartan alone decreased the systolic blood pressure (SBP). However, their combination significantly lowered the SBP from the 3<sup>rd</sup> week, and there was a positive correlation between this reduction in blood pressure and the improvement in arterial endothelium-dependent relaxation. In addition, the combination therapy (1:20) decreased both the cardiac mass and left ventricular weight to a greater extent than with either amlodipine or losartan alone. The collagen content in the cardiac tissue was also significantly lower after the 4-week combination therapy (1:10). These results suggest that the combined use of amlodipine and losartan might be more effective in treating hypertension than a monotherapy.

**Key words:** Amlodipine, Losartan, Combination, Spontaneously hypertensive rat, Systolic blood pressure

### INTRODUCTION

Hypertension is one of the major risk factors for cardiovascular complications and can cause cerebrovascular and renal damage if not properly controlled. The optimal blood pressure is < 120/80 mmHg and the recommended blood pressure goal is <130/85mmHg, which is lower than the previous limit of 140/90mmHg (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003; Chobanian et al., 2003). However, in achieving these more strict blood pressure limits, there are some limitations in monotherapy within the framework of efficacy. In practice, < 50% of hypertensive patients reach their optimal blood pressure with monotherapy using diuretics,  $\beta$ -block-

ers, ACEI (angiotensin-converting enzyme inhibitor), and CCB (calcium channel blocker) (Materson et al., 1993). Moreover, the response rate can fall to almost 10% when the half-maximal doses are used (Lindholm et al., 2002).

Therefore, various combinations of antihypertensive agents have been marketed, and recent clinical guidelines for the treatment of hypertension recommend a combination therapy for appropriate blood pressure control (Chobanian et al., 2003; Whitworth, 2003; Gavras and Rosenthal, 2004; Rosenthal and Gavras, 2006). The advantage of a first-line treatment for hypertension with combination therapy compared with monotherapy are as follows: 1) there is some synergistic action when using two or more drugs that have different mechanisms of action – i.e. the blood pressure is controlled more effectively and the hypertension related complications are prevented, and 2) the number and severity of side effects can be reduced using low-dose range, thereby optimizing the patient's compliance (European Society

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of Hypertension-European Society of Cardiology Guidelines Committee, 2003).

Among the many combinations of antihypertensive drugs, it has been demonstrated that the calcium channel blocker (CCB) and angiotensin II receptor type-1 blocker (ARB) combination is useful for lowering blood pressure and preventing an impairment of various organs such as the heart and kidney (Yao et al., 2003a; Okuda et al., 2005; Yao et al., 2003b; Kohlmann et al., 2006). Therefore, a combination of dihydropyridine CCB (Amlodipine) and ARB (Losartan) is expected to be a promising option for the treatment of hypertension. This study assessed the therapeutic value of an amlodipine and losartan combination on both blood pressure reduction and organ protection in a SHR model.

## MATERIALS AND METHODS

### Animals

Male spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats, 12 weeks of age (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu, Japan), were used in this study. The rats were kept at  $23\pm 2^{\circ}\text{C}$  under a 12-h light-dark cycle, and allowed access to a commercial chow (5L79 diet, Labdiet, Indiana, USA) and tap water *ad libitum*. This study was performed in accordance with the institutional Standard Operation Procedure for Animal Care and Experiments (SOP-ANC) of the Dong-A Pharmaceutical Company and with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

### Drugs

Amlodipine orotate (Lot No. 6301) and losartan (Lot No. 6501) were synthesized at Dong-A Pharmaceutical Company Research Laboratories. The other chemicals were of reagent grade and purchased from commercial sources.

### Experimental protocols

The rats were divided into 6 treatment groups (5 rats in each group) as follows: (i) untreated age-matched WKY rats; (ii) vehicle-treated SHR-SP used as the control group; (iii) amlodipine orotate 3 mg/kg/day; (iv) losartan 30 mg/kg/day; (v) amlodipine 3 mg/kg/day combined with losartan 30 mg/kg/day (1:10 ratio); (vi) amlodipine 3 mg/kg/day combined with losartan 60 mg/kg/day (1:20 ratio). The drugs were suspended in 1% hydroxypropylmethylcellulose (HPMC) in a volume of 5 mg/kg body weight and administered orally over a four-week period.

### Body weight and blood pressure measurement

The body weight and systolic blood pressure (SBP) were measured at weekly intervals from the baseline period until sacrifice. The SBP was monitored indirectly using tail-cuff plethysmography (L120; IITC Life Science, San Diego, USA). Briefly, the rats were kept in a box warmed to  $38^{\circ}\text{C}$  for 10 min and then placed in a holder. The SBP was measured as the internal pressure of a latex cuff fixed around the tail of the animals, and the average of three readings was used for further analysis.

### Endothelial function analysis

The rats were sacrificed at the end of the treatment period, and the thoracic aorta was excised immediately and placed in a modified Krebs-Henseleit solution, containing (mmol/L): NaCl 118.0, KCl 45,  $\text{MgSO}_4$  1.2,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{NaHCO}_3$  25.0, glucose 11.0. The aortic tissue was dissected from the adherent fat and connective tissues, and the arteries were cut into 2-3 mm rings. Extreme care was taken to avoid stretching the arteries during the dissection. The contractile force was measured by mounting the aortic rings in 15 ml organ baths containing Krebs-Henseleit solution under a basal tension of 2 g and attaching then to a force displacement transducer (GRASS TELEFACTOR, Astro-Med, Inc., USA). The data was recorded on a polygraph (ML785 PowerLab, ADInstruments). The tissue bath was maintained at  $37^{\circ}\text{C}$  and bubbled with a 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  gas mixture. Each preparation was allowed at least 60 min to stabilize before performing the experiment. During this period the incubation medium was changed every 15 min. The presence of an endothelium was confirmed by the occurrence of relaxation in the rings contracted with norepinephrine ( $10^{-5}$  mol/L) in response to acetylcholine ( $10^{-6}$  mol/L). After washing and re-equilibration, the aortic rings were precontracted with norepinephrine ( $10^{-5}$  mol/L). When the contractile response was stable, the endothelium-dependent relaxation was measured by adding acetylcholine at increasing cumulative concentrations ( $10^{-9}$  to  $10^{-5}$  mol/L). The relaxation is expressed as the percentage decrease in the maximum tension obtained using the norepinephrine-induced contraction.

### Evaluation of cardiac hypertrophy

At the end of experiment, all the animals were sacrificed by a cervical dislocation. The isolated heart was washed out with warmed saline to eliminate the excess blood, and then fixed in 10% neutralized formalin solution. After fixation, the heart

was weighed, and dissected into the left ventricle, inter ventricular septum and the right ventricle, which were then weighed individually. The portion of the middle one-third near the apex was embedded in paraffin and stained using Masson's trichrome technique (Sigma Diagnostics, St. Louis, Mo. USA) to determine the collagen level. The areas of collagen were determined and added to obtain the total square using an Axioskop 2 microscope (Zeiss Co. Ltd., Germany) and an image-analyzer (Image-pro plus, media cybernetics USA).

### Statistical analysis

The data is expressed as the mean  $\pm$  SD. One-way analysis of variance (ANOVA) followed by a Dunnett's test was used to determine the significance of the difference between the different groups. A  $p$  value  $<0.05$  was considered significant.

## RESULTS

### Effects of amlodipine and losartan alone, and their combination on the systolic blood pressure

Fig. 1 shows the hypotensive effects in SHR of each drug and their combination. At the beginning of the experiment, the SBP in the SHR was almost double that in the normotensive WKY rats. Before administering the drugs, there were no significant differences between the SHR groups. Although 3 mg/kg amlodipine and 30 mg/kg losartan alone lowered the SBP after 3 and 4 weeks treatment respectively, their combination significantly lowered the SBP from the 3<sup>rd</sup> week. Therefore, the combina-

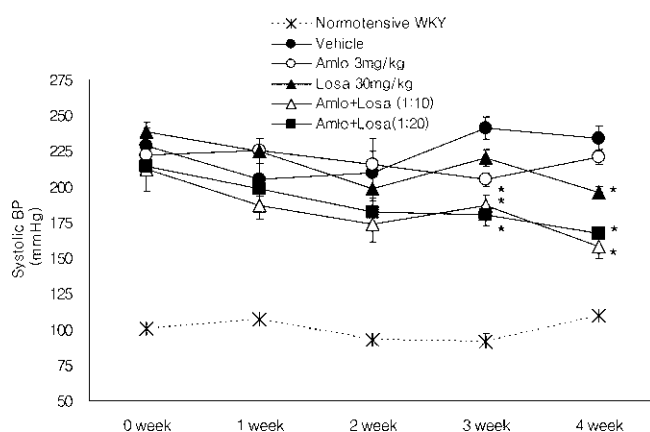


**Fig. 1.** The changes in blood pressure by the amlodipine and/or losartan treatment. The systolic pressure was measured every week for 4 weeks. \*significantly different from the vehicle-treated group,  $P<0.05$ . The data is expressed as the mean $\pm$ SD.

tion of amlodipine and losartan decreased the blood pressure of the SHR more potently than either amlodipine or losartan alone.

