AMの主要な作用は血管拡張による血圧低下作用であり，血管平滑筋への直接作用が主体であるが，一部一酸化窒素（NO）分泌を介する作用もある．AMの発現は全身の血管系や心臓，腎臓，副腎，肺などの重要臓器に広く認められ，AM受容体も同様に広く分布している．このため，AMは非常に多様な作用を示すが，全体としては血圧を下げ，体液量を減少させる方向に作用する．

また，細胞増殖や炎症にも関与し，循環器疾患においては主に酸化ストレスに対抗して，臓器障害や動脈硬化を抑制し臓器保護的に作用する．ただし，腫瘍細胞に対しては細胞増殖促進作用を有しており，状況によっては細胞増殖因子ともなる．強い炎症が存在する場合は抗炎症因子として作用し，特に敗血症ではAMの著明な上昇が認められる．一方で，基礎状態のマクロファージに対しては炎症促進的にも作用する（IL-6等の産生亢進）．最近，われわれは炎症のないヒトにAMを長時間投与すると，軽度だがIL-6を介してCRP（C-reactive protein）上昇が起こることを確認している[3]．

## 2 アドレノメデュリンの内分泌系への作用

AMは血圧を下げ，体液量を減らす方向に働くため，下垂体からのACTH（副腎皮質刺激ホルモン）やAVP（アルギニンバソプレシン）分泌を抑制し，心臓からのANP（心房性ナトリウム利尿ペプチド）分泌を促進し，血管壁ではエンドセリンの産生や効果を抑制し，一酸化窒素（NO）産生を促進する．レニン・アンジオテンシン・アルドステロン系の作用にも拮抗し，血圧だけでなくアンジオテンシンII（A-II）による心臓，腎臓，血管（動脈硬化促進）などの臓器障害を抑制する[1]．しかし，AMを持続静注した場合，血漿レニン活性が抑制されるわけではなく，むしろ血圧低下に対する反作用としてレニン活性は上昇する．AMには腎交感神経抑制作用なども報告されているが，AMを持続静注した場合，血中カテコラミンも反応性に上昇する．レニンやカテコラミンの上昇反応は，AM投与時とCa拮抗薬のニカルジピン投与時で全く同等であり，AMがこれらの分泌を直接抑制するとは考えにくい[4]．

### 1）アドレノメデュリンとアルドステロンの相互関係

AMはもともと褐色細胞腫から分離精製され，その名前からも明らかなように副腎髄質に強く発現している．ところが，アルドステロンを産生する副腎皮質球状層（zona glomerulosa：ZG細胞）にも同程度の発現が認められる（**図1**）．さらに，原発性アルドステロン症（primary aldosteronism：PA）の腺腫細胞（Conn細胞）にも発現が確認されている[5]．副腎皮質球状層および腺腫の細胞にはAMの受容体も発現しており，AMがアルドステロン分泌調節に重要な役割を果たしていることが推察される．大まかにAMはアルドステロン分泌抑制的に働くとされているが，両者の関係は単純ではない（**表**）：ZG細胞だけでなくConn細胞においても，AMは細胞増殖促進とアポトーシス抑制によりアルドステロン分泌細胞の増殖・維持に重要な働きをしており[5][6]，細胞レベルでアルドステロンの基礎分泌は抑制しない[7][8]．ZG細胞に関しては，ごく軽度であるが，アルドステロン分泌を促進するとのデータもある[9]．一方，A-IIや$K^+$によるアルドステロン分泌亢進に対しては，AMは抑制的に作用する[7][8][10]．ACTH刺激によるアルドステロン分泌への抑制はないか，あっても軽度である[7][10]．以上をまとめると，AMはアルドステロンの基礎分泌を維持するとともに，過剰な分泌（オーバーシュート）を抑制することで，アルドステロンの適正な分泌を調整していると考えられる．