### Effects on Ach-induced aorta relaxation

Fig. 2 shows the vasodilatation response of the thoracic aorta to cumulative doses of acetylcholine. The  $EC_{50}$  of the SHR rats was almost 45 times higher than that of the normotensive WKY rats ( $1.483 \mu\text{mol/L}$  vs  $0.033 \mu\text{mol/L}$ , respectively). Treatment with losartan alone or combined with amlodipine significantly ( $p<0.05$ ) improved the endothelium-dependent relaxations compared with the vehicle treated animals. However, there was a similar level of endothelium-dependent relaxation between the combination ratios of these two drugs ( $EC_{50}$  of Ach in 1:10 and 1:20 combination ratio was  $0.226$  and  $0.209 \mu\text{mol/L}$ , respectively).

### Effects on body weight and cardiac hypertrophy

Table I shows the body weight, heart and left ventricular weight of all the experimental groups. There were no significant differences in body weight observed during the entire experimental period. The relative heart and left ventricular weight were significantly ( $p<0.05$ ) higher in the SHR rats (32 and 42%, respectively) than in the normotensive rats. This increase in heart weight was attenuated significantly by losartan alone and when combined with amlodipine (1:20 ratio). The degree of decrease in the cardiac weight was 14.4% in losartan alone

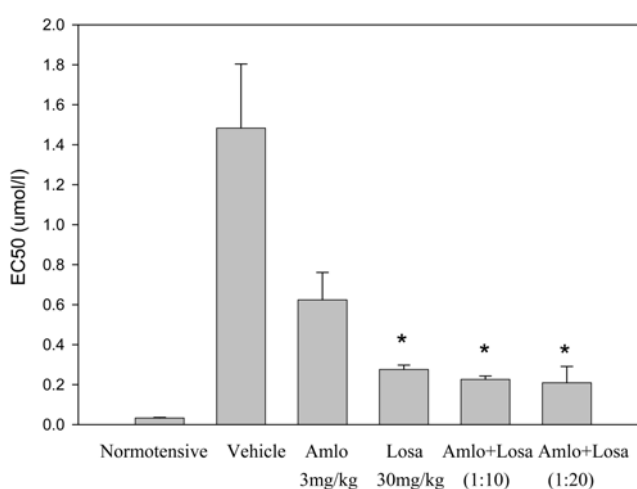


**Fig. 2.** Effect of amlodipine and/or losartan on the acetylcholine-induced thoracic aorta relaxation. \*significantly different from the vehicle-treated group,  $P<0.05$ .  $EC_{50}$ , effective concentration of acetylcholine that induced a 50% relaxation of the pre-contracted thoracic aorta. The data is expressed as the mean $\pm$ SEM.

**Table I.** Body weight, cardiac weight, and left ventricular weight in each group of SHR

|                                    | BW (g)     | Heart (mg/g BW)          | Left ventricle (mg/g BW) |
|------------------------------------|------------|--------------------------|--------------------------|
| Normotensive                       | 370.8±19.7 | 3.49±0.05                | 1.54±0.16                |
| Vehicle                            | 337.8±15.6 | 4.59±0.39 <sup>a</sup>   | 2.19±0.14 <sup>a</sup>   |
| Aml 3 mg/kg                        | 339.8±17.2 | 4.38±0.28 <sup>a</sup>   | 2.28±0.20 <sup>a</sup>   |
| Losa 30 mg/kg                      | 344.6±12.0 | 3.93±0.06 <sup>a,b</sup> | 1.95±0.11 <sup>a</sup>   |
| Aml 3 mg/kg + Losa 30 mg/kg (1:10) | 326.0±18.6 | 4.17±0.12 <sup>a</sup>   | 2.08±0.14 <sup>a</sup>   |
| Aml 3 mg/kg + Losa 60 mg/kg (1:20) | 327.0±15.8 | 3.75±0.16 <sup>a,b</sup> | 1.85±0.07 <sup>b</sup>   |

Aml, amlodipine; Losa, losartan; BW, body weight.

<sup>a</sup>significantly different from normotensive rats,  $p < 0.05$ .

<sup>b</sup>significantly different from vehicle-treated group,  $p < 0.05$ .

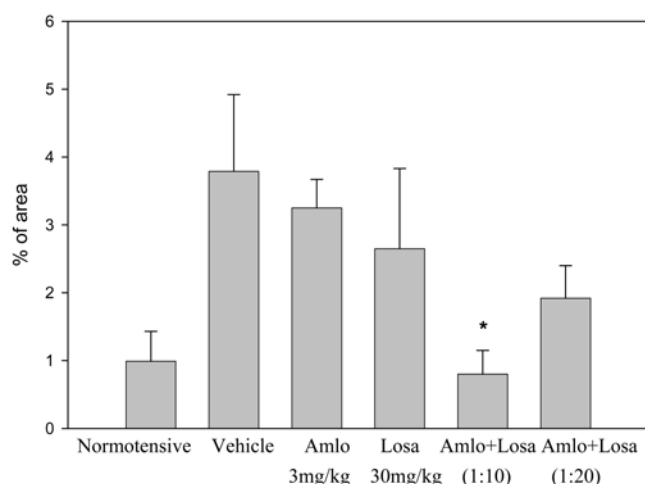


**Fig. 3.** Effect of amlodipine and/or losartan on the cardiac collagen content. The results are presented as a percentage of the collagen positive area per total area. \*significantly different from the vehicle-treated group,  $P < 0.05$ .

group and 18.3% in amlodipine plus losartan (1:20) group. In addition, only the combination of amlodipine with losartan (1:20) treatment significantly (15.5%,  $p < 0.05$ ) lowered the relative left ventricular weight compared with the vehicle.

#### Effects on cardiac morphometry

Fig. 3 shows the changes in the collagen content in the cardiac tissue. The collagen content of the vehicle treated group was 4 times higher than in the normotensive WKY rat. Amlodipine combined with losartan (1:10 ratio) significantly ( $p < 0.05$ ) decreased this increase in collagen content to almost the level of the vehicle group. Fig. 4 shows the representative photographs of the left ventricle from the normotensive WKY rats, vehicle treated SHR, and SHR treated with amlodipine combined with losartan (1:10).

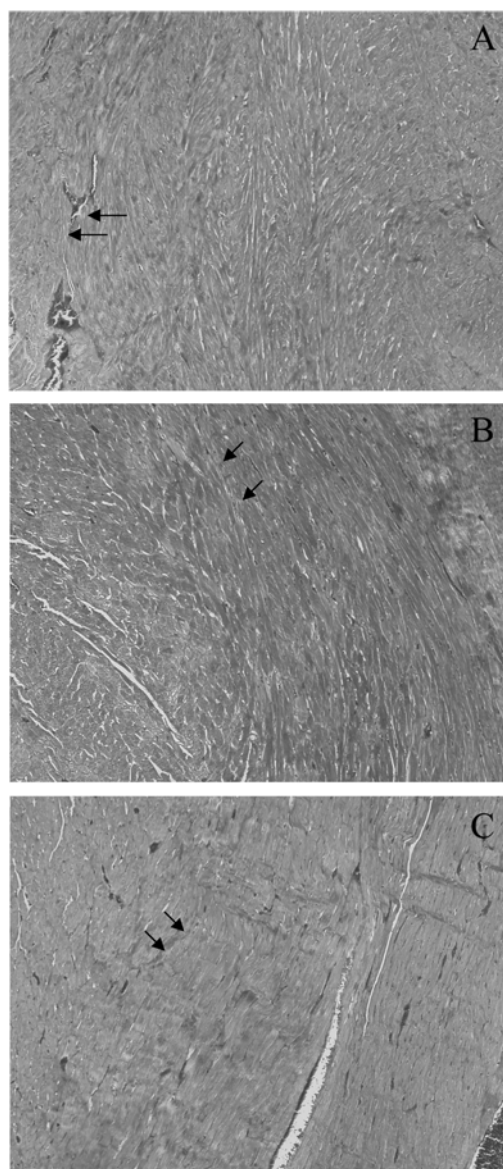


**Fig. 4.** Representative cardiac collagen content in each group. A, normotensive WKY rat; B, vehicle-treated SHR; C, Aml 3 mg/kg combination with Losa 30 mg/kg (1:10). The interstitial collagen fibers staining blue (arrows) were combined using a computer-assisted analysis system.

## DISCUSSION

In a previous study, the efficacy and tolerability of a fixed-combination of amlodipine and losartan (1:20) was evaluated in 204 Brazilian patients (Kohlmann et al., 2006). Their study demonstrated that the combined use of amlodipine and losartan has a high antihypertensive efficacy, which can be sustained for a long-time. In Indian post-marketing surveillance recorded from 728 patients, most of the patients prescribed the losartan-amlodipine combination (93.8%) showed a good to excellent response (Gokhale et al., 2002).

Based on this clinically proven combination ratio, the present study further evaluated the combination ratio to determine the rationale for the most appropriate combination ratio. These observations provide evidence that the lower losartan combination had as much effect on lowering the blood pressure and alleviating the related symptoms as the established combination ratio. These results also reconfirmed that the amlodipine-losartan combination has a larger hypotensive effect in the hypertension animal model compared with either of the antihypertensives used alone. In addition, the combined treatment with amlodipine and losartan also alleviated the cardiac hypertrophy and endothelial dysfunction more effectively than the monotherapy.

Recent studies have shown that amlodipine reduces the extent of left ventricular hypertrophy and regressed collagen deposition (Tomassoni et al., 2003), which persisted after treatment withdrawal (Sevilla et al., 2004). This beneficial effect of amlodipine can be explained by the inhibition of calcium entry in the myocytes (Sevilla et al., 2004). Losartan also has a similar beneficial effect on the heart, which affects not only the myocytes but also the production of collagen and other matrix tissues (Makino et al., 1997).

Evidence from experimental and clinical studies suggests that a multitherapy-based antihypertensive regimen, which includes inhibitors of the rennin-angiotensin system and calcium channel antagonists, can improve the endothelial function (Schulman et al., 2005). It was reported that in addition to its well-known antihypertensive effect, amlodipine improves the endothelial function in patients with arterial hypertension (On et al., 2002). Basically, the molecular mechanism of this effect can be explained by its antioxidative effect (Franzoni et al., 2004). Amlodipine prolongs the half-life of NO through its reactive oxygen species (ROS) scavenging capacity, accompanied by the NO formation enhancing activity

(Berkels et al., 2004), which can improve the NO bioavailability. The improvement in the endothelial function by losartan appears to be dependent on the increased relaxant factor such as NO availability (Maeso et al., 1998) similar to that of amlodipine, and the normalization of the constricting factor such as endothelin-1 content (d'Uscio et al., 1998).

Based on these results, it is concluded that the combined treatment of amlodipine and losartan is more effective in treating hypertension and cardiovascular disease than a monotherapy. Furthermore, it was confirmed that a lower losartan ratio (1:10) than the clinically proven combination ratio has a similar effect in the treatment of cardiovascular hypertrophy and remodeling.

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# Role of Combination Therapy in the Treatment of Hypertension: Focus on Valsartan plus Amlodipine

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## ABSTRACT

Hypertension control is rare in clinical practice, particularly in high-risk patients. A large factor is therapeutic inertia deriving from poorly prescribed lifestyle changes, excess monotherapy use, and scarce on-treatment modifications. The use of drug combinations significantly improves blood pressure (BP) control; in particular, fixed combinations improve therapy without increasing daily pill intake, thereby favouring patient compliance and therapy continuation.

The most widely used fixed combination is based on thiazide diuretics added to either angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs). Several large-scale clinical trials have been conducted showing that these combinations are effective in lowering BP. However, thiazide diuretics can reduce the metabolic benefits derived from renin–angiotensin–aldosterone system (RAAS) inhibitors in high metabolic risk patients. In contrast, ACE inhibitors or ARBs combined with dihydropyridine calcium channel antagonists (DHPCAs) exert a marked antihypertensive effect without decreasing metabolic protection by RAAS blockade. In the recent JIKEI heart study, ~60% of patients affected by hypertension, heart failure, coronary heart disease or their combination in the valsartan arm were simultaneously treated with DHPCAs. Of note, a 39% reduction in the primary endpoint of combined morbidity and mortality was reported in the valsartan compared with the non-valsartan arm. Furthermore, in a recent multinational study, 83% of 3161 hypertensive patients treated with valsartan and the DHCPA amlodipine reported a

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controlled BP after 8 weeks of treatment. As expected, amlodipine did not negatively influence the metabolic profile of patients, thereby supporting the role of ARB+DHPCA combinations as effective and promising tools in hypertension treatment.

In summary, the combination of ARBs with DHPCAs is an effective strategy in hypertension treatment through synergy between their antihypertensive and vascular protective actions, persistent metabolic benefits deriving from RAAS inhibition, and reduced incidence of side effects.

**Keywords:** amlodipine; angiotensin-converting enzyme inhibitors; angiotensin receptor antagonists; dihydropyridine calcium channel antagonists; hypertension; treatment; valsartan

## BLOOD PRESSURE CONTROL IN TREATED HYPERTENSIVE PATIENTS

In the most recent National Health and Nutrition Survey (NHANES 2003–2004) overall age-adjusted prevalence of hypertension in US residents was 29.3%.<sup>1</sup> The percentage of these patients who reached target blood pressure (BP) levels was 33.1% for all hypertensive patients and 63.9% for those receiving antihypertensive treatment.<sup>1</sup> The number of patients treated with antihypertensives who reached target BP levels has not changed from the 2001–2002 survey, even though more patients received antihypertensive therapy.<sup>1,2</sup> Compared with the US, the prevalence of hypertension is even higher in European countries (44%).<sup>3</sup> Despite this, hypertension treatment is less frequent in Europe (26.8%), ranging from 26% in Germany to 32% in Italy.<sup>3</sup> Therefore, it is not surprising to note that only 8% of the whole hypertensive population in Europe reported a BP of <140/90 mmHg.<sup>3</sup> Thus, it is evident that BP control in hypertensive patients is still

far from ideal in both the US and Europe, particularly in patients who are receiving antihypertensive medication.

Concordant with the above surveys, a recent pooled analysis conducted in 52,715 Italian hypertensive patients reported stable hypertension treatment in 69% of patients ( $n=36,556$ ). Mean systolic (SBP) and diastolic blood pressure (DBP) were  $148\pm 9$  mmHg and  $90\pm 5$  mmHg, respectively,<sup>4</sup> indicating that the mean SBP, rather than DBP, was abnormal. BP stratification was obtained in 40,829 individuals and demonstrated that about one third (32.6%) of uncontrolled hypertensives had mean BP of >160/100 mmHg. Thus – also based on the reported prevalence of other concomitant risk factors and target organ damage in the same analysis<sup>4</sup> – it seems evident that most Italian hypertensive patients who received antihypertensive treatment for a high or very-high cardiovascular risk profile had an unchanged risk profile at the end of treatment.

In keeping with the above, similar findings have been found in a recent survey conducted in our unit. Indeed, in con-

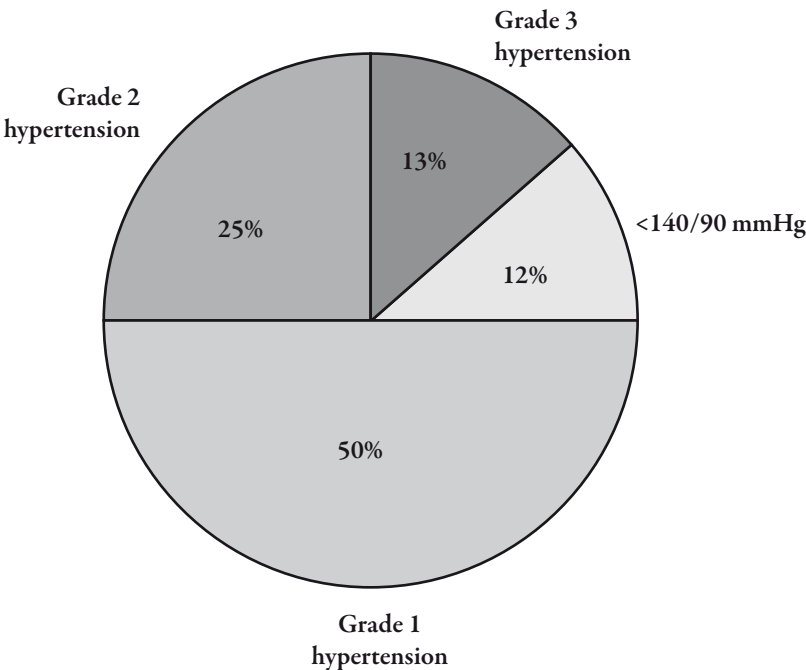
secutive outpatients who referred for the first time, following treatment by specialists or general practitioners, we observed that only 12% of patients had BP levels of <140/90 mmHg, while 38% had grade 2/3 hypertension despite being often prescribed antihypertensive medication (Figure 1). Even more perplexing, the vast majority of these patients were rarely affected by hypertension alone and usually presented with at least one additional cardiovascular risk factor (20% had  $\geq 2$  additional risk factors). Thus, it appears that a lack of BP control is not limited to low-risk patients, but also to those who have an increasing constellation of risk factors known as the metabolic syndrome.<sup>5</sup> In line with this hypothesis, the recent European Action on Secondary Prevention Through Intervention to Reduce Events (EUROASPIRE)

III survey was conducted in 22 European countries and demonstrated that the majority of patients with known atherosclerotic disease were still not achieving BP, lipid and glucose targets.<sup>6</sup> Therefore, according to these data, it is likely that approaches to cardiovascular disease prevention need to be revised and adjusted.

**LACK OF HYPERTENSION  
CONTROL IN TREATED  
HYPERTENSIVE PATIENTS:  
A CALL TO ACTION**

Lack of hypertension control is mainly responsible for the persistently elevated hypertension-related cardiovascular morbidity and mortality observed in treated patients.<sup>1-4</sup> Such effects persist despite several antihypertensive agents being

**Figure 1.** First visits in a hypertension and cardiovascular prevention outpatient unit in patients previously followed in other settings by either specialists or general practitioners.



available, and effective lifestyle-based antihypertensive strategies having clearly demonstrated effective BP reduction in the general hypertensive population<sup>7</sup> as well as in hypertensives with additional cardiovascular risk factors.<sup>8</sup> Additional interventions suggested to improve BP control have often been debated and include education programmes aiming to encourage and then maintain lifestyle changes in the long run, evaluation of physician approaches to cardiovascular risk factor control by quality and not economic indicators, implementation of simplified guidelines for patients and general practitioners, counselling with patients to improve awareness and collaboration in the management of hypertension, and increased use of home BP monitoring.<sup>7–12</sup> The main aim of all of these measures is to reduce therapeutic inertia,<sup>9,10</sup> i.e. the main culprit responsible for the lack of BP control in the vast majority of treated hypertensive individuals.