$K^+$刺激によるアルドステロン分泌亢進に対するAMの抑制効果は，AMやCGRPアナログにより阻害され

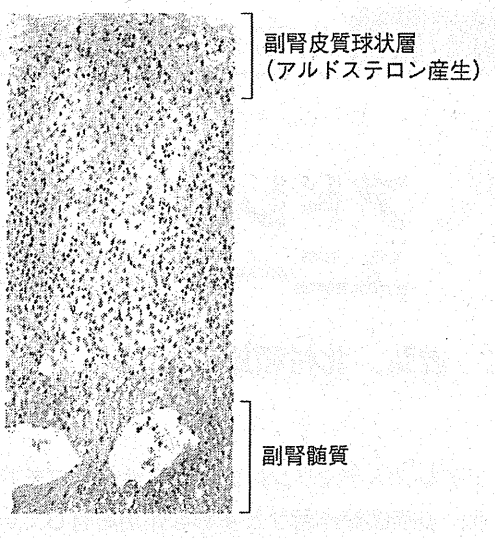


**図1　副腎におけるアドレノメデュリンの局在**
（巻頭カラー図1参照）

**表　アドレノメデュリンのアルドステロン分泌への影響**

| | | 基礎分泌 | 刺激後の分泌 | | | 細胞増殖 |
|---|---|---|---|---|---|---|
| | | | A-II | ACTH | $K^+$ | |
| ZG細胞 | 細胞実験 | →～↗ | ↓ | →～↓ | ↓ | ↑ |
| | 動物実験 | →～↘ | ↓ | ↘ | ↓ | ND |
| | ヒト | →～↘ | →～↓ | → | ND | ND |
| Conn細胞 | 細胞実験 | → | ↓ | ND | ND | ↑ |
| | ヒト | ↓ | ND | ND | ND | ND |

ND：検討なし

ることから，先に述べた受容体を介する作用と考えられる[11]．AMが受容体と結合した後，Caチャネルを介する細胞内への$Ca^{2+}$流入を阻害することで，アルドステロン分泌を抑制すると報告されている[11]．一部，NOを介する作用があるとの報告もあるが，機序が完全に解明されたわけではない．

実験動物やヒトにAMを静注した場合，実験条件によって，血中アルドステロン濃度は変化しないか，軽度低下する[12]．具体的には，食塩制限などで事前にアルドステロン濃度を高くしておくとAM投与によりアルドステロンは低下しやすく[13]，心不全患者などもともとアルドステロン濃度が高い患者でも低下しやすい．一方，アルドステロン濃度が正常の場合，十分な量のAMを長時間投与しないとアルドステロンは低下しない[4) 12) 13)]．これには，AM投与によりレニン活性が上昇し，アルドステロン分泌が刺激されることも関与していると考えられる．Conn細胞では，AMによるアルドステロン抑制作用が強く表れ，感受性が高いことが報告されている[8]．われわれの試験では，AM持続静注によりアルドステロン値が正常な対象者では中等度の，PA患者では強いアルドステロン分泌抑制効果が認められた[3]（**図2**）．本試験でAMはACTH-コルチゾール系には全く影響を与えておらず，PA患者におけるAMによる選択的アルドステロン抑制効果は，PAの診断に応用できる可能性がある．

血中AM濃度と血圧の相関は非常に弱く，臓器障害のない高血圧患者の血中AM濃度上昇はごく軽度である[1]．一方，PA患者ではAM濃度は明らかに上昇しており，手術で腺腫を取り除くとAMは低下する[3) 14)]．面白いことに，手術で摘出したPA患者の腺腫の直径と血中AM濃度が相関していた[14]．一方，ANPも正常