In this context, one of the most important tools in the fight against therapeutic inertia is represented by combination treatments.<sup>11,12</sup> Fixed combination therapies are particularly able to significantly increase patient compliance and long-term stay on-treatment, reduce side effects and (when appropriately chosen) allow the achievement of lower SBP and DBP levels.<sup>13</sup>

## COMBINATION TREATMENT IN ESSENTIAL HYPERTENSION

While initial guidelines on BP control emphasised the role of monotherapy, in recent years, clinical trials and daily practice experience have suggested that

such an approach is not effective in the majority of patients.<sup>14–16</sup> In keeping with the above, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of Blood Pressure (JNC 7) suggests that antihypertensive therapy should start with two drugs when a patient presents with a BP of >20 mmHg above SBP and/or >10 mmHg above DBP target.<sup>17</sup> Furthermore, the more recently available European guidelines<sup>18</sup> clearly state that the majority of hypertensive patients enrolled in trials start on a single agent but end up on combination therapy. Combination treatment is particularly encouraged in patients with higher risk profiles, more marked BP elevation and lower BP targets.<sup>18</sup> Thus, statements from different expert panels concluded that combination treatment should be preferred in most hypertensive individuals. An explanatory flow chart – largely based on the European Guidelines<sup>18</sup> – depicting the possible pathways leading to the more appropriate choice is shown in Figure 2.

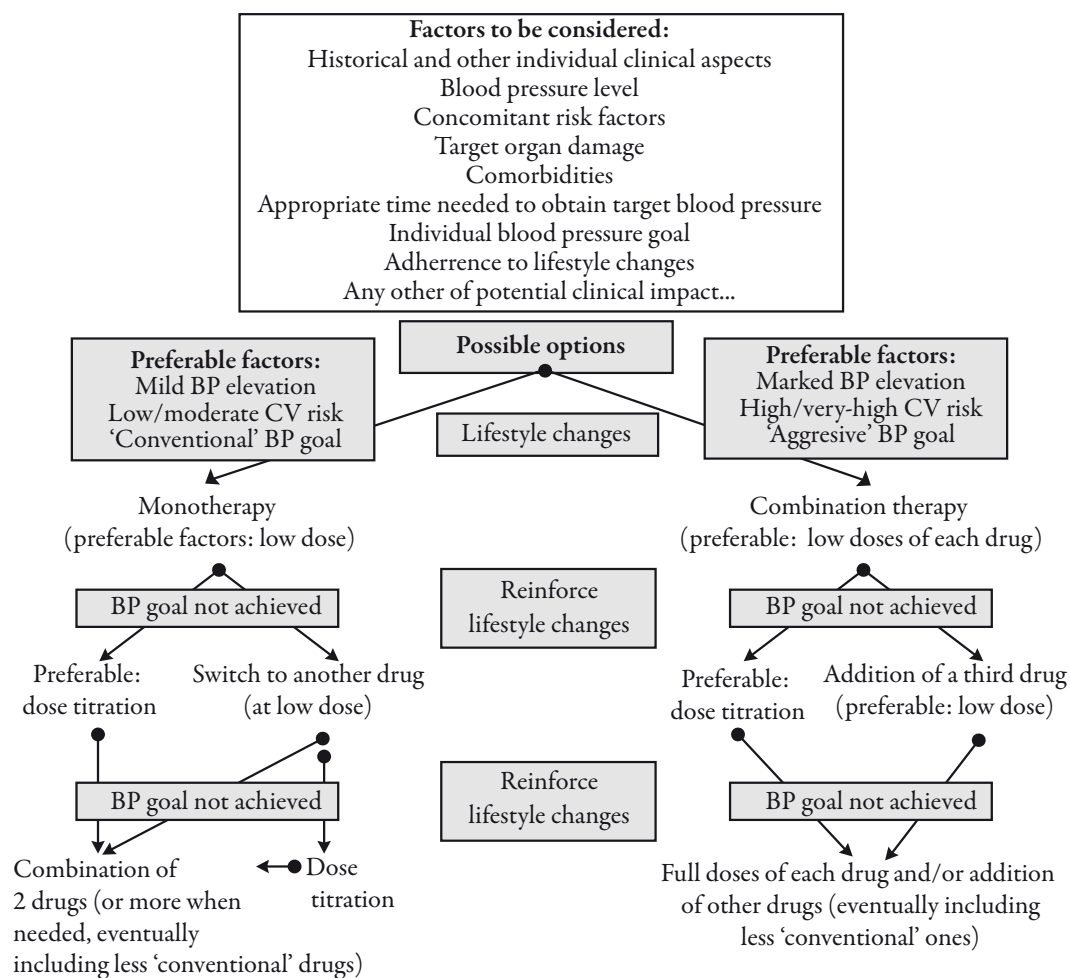
In summary, in contrast to previous approaches, current guidelines support the evidence that combination treatment is often necessary in the vast majority of hypertensive patients. In particular, the same guidelines also indicate that combination of two drugs should be considered as the initial treatment in most cases.

## Combination Therapy Based on Angiotensin Receptor Blockers

Antihypertensive therapies based on angiotensin receptor blockers (ARBs) have been demonstrated to reduce composite outcomes comprising cardiovas-

**Figure 2.** Antihypertensive treatment in the general hypertensive population: a practical flow chart.<sup>18</sup>  
BP=blood pressure; CV=cardiovascular.

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cular and cerebrovascular events;<sup>19</sup> slow down the progression from incipient to overt nephropathy in type 2 diabetic hypertensive patients;<sup>20</sup> reduce the progression of overt renal damage in type 2 diabetic patients;<sup>21</sup> be effective in reducing outcomes in patients with heart failure<sup>22</sup> and after an acute myocardial infarction;<sup>23</sup> and reduce cardiovascular morbidity and mortality in patients affected by either hypertension, coronary

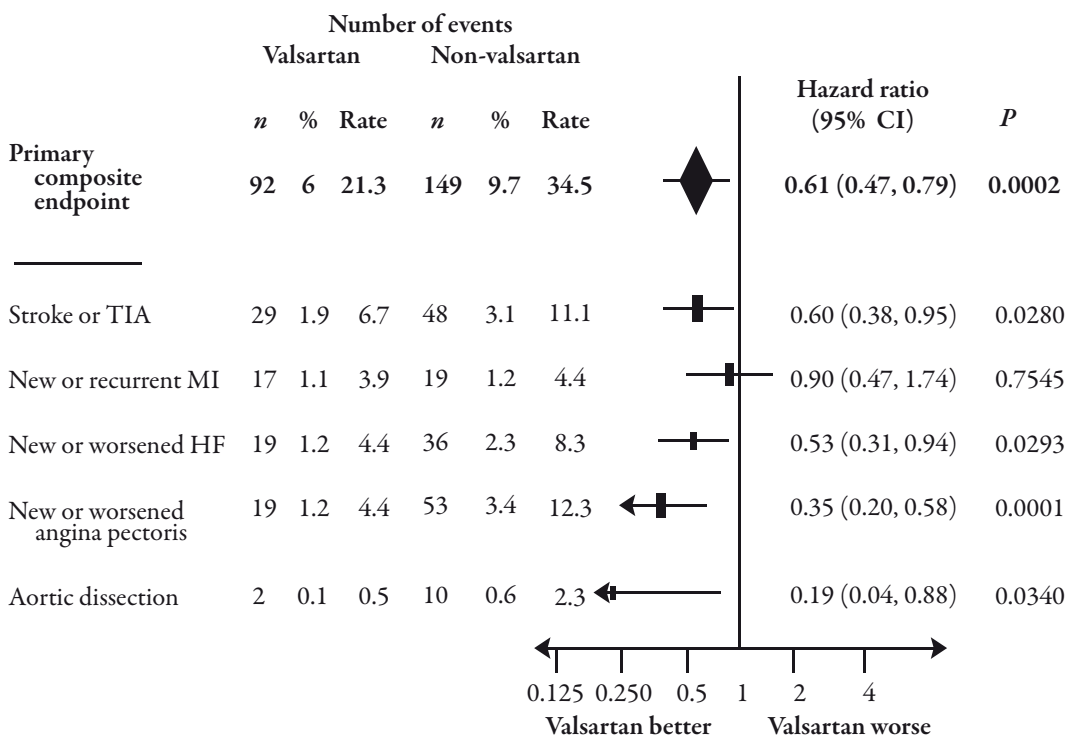
heart disease, heart failure or a combination of these diseases.<sup>24</sup> The majority of patients enrolled in the above trials received the ARB valsartan. Studies conducted in hypertensive individuals clearly showed that the ability of valsartan to reduce SBP and DBP levels was dose-dependent, with progressive BP decrements from 40 mg to 320 mg daily.<sup>25</sup> Accordingly, in the recent JIKEI heart study<sup>24</sup> SBP/DBP levels after a mean follow-up period of 3.1 years

(range, 1–3.9 years) were 131/77 mmHg in a cohort of 1541 Japanese patients at high cardiovascular risk randomised to receive valsartan treatment instead of placebo ( $n=1540$  patients), in both cases in addition to conventional treatment. Therefore, achieved BP in the JIKEI heart study was markedly below the threshold of 140/90 mmHg and very close to the levels of <130/80 mmHg that have been recommended by more recent European guidelines in high-risk individuals.<sup>18</sup> Achievement of these BP levels was accompanied by significant reductions of the primary composite and secondary endpoints in patients taking valsartan at

a dose ranging from 40 to 160 mg once daily (mean dose, 76 mg daily) compared with those taking placebo (Figure 3). Of note, at the end of the JIKEI heart study about two thirds of patients were on simultaneous treatment with a dihydropyridine calcium channel antagonist (DHPA), one third with an angiotensin-converting enzyme (ACE) inhibitor, one third with a beta blocker and 10% with a diuretic.<sup>24</sup> Thus, based on the JIKEI heart study findings it is evident that most high-risk patients require the prescription of ARBs in appropriate combination with another antihypertensive treatment to obtain optimal BP control.

**Figure 3.** Reduction in incidence of primary and secondary endpoints in valsartan- and non-valsartan-treated patients in the JIKEI heart study.<sup>24</sup> CI=confidence interval; HF=heart failure; MI=myocardial infarction; TIA=transient ischaemic attack.

*Reprinted from The Lancet, Vol 369, Mochizuki S, et al. Valsartan in a Japanese population with hypertension and other cardiovascular disease (JIKEI heart study): a randomised, open-label, blinded endpoint morbidity-mortality study. Pages 1431–1439. Copyright 2007, with permission from Elsevier.*



Thiazide diuretics have historically represented the most widely used combination drug with ARB-based therapy.<sup>26</sup> ARBs provide enhanced activity when combined with 12.5 or 25 mg hydrochlorothiazide (HCTZ), thereby providing an important option for tighter BP control.<sup>27</sup> In turn, response to HCTZ is also enhanced by the use of ARBs and patients exhibiting a low response to HCTZ monotherapy often report significant BP reductions when the diuretic is combined with an ARB.<sup>27</sup>

In keeping with this, in the recent COMparative Study of efficacy of Irbesartan/HCTZ with valsartan/HCTZ using home blood pressure Monitoring in the treatment of mild-to-moderate hypertension (COSIMA), the addition of ARBs was able to induce further BP reductions in 464 patients unresponsive to previous diuretic monotherapy.<sup>28</sup> However, in the COSIMA study patients received 12.5 mg HCTZ daily as additional treatment to the ARBs irbesartan (150 mg daily) or valsartan (80 mg daily).<sup>27</sup> In clinical practice, most patients require the use of higher doses of HCTZ, i.e. 25 mg daily, reporting greater BP reduction but also potential increments in thiazide-related side effects. Thus, in the INCLUSIVE trial<sup>29</sup> patients ( $n=844$ ) who still had uncontrolled SBP levels following 4 weeks of treatment with monotherapy were treated sequentially with placebo (4–5 weeks), HCTZ 12.5 mg once daily (2 weeks), irbesartan 150 mg/HCTZ 12.5 mg once daily (8 weeks), and finally with irbesartan 300 mg/HCTZ 25 mg (8 weeks). The mean change in SBP from placebo phase to week 18 (intention-to-treat,  $n=736$  patients) was  $-21.5\pm$

$14.3$  mmHg ( $P<0.001$ ), while the mean change in DBP was  $-10.4\pm 8.7$  mmHg ( $P<0.001$ ). Overall, 77% (95% confidence intervals [CI]: 74%, 80%) of patients achieved a SBP of  $<140$  mmHg, 83% (95% CI: 80%, 86%) achieved a DBP of  $<90$  mmHg, and 69% (95% CI: 66%, 72%) achieved dual SBP/DBP goals. Although combination treatment was well tolerated, only one patient manifested with hypotension with the addition of low-dose HCTZ (12.5 mg) versus eight patients among those receiving the full daily dose (i.e. 25 mg). Similarly, three versus eight patients reported fatigue in the 12.5 mg compared with the 25 mg HCTZ groups, respectively.<sup>29</sup> Thus, the use of HCTZ 25 mg daily enhanced BP response to irbesartan and was effective, although possibly associated with very modestly worse tolerability.

However, the possibility that diuretics, in general, and HCTZ, in particular, might counteract, at least in part, some of the benefits deriving from renin–angiotensin–aldosterone system (RAAS) inhibitors is supported by two recent post-hoc analyses of the VALUE trial.<sup>30–32</sup> In the first of these analyses, valsartan, compared with amlodipine, was able to significantly reduce the incidence of new-onset type 2 diabetes mellitus in hypertensive patients at high cardiovascular risk.<sup>31</sup> Significant protection was still evident in valsartan-treated hypertensives when a more recent definition of fasting hyperglycaemia was used, as well as in patients taking diuretics and/or beta blockers in addition to ARB treatment.<sup>31</sup> However, new-onset type 2 diabetes mellitus occurred in 15.8% of these latter patients compared with 9.8%

of valsartan-treated patients as a whole. Thus, metabolic protection by valsartan persisted, but was mildly reduced by the addition of beta blockers and/or diuretics. Concordantly, the second post-hoc analysis<sup>32</sup> of the VALUE trial showed that new-onset type 2 diabetes mellitus had detrimental effects on cardiovascular prognosis, inducing an increment in the hazard ratio for total cardiac morbidity of 1.43 (95% CI: 1.16, 1.77;  $P=0.0008$ ), supporting the notion that reduced metabolic protection induced by beta blockers and/or diuretics added to previous ARB treatment might negatively influence, at least in part, individual cardiovascular prognosis.

### Rationale for the Combination of ARBs with DHPACs

Apart from the evident cardiovascular benefits deriving from the combination of the ARB valsartan with calcium antagonists coming from the recent JIKEI heart study,<sup>24</sup> the possibility that simultaneous angiotensin II type 1 ( $AT_1$ ) receptor and voltage-dependent calcium channel blockade may result in a marked synergy with regard to individual BP-lowering action and vascular protective properties derives from different experimental studies.

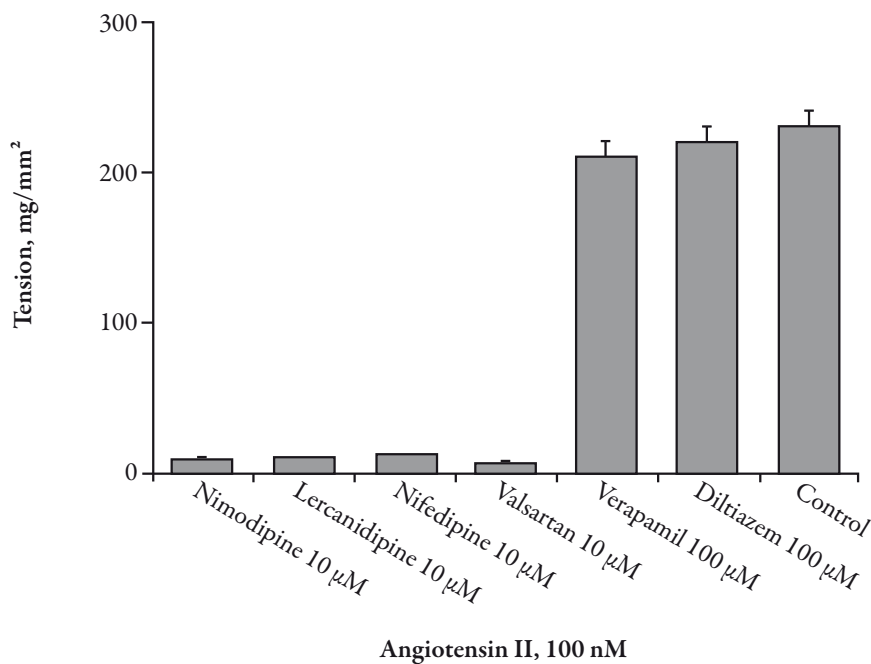
As is known, the  $AT_1$  receptor plays an important role in the structural and functional integrity of the arterial wall.<sup>33</sup> Most of the  $AT_1$ -mediated vascular effects, including vascular contraction, are dependent on activation of a number of intracellular pathways and cytosolic free  $Ca^{++}$  concentration which, in turn, is increased by angiotensin II via the same receptor.<sup>34,35</sup> Although based on cytosolic free  $Ca^{++}$  con-

centration and then also on  $AT_1$ -mediated  $Ca^{++}$  mobilisation from the intracellular storage pool,<sup>36</sup> increments in cytosolic free  $Ca^{++}$  in angiotensin II-stimulated vascular smooth cells mainly depend on external  $Ca^{++}$  influx.<sup>34</sup> Angiotensin II-induced  $Ca^{++}$  influx in vascular smooth muscle cells involves dihydropyridine-sensitive calcium channels, receptor-gated calcium channels, dihydropyridine-insensitive cation channels,  $Ca^{++}$ -operated  $Ca^{++}$  release and membrane-bound  $Na^+/Ca^{++}$  exchanger.<sup>34,37,38</sup> However, external calcium influx across the vascular smooth muscle cell membrane seems to be mainly related to dihydropyridine-sensitive calcium channels since angiotensin II was unable to induce contraction of isolated aortic rings from male Sprague-Dawley rats in calcium-free Krebs.<sup>39</sup> Of note, the vascular response to angiotensin II was less evident when aortic endothelium was removed and more pronounced in rats made diabetic by streptozotocin than in non-diabetic rats,<sup>39</sup> thereby suggesting hyperglycaemia enhances the vascular responsiveness to  $AT_1$  blockade. Even more interestingly, angiotensin II-induced vascular contraction in the presence of external calcium was abolished by the  $AT_1$  receptor inhibitor valsartan as well as by DHPACs (Figure 4), thereby indicating  $AT_1$ -mediated dihydropyridine-sensitive calcium channel activation was the main mechanism responsible for  $AT_1$ -induced cytosolic free  $Ca^{++}$  increment and the consequent aortic ring contraction. Also, non-DHPACs were unable to reduce the vascular response to angiotensin II in the presence of external calcium (Figure 4). Thus, it is evident that angiotensin II-induced vascular contraction is mediated by the  $AT_1$  receptor, but also by the activa-