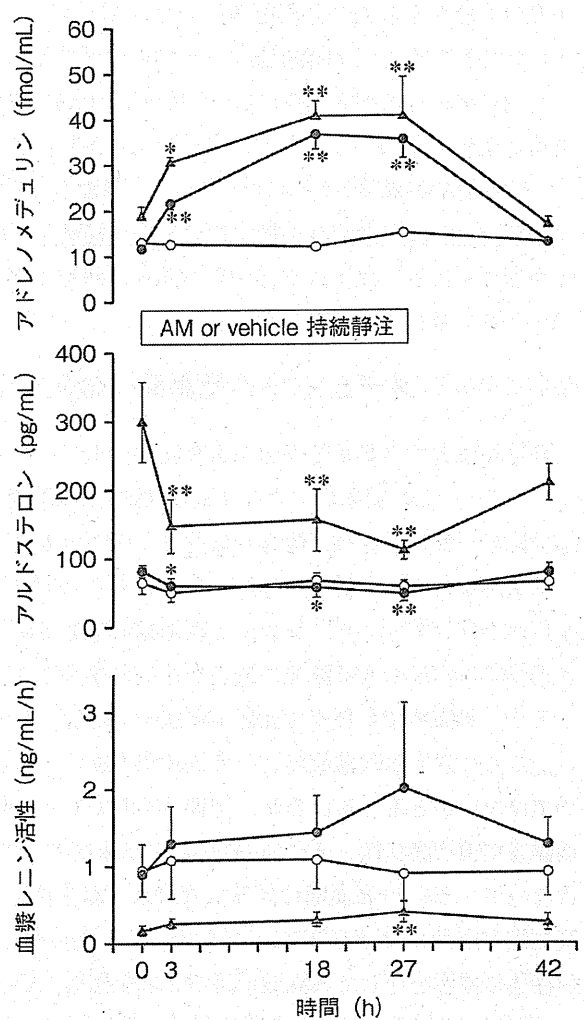


**図2　アドレノメデュリンのアルドステロン抑制作用**
原発性アルドステロン症5名（▲：AM投与）と本態性高血圧患者7名（●：AM投与，○：vehicle投与）にAMを持続静注（2.5pmol/min/kg，27時間）したときの，アルドステロンとレニンの反応

副腎からのアルドステロン分泌を抑制するが，PAの腺腫にはANP受容体がなく，PA患者にANPを投与してもアルドステロンは抑制されない[15]．PA患者のアル

ドステロンを強力に抑制する内因性物質としてはAM以外に報告がなく，PAにおいてAMがアルドステロン分泌調整（たぶん抑制的調整）に重要な因子となっていることは疑いない．

分泌だけでなく，標的臓器においてもAMとアルドステロンは相互に関連を持っている．アルドステロンは心臓や血管において細胞増殖，線維化などのリモデリングを促進し，酸化ストレスを増加させる作用（アルドステロンの腎外作用※1）があるが，AMはこれらの作用を抑制する．一方で，アルドステロンは血管平滑筋や心筋からのAM分泌を増加させることが明らかとなっており[16)17)]，標的臓器においてAMとアルドステロンは拮抗的な平衡関係にある．PA患者で血中AM濃度が上昇するのは，アルドステロンによる末梢でのAM産生増加が原因かもしれない．また，増加したAMは副腎でのアルドステロン産生を部分的に抑制している可能性もある．以上のことから，AMは抑制的アルドステロン調整因子と考えられる．

## ③ アドレノメデュリンの代謝系への作用

糖尿病患者で，必ずしも血中AM濃度は上昇していない[2)]．しかし，糖尿病による合併症（腎症，網膜症，神経障害—特に自律神経障害）の程度とAM血中濃度には関連があり，合併症が進行した患者ではAM血中濃度が上昇している[18)]．また，1型糖尿病患者では心血管障害の程度とAM濃度に関連があると報告されている[19)]．糖尿病では種々の血圧上昇因子が増加しており，これに対する代償反応としてAMが増加しているのではないかと考えられるが，詳細は不明である．断面調査で糖代謝状態とAM血中濃度間に直接的な関係はないが，著しい高血糖にするとAM濃度は上昇し，高血糖が血管におけるPKC（プロテインキナーゼC）依存性の機序を介してAM遺伝子の発現を増加させるとの報告がある[20)]．また，糖尿病患者で高インスリン血症状態にするとAM濃度が上昇することも確認されている[21)]．AMとその受容体は膵臓のランゲルハンス島β細胞にも発現しており，AMはインスリン分泌を抑制する[22)]．よって，AM投与下に経口糖負荷試験を行うと，コントロールと比較して，血中インスリン濃度は低下し，血糖値は高くなる．糖尿病で血中AM濃度が高い患者では，AMが糖代謝を悪くしている可能性があり，糖尿病患者でAMは良い作用だけをしているとは限らない．推測の域を出ないが，PA患者では糖代謝が障害されている場合が多く，低K血症によるインスリン分泌障害が原因とされているが，増加したAMによるインスリン分泌抑制も関係があるかもしれない．

インスリン抵抗性は，メタボリックシンドローム（MS）の重要な基礎病態であり，一連の病気進行の上流に位置している．肥満がインスリン感受性を低下させる主因であることは周知の事実であるが，レニン・アンジオテンシン系もインスリン感受性を低下させる重要な因子である．このことは，MSや糖尿病を伴う高血圧患者にARBが推奨される根拠ともなっている．マウスにA-IIを持続的に投与すると酸化ストレスが増加してインスリン抵抗性が出現するが，AMノックアウトマウス（ヘテロ体）ではより強いインスリン抵抗性が出現した[23)]．さらに，このマウスでは加齢により自然にインスリン抵抗性が出現し，酸化ストレスも増加していた[24)]．加えて，このマウスに抗酸化剤を投与すると，インスリン抵抗性が改善した[23)24)]．総合すると，AMは血中インスリン濃度を低下させるとともに，酸化ストレスを減らすことで，インスリン抵抗性を改善すると考えられる．

それでは，インスリン抵抗性がある状態でのAMの効果はどうなのだろうか．肥満ラット（fa/fa Zucker rat）では，外因性に投与したAMに対する感受性が低下していた[25)]．われわれは，糖尿病患者を含む対象者にAMを持続静注したところ，インスリン抵抗性の指標であるHOMA indexが高い対象者でのみ，AMによる動脈stiffness※2指標の改善効果が低いことを確認した[4)]（**図3**）．AMとインスリンは拮抗関係にあり，AMはインスリン抵抗性を改善する作用があるものの，す

**※1 アルドステロンの腎外作用**
アルドステロンは，ミネラルコルチコイド受容体（MR）を介した直接作用により，心血管系の炎症を惹起し，最終的に組織の線維化を起こす．MR阻害薬はこれを抑制し，心不全患者などの生命予後を改善する．

**※2 動脈stiffness**
大動脈壁の硬化は，大動脈による血圧緩衝作用を減弱させ，収縮期高血圧をきたす．独立した生命予後規定因子である．脈波伝播速度（PWV）やaugmentation indexが簡便な指標として普及している．

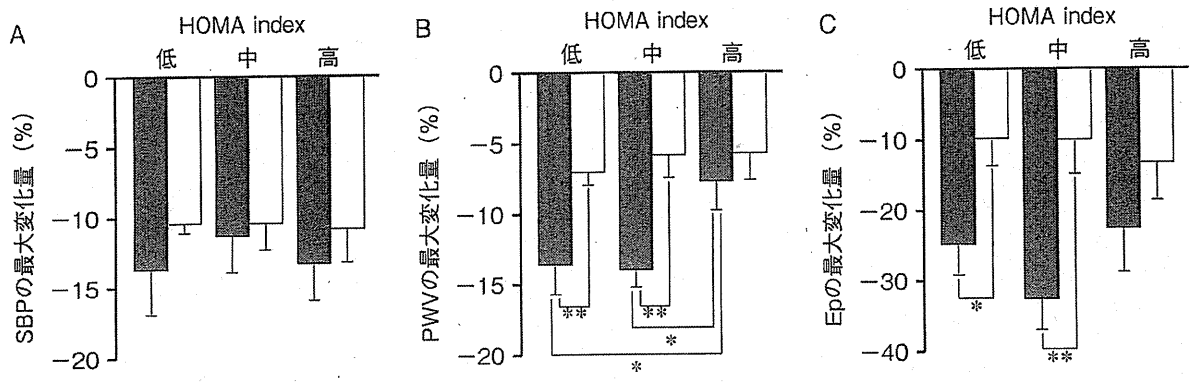


**図3　インスリン抵抗性とアドレノメデュリンの作用**
28名の対象者にAM（■：5 pmol/min/kg）またはニカルジピン（□：1～1.5 μg/min/kg）を1時間静注したときの，収縮期血圧（SBP），脈波伝播速度（PWV），頸動脈弾性特性（Ep）の最大変化量．降圧量を合わせると，AMの方がPWVなどの改善効果が強い．しかし，HOMA indexで3等分すると，インスリン抵抗性を有する群（高，HOMA index≧2.0）のみAMの効果が減弱し，ニカルジピンと差がなくなった