**Figure 4.** Effect of dihydropyridine and non-dihydropyridine calcium channel antagonists on angiotensin II-induced contractions in isolated thoracic rat aorta ( $n=10$ ).<sup>39</sup>

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*functional coupling in supersensitivity to angiotensin II in diabetic rats.* Cardiovasc Res. 2005;65:374–386.



tion of dihydropyridine-sensitive calcium channels. Based on these in-vitro findings, it is also evident that simultaneous AT<sub>1</sub> receptor and dihydropyridine-sensitive calcium channel blockade may manifest with a significant synergy in clinical practice, by acting on the same pathways leading to cytosolic free Ca<sup>++</sup> increase and then reciprocally potentiating their vascular dilative and protective properties (Figure 5).

In addition to the above, valsartan could also potentiate the BP-lowering effects of amlodipine by reducing sympathetic tone. Indeed, while amlodipine induces a modest adrenergic activation,<sup>40</sup> ARBs can reduce the adrenergic tone, particularly by inhibiting  $\alpha_1$ -adrenoceptor activity.<sup>40,41</sup> Thus, the hypotensive actions of valsartan can also be related to sympathetic tone inhibition. This latter effect

of valsartan may be of particular clinical relevance since it could abolish the adrenergic tone increment that can be encountered in patients treated with DHPCAs.

### Antihypertensive Efficacy of the Valsartan and Amlodipine Combination

Data supporting the existence of a marked synergy between valsartan and DHPCAs derive from several sources of in-vivo evidence. The majority of valsartan-treated patients participating in the JIKEI heart study were simultaneously treated with DHPCAs, and this study reported a significant reduction in both primary and secondary cardiovascular endpoints (Figure 3).<sup>24</sup> In addition, in a multinational, multicentre, double-blind, placebo-controlled, parallel-group trial

# Vasoconstrictive role of angiotensin II: co-operative antagonism by AT<sub>1</sub> receptor and dihydropyridine Ca<sup>++</sup> blockers

A

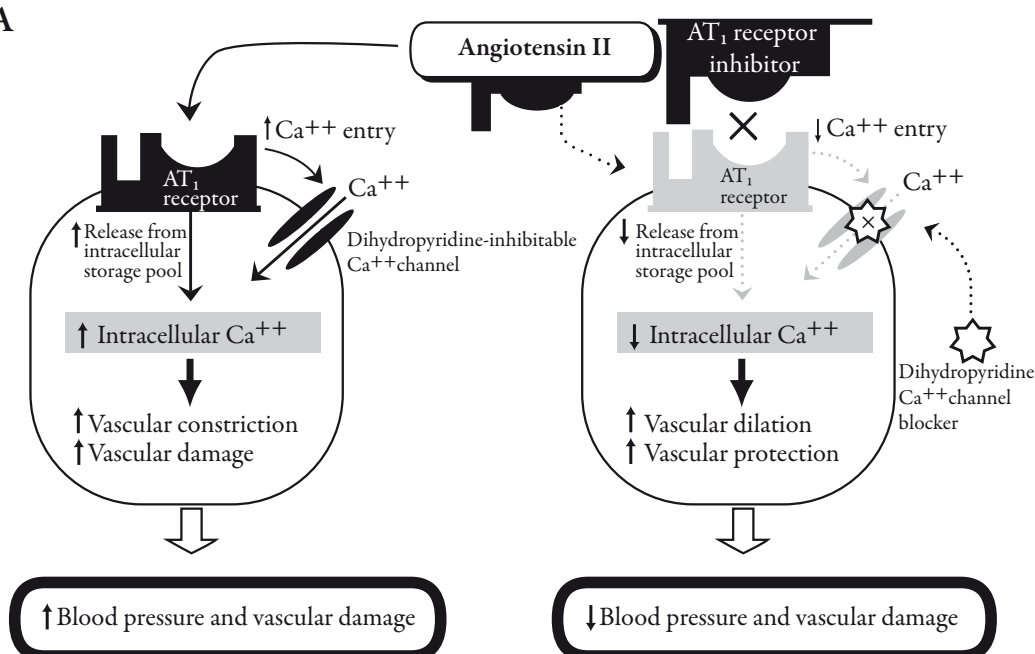


**Figure 5A.** Vasoconstriction is dependent on intracellular Ca<sup>++</sup> availability. Angiotensin II induces vasoconstriction by augmenting intracellular Ca<sup>++</sup> availability. In turn, this latter depends on angiotensin II type 1 (AT<sub>1</sub>)-mediated Ca<sup>++</sup> release from intracellular storage pool and Ca<sup>++</sup> entry through dihydropyridine-inhibitable channels (left size). Thus, AT<sub>1</sub> receptor and dihydropyridine Ca<sup>++</sup> channel blockers co-operate in inducing vasodilation and vascular protection (right size).

## Co-operative antagonism by AT<sub>1</sub> receptor and dihydropyridine Ca<sup>++</sup> blockers : ↑vascular protection and ↓side effects

**B**

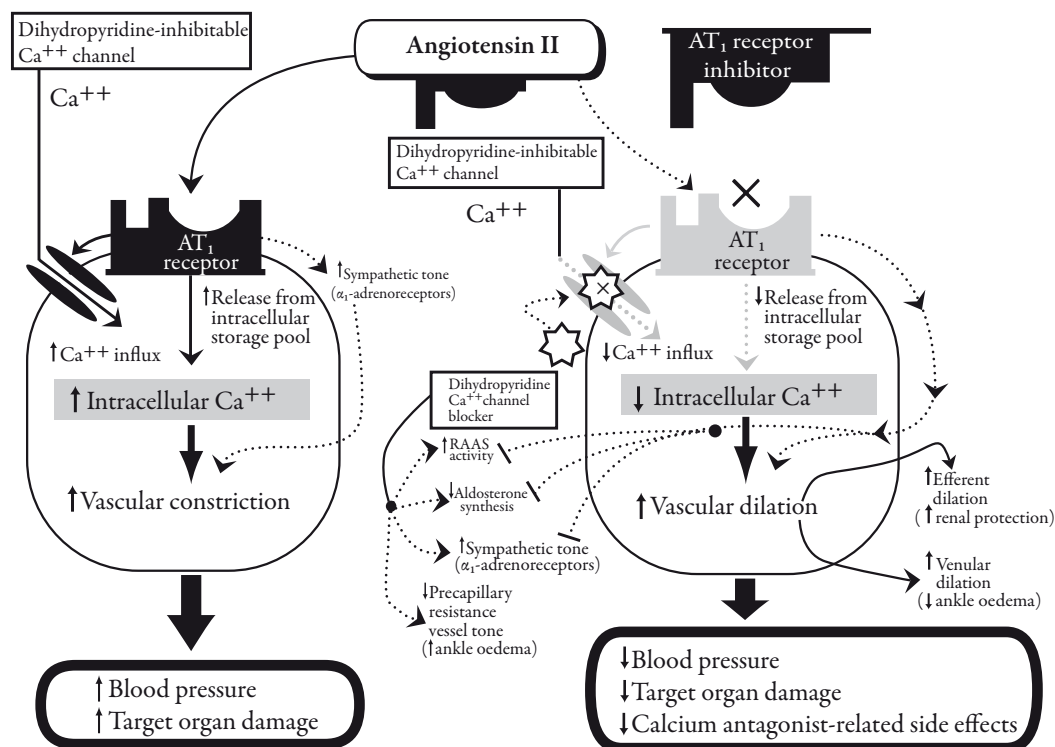


**Figure 5B.** Vasoconstriction is dependent on intracellular Ca<sup>++</sup> availability. Angiotensin II induces vasoconstriction by augmenting intracellular Ca<sup>++</sup> availability. In turn, this latter depends on angiotensin II type 1 (AT<sub>1</sub>)-mediated Ca<sup>++</sup> release from intracellular storage pool and Ca<sup>++</sup> entry through dihydropyridine-inhibitable channels (left size). Thus, complementary antihypertensive actions exerted by AT<sub>1</sub> receptor and dihydropyridine Ca<sup>++</sup> channel antagonists also include a decrement in α<sub>1</sub>-adrenergic tone, thereby counteracting its mild increase induced by dihydropyridine Ca<sup>++</sup> channel antagonists (right size). Combination of AT<sub>1</sub> receptor and dihydropyridine Ca<sup>++</sup> channel antagonists also modulates cutaneous microcirculation and thereby reduces ankle oedema due to the use of Ca<sup>++</sup> channel antagonists as monotherapy. Similar complementary action on intrarenal resistance vessels leads to increased renal protection. Finally, the modest renin–angiotensin–aldosterone system (RAAS) activation that may also be consequent to the use of dihydropyridine calcium antagonists is also blunted by AT<sub>1</sub> blockade. In turn, both this latter and calcium channel blockers directly reduce aldosterone synthesis and secretion.