でにインスリン抵抗性が存在する状態では，AMによる抗動脈硬化作用は減弱していることになる．インスリン抵抗性を基礎とする病態では，AMの活性を十分増強してインスリン抵抗性を解消すれば，AM自体の有益な効果も向上し，全体的に病態を改善することが期待でき，新たな治療ターゲットとなるかもしれない．

## おわりに

AMの臨床応用として，肺高血圧への吸入療法，心筋梗塞後の治療（リモデリングを抑制することで心機能維持を期待），炎症性腸疾患に対する治療（抗炎症作用を期待），閉塞性動脈硬化症に対する治療（血管新生促進作用を期待）などが試されている．しかし，本稿で記したように，AMには他にも多彩な作用があり，内分泌・代謝分野での活用も期待したい．

## 文献


1）北村和雄：Heart View, 13（増刊号）：69-75, 2009
2）Gibbons, C. et al.：Mol. Endocrinol., 21：783-796, 2007
3）Kita, T. et al.：Hypertens. Res., 33：374-379, 2010
4）Kita, T. et al.：Hypertens. Res., 33：314-319, 2010
5）Forneris, M. et al.：Int. J. Mol. Med., 8：675-679, 2001
6）Rossi, G. P. et al.：Hypertens. Res., 26：S85-S92, 2003
7）Yamaguchi, T. et al.：Life Sci., 56：379-387, 1995
8）Andreis, P. G. et al.：J. Clin. Endocrinol. Metab., 83：253-257, 1998
9）Kapas, A. et al.：J. Endocrinol., 156：477-484, 1998
10）Salemi, R. et al.：J. Endocrinol., 166：389-399, 2000
11）Mazzocchi, G. et al.：Peptides, 20：1479-1487, 1999
12）Charles, C. J. et al.：Regul. Pept., 112：41-49, 2003
13）Yamaguchi, T. et al.：Hypertension, 28：308-314, 1996
14）Letizia, C. et al.：Blood Press., 7：19-23, 1998
15）Rocco, S. et al.：Am. J. Hypertens., 3：668-673, 1990
16）Jiang, W. et al.：J. Hypertens., 22：1953-1961, 2004
17）Jiang, W. et al.：Biochim. Biophys. Acta, 1690：265-275, 2004
18）Nakamura, T. et al.：Endocr. J., 45：241-246, 1998
19）Garcia-Unzueta, M. T. et al.：Diabetes Care, 21：999-1003, 1998
20）Hayashi, M. et al.：Biochem. Biophys. Res. Commun., 258：453-456, 1999
21）Katsuki, A. et al.：Eur. J. Endocrinol., 147：71-75, 2002
22）Martinez, A. et al.：Endocrinology, 137：2626-2632, 1996
23）Xing, G. et al.：Endocrinology, 145：3647-3651, 2004
24）Shimosawa, T. et al.：Hypertension, 41：1080-1085, 2003
25）Chan, C. B. & Johnson, K. J.：Can. J. Physiol. Pharmacol., 75：1138-1141, 1997


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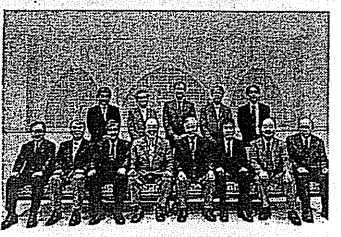
# 九州11大学循環器フォーラム会報

2011年 第7回

2011年6月25日(土)、福岡市のホテルニューオータニ博多にて、第7回九州11大学循環器フォーラムが開催されました。多数の先生方にご参加頂き、盛大に研究会が開催されました。

**第7回 九州11大学循環器フォーラム**
**循環器医専門医のプロフェッショナルリズム**
**日時:平成23年6月25日(土)18:00~**
**(循環器地方会終了後)**
**場所:ホテルニューオータニ博多**

- 第一部　教育講演
- 第二部　特別講演
- 第三部　新臨床研修医制度によって何がどう変化したか?
  各大学がいかに取り組んでいるのか?