Philipp et al.<sup>25</sup> recently evaluated 1911 essential hypertensive patients who were randomised to receive either amlodipine 2.5 or 5.0 mg once daily, valsartan 40 to 320 mg once daily, the combination of amlodipine 2.5 or 5.0 mg with valsartan 40 to 320 mg once daily, or placebo over a period of 8 weeks. Cumulative analysis of data showed that 1022 patients were randomised to amlodipine plus valsartan, 507 to valsartan, 254 to amlodipine, and 128 to placebo treatments. According to in-vitro findings, clinical results showed an evident dose response for all combinations, the highest response rate being associated with the highest dose of com-

bination therapy (amlodipine 5 mg plus valsartan 320 mg; 91.3%) (Figure 6A).

Additionally, Philipp et al.<sup>25</sup> also evaluated 1250 hypertensive patients, who were randomised to a similar valsartan dose but a higher amlodipine dose over a period of 8 weeks. Interestingly, BP decrements were again found to be dose-dependent but the two doses of combination therapy were associated with similar response rates (amlodipine 10 mg plus valsartan 160 mg, 88.5%; amlodipine 10 mg plus valsartan 320 mg, 87.5%) (Figure 6B). This suggests that the commonly available/used doses of both valsartan and amlodipine are characterised

**Figure 6.** Effect of combined valsartan and amlodipine therapy on systolic (SBP) and diastolic blood pressure (DBP) of patients with mild-to-moderate essential hypertension.<sup>25</sup>

*Reprinted from Clinical Therapeutics, Vol 29. Philipp T, et al. Two multicenter, 8-week, randomized, double-blind, placebo-controlled, parallel-group studies evaluating the efficacy and tolerability of amlodipine and valsartan in combination and as monotherapy in adult patients with mild to moderate essential hypertension. Pages 563–580. Copyright 2007, with permission from Excerpta Medica, Inc.*

(A) Least-squares mean reduction in (i) sitting DBP and (ii) sitting SBP at the end of Study 1. The standard errors of the means for all reductions in part (i) ranged from 0.65 to 0.67 mmHg; in part (ii), they ranged from 1.03 to 1.06 mmHg.

\* $P < 0.05$  vs placebo; † $P < 0.05$  vs the same dose of valsartan monotherapy; ‡ $P < 0.05$  vs the same dose of amlodipine monotherapy.

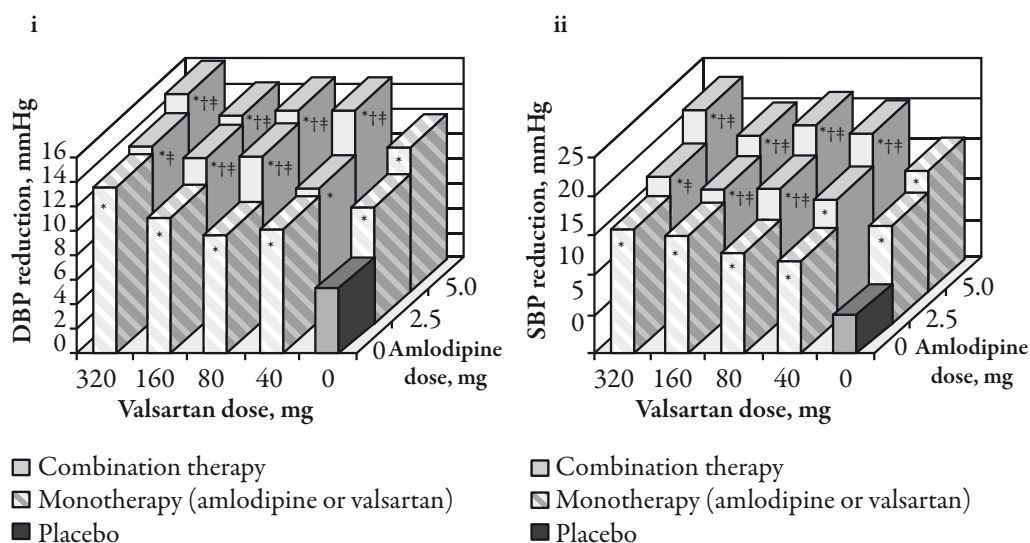
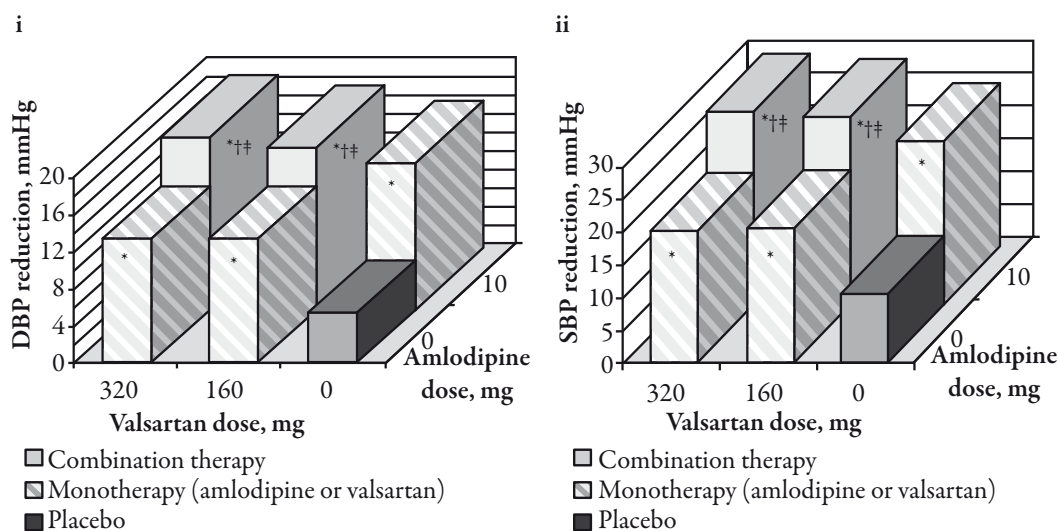


**Figure 6.** (Continued)

Reprinted from *Clinical Therapeutics*, Vol 29. Philipp T, et al. Two multicenter, 8-week, randomized, double-blind, placebo-controlled, parallel-group studies evaluating the efficacy and tolerability of amlodipine and valsartan in combination and as monotherapy in adult patients with mild to moderate essential hypertension. Pages 563–580. Copyright 2007, with permission from Excerpta Medica, Inc.

(B) Least-squares mean reduction in (i) sitting DBP and (ii) sitting SBP at the end of Study 2. The standard errors of the means for all reductions in part (i) ranged from 0.62 to 0.63 mmHg; in part (ii), they ranged from 0.96 to 0.97 mmHg.

\* $P < 0.05$  vs placebo; † $P < 0.05$  vs the same dose of valsartan monotherapy; ‡ $P < 0.05$  vs the same dose of amlodipine monotherapy.



by optimal BP responses. Also, comparisons of two different combination regimens based on either the ACE inhibitor lisinopril 10–20 mg plus HCTZ 12.5 mg, or valsartan 160 mg plus amlodipine 5–10 mg showed a marked BP response in hypertensive patients treated with the second combination (SBP –35.8 mmHg after 6 weeks, DBP –28.6 mmHg after 6 weeks;  $P < 0.001$  vs baseline in both cases).<sup>42</sup> Therefore, controlled clinical trials indicate that the combination of amlodipine plus valsartan is associated with significantly greater BP reductions from baseline compared with amlodipine or valsartan monotherapy. In keeping with this, 77.9% of pa-

tients in the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial, who were randomised to losartan, received calcium channel blockers and reported a significant decrease in the pre-defined renal endpoint of worsening of kidney failure,<sup>43</sup> thereby indicating that combination of ARBs with DHPCAs can also be used in high-risk conditions, including type 2 diabetes mellitus with overt renal damage. A review of available data supports the notion that the combination of RAAS and calcium channel antagonists can be very effective in the treatment of hypertension and further improve BP control.<sup>44</sup>

# Safety Profile of the Valsartan and Amlodipine Combination

Some post-hoc evidence from the VALUE trial suggests that the combination of valsartan with diuretics and/or beta blockers modestly attenuates the metabolic benefits derived from RAAS blockade.<sup>31,32</sup> Obviously, this does not mean that a low-dose thiazide cannot be added in principle to RAAS inhibitors, and this also applies to other diabetogenic drugs, i.e. beta blockers. Indeed, it is obvious that the use of diuretics and/or beta blockers is often necessary in hypertensive patients. However, thiazides and beta blockers may limit the metabolic benefits that are known to derive from RAAS blocking agents. Their use, at the higher doses, should be prescribed only when necessary in patients with metabolic disturbances or at high risk for developing type 2 diabetes mellitus. Therefore,