## 第一部　教育講演

### 「降圧因子アドレノメデュリンの新たな可能性」

北村　和雄 先生 (宮崎大学医学部内科学講座循環体液制御学分野 教授)

1993年、ヒト副腎髄質由来の褐色細胞種より発見されたアドレノメデュリンは、強力な降圧作用を有する生理活性ペプチドで、副腎の他、心房や肺などの組織で高濃度存在します。とりわけ血管内皮からはエンドセリンと同程度分泌され、強力な血管新生作用、血管拡張作用等を有する事が分かってきました。

閉塞性動脈硬化症実験モデル動物においては、アドレノメデュリンの投与と抹消血単核細胞移植を併用することによって血管新生効果の上がることが分かり、当大学では今年から抹消血単核球移植にアドレノメデュリン持続静注療法する併用する臨床研究を開始しました。炎症性疾患に対してもアドレノメデュリンを投与する事により、効果が上がる事も分かってきたので、難治性炎症性腸疾患の治療薬としての特許申請も行い、実用化をめざしています。

従来は、優れた業績を基に研究資金と人材の獲得・育成が可能となり、さらに業績をあげるという良い循環ができていました。しかし、新臨床研究制度が導入されてからは人材の確保が難しくなり、それに伴い業績も上がりにくくなり、研究資金の獲得ができなくなるという悪循環に陥っている所が増えてくるのではと危惧しています。

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## 第二部　特別講演

### 「臨床研修医制度の今後について」

田原　克志 先生 (厚生労働省医政局医師臨床研修推進室 室長)

臨床研修医制度は平成16年から施行され、今年8年目です。医師不足など様々な問題が顕在化したため、臨床研修の質の向上と医師不足への対応を図ることを主眼として見直しを行い、平成22年度の研修から適用しています。

見直しの内容は研修プログラムの基準を弾力化すること、臨床研修病院の基準を強化すること、そして募集定員を見直し都会に研修医が集中するのを防ぐという3つの柱があります。プログラムの見直しについては必修科目を絞り、将来専門を希望する診療科を中心とした研修ができるようになりました。また臨床研修病院の基準を強化した事によって病院の数を絞り、研修希望者の数と合わせて定員を削減することができました。採用実績を見ると、臨床研修制度が始まる前よりも都市部の研修医は減り、その他の地方の方が増えています。

現在、全臨床研修修了者に対するアンケート調査のほか、制度導入前後の研修医の地域分布や移動状況について様々な調査を行っています。今後、こういったデータを題材にした入念な議論と積極的な情報発信が必要だと思います。

司会　今泉　勉 先生
(久留米大学医学部
心臓・血管内科部門 主任教授)

## 第三部　新臨床研修医制度によって何がどう変化したか？<br>各大学がいかに取り組んでいるのか？

司会　小川　久雄 先生
（熊本大学大学院
生命科学研究部循環器病態学 教授）

### 朔　啓二郎 先生（福岡大学医学部心臓・血管内科学 主任教授）

福岡大学病院は、地下鉄を降りると病院の受付に出られる新しい新診療棟ができ、非常に新しくなったというインパクトがあります。マッチングについては100%マッチしていますが、その後国家試験に落ちるところが１つの問題です。当大病院の研修医は、九州で一番給料が安かったので、ある程度是正していただいたのですが、医学部生の話を聞くと、少しでも高いところに行きたいという意見があります。やはり一定の給料は必要で、研修病院の質をきちんとキープする評価機構をもっとしっかり働かせるべきだと思います。

### 尾辻　　豊 先生（産業医科大学第二内科学 教授）

研修制度に関して言えば、産業医科大学は破綻的な危機を迎えています。40人ぐらいの定数でスタートしたのですが現在10人、さらに減る傾向にあります。都会の研修医を減らし、地方の研修医を増やす政策や研修の質が高くない（人気がない）病院の定数を減らす政策は理解できますが、研修の質が高い病院の定数を増やせるシステムを考えていただければと思います。また現在の研修制度では優秀な研修医は医師として成長出来ますが、優秀でない研修医はどこでもお客さん扱いになり成長出来ないのではないかということを危惧しています。

### 野出　孝一 先生（佐賀大学医学部内科学 教授）

国立大学が法人化し経営タスクが多くなったところに相まって、指導医の業務が増えたという印象です。研修医は２、３ヶ月のローテートなので実際は指導医が主治医として働くこともあり、同時に循環器内科に興味を持たせるという役目もあるので、かなり責任が増えた感があります。この研修制度を評価するべきは、研修医とともに、税金の支払い者である国民、住民です。

地域の開業医や公的病院の医師などの現場の意見を吸い上げていくことが、研修医制度改革には必要です。オーバーロールで解析された地域格差、診療価格差など数字だけで表す事のできない現場の声を反映させてほしいところです。

### 前村　浩二 先生（長崎大学大学院循環病態制御内科学 教授）

長崎県では、医師を長崎大学からの派遣にかなり依存していますが、現在では大学の入局者が80数名からおよそ半数に減ったため派遣先を維持できず、各地から引き揚げざるを得ない状況です。研修医の数も一時マッチングが30人台になり、研修内容の充実、研修環境の向上、広報に取り組んできました。プログラムを充実させ、メンター制度のほか海外留学も行っています。またコメディカルチームに業務のかなりの部分を移し、給料の面や研修医室の環境も改善しました。webでの情報発信も積極的に行い、ようやくマッチングが50人台に回復しました。評価の際は、臨床研修制度は本当に国民が望む医師が育つ制度なのか、独立的に議論される必要があると思います。

### 大屋　祐輔 先生（琉球大学大学院 循環器・腎臓・神経内科学 教授）

琉球大学では当初から臨床研修制度の理念にのっとった研修プログラムを作り、指導医を育ててきました。プログラムは非常に自由度が高く個々な研修ができ、メンター制度もあります。自由できるプログラムなのに学生が入らない。その理由をずっと探ってきましたが、要因は複合的で難しい、因るは精神論だという結論に達し、いま「I LOVE琉大運動」に取り組んでいます。精神論とはつまりプロフェッショナリズムです。医師、循環器内科医としての使命、地域や国への貢献の尊さなどを学生に説き続けることが大切だと思っています。

### 岸川　哲典 先生（大分大学医学部臨床検査・診断学講座 教授）

大分大学では様々なプログラムのほかドクターヘリや救急の体験、ロボット導入の予定など様々なアピールをしてきた結果、定員64に対してマッチ者が44と7割弱になりました。平成16年当時に比べると少しよくなりましたが、旧制度には追いついていません。最近、大学への回帰傾向は感じられますが、根拠の乏しい選択によって一般の総合病院を希望する傾向が強く、予断を許さない状況です。細切れにいろんな科を回る状況では、2年終わっても十分な研修の成果をあげられているか気になっております。

### 砂川　賢二 先生（九州大学大学院循環器内科学 教授）

新研修医制度は何を目指したのかが問われているのではないかと思います。これからの日本の医療制度、カルチャーまで包括する長いレンジで考えていなかったため、今ほつれが見え始めたのではないかと感じています。我々の大学では、初期研修も後期研修も以前と変わらない人数が入ってきています。ただ大きく違うのは、いわゆる臨床志向の人が明らかに増え、非常に重要な基礎研究に情熱を持つ若手が減少していることです。国の将来を考えた時、現在の医師不足についての議論だけではなく、将来の医療を担う有能な研究者をいかに育てていくのかという長い視点の制度設計が大切だと思います。



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**第7回　九州11大学循環器フォーラム**（五十音順）

**当番世話人**
今泉　勉　久留米大学医学部内科学講座 心臓・血管内科部門　主任教授

**代表世話人**
小川　久雄　熊本大学大学院 生命科学研究部循環器病態学　教授
鄭　忠和　鹿児島大学大学院 循環器・呼吸器・代謝内科学　教授

**顧　問**
瀬下　修一　琉球大学　名誉教授
矢野　捷介　長崎国際大学　学部長

**世話人**
今泉　勉　久留米大学医学部心臓・血管内科部門　主任教授
大屋　祐輔　琉球大学大学院 循環器・腎臓・神経内科学　教授
尾辻　豊　産業医科大学第二内科学　教授
北村　和雄　宮崎大学医学部循環体液制御学分野　教授
岸川　哲典　大分大学医学部臨床検査・診断学講座　教授
朔　啓二郎　福岡大学医学部心臓・血管内科学　主任教授
砂川　賢二　九州大学大学院循環器内科学　教授
野出　孝一　佐賀大学医学部内科学　教授
前村　浩二　長崎大学大学院循環病態制御内科学　教授