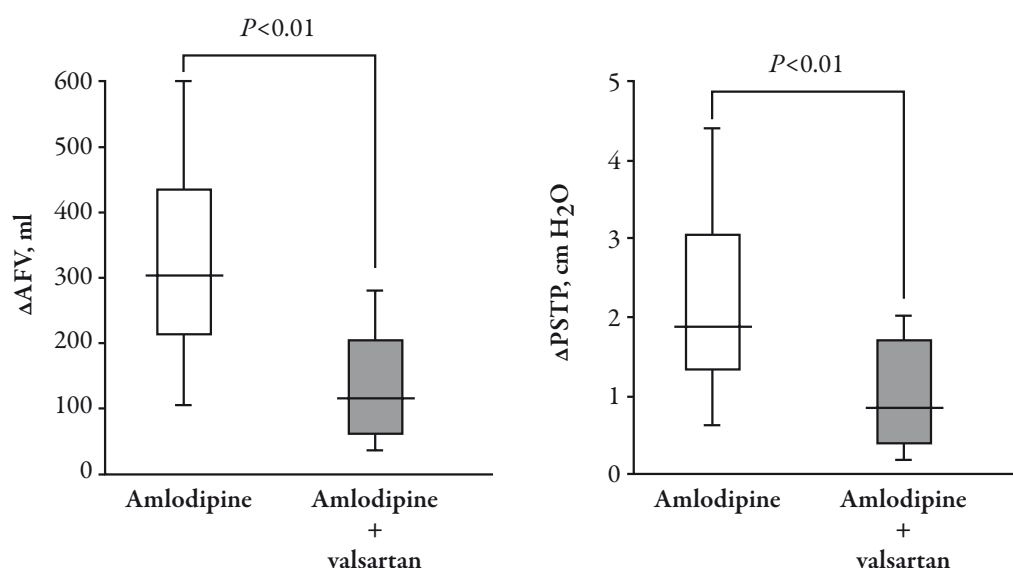
combinations of ARBs and DHPCAs appear the more rational choice in these patients when monotherapy is not able to normalise BP.

Apart from the metabolic aspect, it is of particular interest to note that the two studies evaluating the antihypertensive efficacy of valsartan plus amlodipine combinations<sup>25,42</sup> also showed that side effects were modest and occurred to a lesser extent in patients taking valsartan plus amlodipine compared with those on amlodipine alone.<sup>25</sup> In particular, the incidence of ankle oedema was lower (5.4% vs 8.7%, respectively;  $P=0.014$ ). The incidence of headache was also lower in patients treated with valsartan plus amlodipine compared with those on amlodipine alone (4.3% vs 7.6%, respectively;  $P<0.01$ ).

The reasons leading to the reduced occurrence of the more common side ef-

**Figure 7.** Effects of amlodipine (10 mg once daily) monotherapy or valsartan (160 mg once daily) plus amlodipine (10 mg once daily) on ankle foot volume (left) and pretibial subcutaneous tissue pressure (right) in 80 hypertensive patients.<sup>49</sup>

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fects observed in patients treated with DHPCAs are unclear. However, a certain reduction in ankle oedema has been already reported in two previous studies conducted in hypertensive patients treated with amlodipine in combination with ACE inhibitors<sup>45,46</sup> and in a previous observation based on the evaluation of ankle oedema by the use of the water displacement technique, i.e. the ancient Archimedes principle.<sup>47</sup> Furthermore, evaluation of skin blood flow by laser Doppler flowmetry and of leg weight in hypertensive patients treated with amlodipine showed that enalapril restrained fluid extravasation and increment in leg weight induced by amlodipine, probably because of enalapril-related venodilation.<sup>48</sup> Similar findings might be taken into account to explain the protection against amlodipine-related ankle oedema that was exerted by valsartan.<sup>40</sup> In accordance with this, a recent study measured two objective measures of ankle oedema, i.e. ankle foot volume and pretibial subcutaneous tissue pressure in 80 hypertensive patients who after a 4-week period on placebo were randomised to amlodipine 10 mg, or valsartan 160 mg, or amlodipine 10 mg plus valsartan 160 mg for 6 weeks according to an open-label, blinded-endpoint, crossover design.<sup>49</sup> At the end of the placebo period and of each treatment period, ankle foot volume and pretibial subcutaneous tissue pressure were measured. As expected, amlodipine and valsartan monotherapy significantly reduced SBP (−16.9 and −14.5 mmHg, respectively;  $P<0.01$  vs baseline) and DBP (−12.9 and −10.2 mmHg, respectively;  $P<0.01$  vs baseline) but the reduction

was greater with the combination (−22.9 mmHg for SBP,  $P<0.01$  vs monotherapy; −16.8 mmHg for DBP,  $P<0.01$  vs monotherapy). Amlodipine but not valsartan monotherapy significantly increased both ankle foot volume (amlodipine, +23%;  $P<0.01$  vs baseline) and pretibial subcutaneous tissue pressure (amlodipine, +75.5%;  $P<0.001$  vs baseline). Compared with amlodipine monotherapy, the valsartan/amlodipine combination produced a less marked increase in ankle foot volume (+6.8%;  $P<0.01$  vs amlodipine monotherapy) and pretibial subcutaneous tissue pressure (+23.2%;  $P<0.001$  vs amlodipine monotherapy) (Figure 7). Ankle oedema was clinically evident in 24 patients with amlodipine and in six patients with the combination, thereby supporting a role for valsartan in counteracting the microcirculatory changes responsible for calcium channel blocker-induced oedema formation. Indeed, while angiotensin II is able to induce the constriction not only of arteries but also of veins due to the high amount of  $AT_1$  receptors that are present at the venous level,<sup>50</sup> DHPCAs mainly dilate large and small arteries and reduce precapillary arteriolar resistance (Figure 5).<sup>51</sup> In keeping with this, complementary actions of ARBs and calcium antagonists might also explain the reasons leading to improved renal outcome in the recent RENAAL study,<sup>43</sup> whose patients were treated with losartan in frequent combination with calcium antagonists. In fact, while ARBs reduce systemic BP and preferentially reduce vascular efferent resistance, thereby reducing capillary intraglomerular pressure, blockade of

dihydropyridine-sensitive calcium channels reduces systemic BP and dilates both afferent and efferent arterioles.

In addition to the above explanation, valsartan could have reduced amlodipine-related peripheral oedema because of changes in the sympathetic tone. While amlodipine induces a modest degree of sympathetic activation,<sup>40</sup> ARBs can reduce the adrenergic tone, particularly by inhibiting  $\alpha_1$ -adrenoceptor activity.<sup>40,41</sup> In contrast to this, the selective  $\alpha_1$ -adrenoceptor antagonist doxazosin had very modest effects on cutaneous microcirculation<sup>52</sup> and seems to be ineffective in reducing amlodipine-induced peripheral oedema. Thus, while the hypotensive action of valsartan may be mediated in part by the inhibition of the sympathetic tone in patients with hypertension (Figure 5),<sup>40</sup> such activity is unlikely to be responsible for the observed reduction in ankle oedema in patients treated with valsartan plus amlodipine compared to those treated with amlodipine alone.

## CONCLUSION

Effective reduction in BP is rare in clinical practice, particularly in high-risk hypertensive patients. Clinical trials and epidemiological evidence support the notion that BP decrements within the range of normality significantly improves survival and morbidity. RAAS blockade has been proven to be effective in reducing BP as well as mortality/morbidity outcomes in hypertensive patients. However, ARB monotherapy is not always sufficient to induce an effective BP reduction. In this case, combination therapy is necessary.

In contrast to thiazides, DHPCAs do

not have unfavourable metabolic actions<sup>53</sup> and are able to protect the cardiovascular system not only by reducing BP but also through blood pressure-independent mechanisms. Furthermore, since angiotensin II-induced vasoconstriction depends on external calcium, the addition of DHPCAs to ARB treatment provides additional BP decrements. Accordingly, combination of RAAS blocking agents with DHPCAs has been proven to be particularly useful in the management of hypertension and to reduce cardiovascular, cerebrovascular and renal outcomes even in patients with type 2 diabetes mellitus, overt renal failure, coronary heart disease and heart failure. In addition, due to the inhibition of adrenergic tone and venular dilation, ARBs are also able to significantly reduce ankle oedema, i.e. a minor side effect that can be observed in patients taking DHPCAs and can limit long-term adherence to therapy. Accordingly, the valsartan/amlodipine combination has been demonstrated to induce satisfying BP decrements in more than 80% of a large cohort of hypertensive patients.<sup>25</sup> In this regard, the modest RAAS activation that can be encountered after DHPCA administration<sup>54</sup> can be also suppressed by ARBs.<sup>55</sup>

Based on a recent meta-analysis<sup>56</sup> that stressed the role of fixed combinations in improving long-term adherence to therapy, as well as another study that highlighted the tendency of many hypertension physicians to use approaches that diverge from guideline recommendations,<sup>57</sup> the use of fixed combinations, in general, and of RAAS plus calcium channel blocking agents, in particular, seems to represent an extremely attrac-

tive tool in cardiovascular prevention in essential hypertensive patients. Finally, the combination of ARBs and DHPCAs is also likely to represent a more fruitful opportunity to obtain the more possible advantages from the suggested ancillary properties that might be responsible for the BP-independent benefits deriving from amlodipine and ARB monotherapies.<sup>58</sup>

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