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ORIGINAL ARTICLE

# Hemodynamic and hormonal effects of exogenous adrenomedullin administration in humans and relationship to insulin resistance

Toshihiro Kita, Yoshihiko Suzuki[1] and Kazuo Kitamura

**Although adrenomedullin (AM) is a potent hypotensive peptide that acts mainly as a vasodilative and proliferation inhibitory factor, there have been few hemodynamic studies on AM in humans, especially concerning arterial stiffness and hormonal effects. In addition, AM is a suppressive factor in insulin resistance, suggesting that the effects of AM in a state of insulin resistance are important. To evaluate the effects of AM in humans, 28 participants were intravenously administered AM (5 pmol $min^{-1}$ $kg^{-1}$) for 90 min. They also received a representative vasodilator drug, nicardipine, as a reference drug. Blood pressure, heart rate, pulse wave velocity (PWV) and blood flow were monitored throughout the experiment. Hormonal changes were also monitored by blood tests. The effects of AM were compared with those of nicardipine. In addition, the effects of AM were re-evaluated against insulin resistance state. AM and nicardipine produced the same level of hypotension, but AM showed a more potent ability to increase heart rate, blood flow and cardiac output and reduce PWV. AM and nicardipine similarly stimulated plasma noradrenaline and renin activity. However, in the state of insulin resistance, favorable effects of AM on aortic stiffness were blunted and differences between AM and nicardipine disappeared. Furthermore, there was a significant correlation between maximum changes in the PWV induced by AM and the homeostasis model assessment of insulin resistance index ($r$=0.58, $P$=0.001). Our results suggest that AM may improve arterial stiffness and act as a compensatory factor against arterial sclerosis. Moreover, decreased reactivity of AM may participate in the progression of arterial sclerosis in insulin resistance.**
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**Keywords:** adrenomedullin; insulin resistance; nicardipine; pulse wave velocity

## INTRODUCTION

Adrenomedullin (AM) is a potent hypotensive peptide found ubiquitously in tissues and organs, especially in cardiovascular tissues, the kidneys, lungs and endocrine glands. AM has multiple functions in a wide range of tissues and acts mainly as a vasodilative and proliferation inhibitory factor.[1] AM also has a role in the development of arterial sclerosis as an inflammatory modulator.[2,3] Recently, it was shown that endogenous AM has a protective effect against cardiovascular injury, possibly through the inhibition of oxidative stress.[4] Morphologically, dense manifestation of AM has been detected in macrophages within plaques of atherosclerotic lesions.[5] Shinomiya *et al.*[6] reported an association between plasma AM concentration and carotid atherosclerosis in patients with stroke. Furthermore, we previously reported a relationship between plasma AM levels and pulse wave velocity (PWV), an indicator of arterial stiffness, in patients.[7] In addition, AM may counteract insulin resistance development through an antioxidative stress factor.[8,9] Insulin resistance is well recognized as a major pathogenetic factor of arterial disorders, including hypertension and arterial sclerosis. Accumulating data suggest that AM acts as an important modulator against arterial sclerosis and organ damage.

Exogenous AM administration has been shown to have beneficial effects in various stages of cardiovascular disease. In normotensive and hypertensive subjects, short-term AM infusion produced hypotension through vasodilation and increased cardiac output.[10,11] AM also improved hemodynamics in heart failure patients. Specifically, AM administration reduced arterial pressure and cardiac filling pressure and also increased cardiac output and renal sodium excretion.[12] However, the effect of AM on the arteries, especially large arteries, has not been determined in humans. A sustained increase in arterial stiffness, as demonstrated by increased PWV, is closely correlated with morbidity and mortality in cardiovascular events.[13–15] In this study, we investigated the effect of AM on arterial stiffness in human subjects. In addition, because the effect of AM may be affected by insulin resistance, we evaluated the relationship between the effects of AM and insulin resistance state in these subjects.

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## METHODS

### Study subjects

Twenty-eight subjects that were normotensive ($n$=11), hypertensive ($n$=9) or diabetic ($n$=8) received AM. In addition, all subjects received nicardipine as a reference drug at least 1 week after AM. Subjects with one or more of the following conditions were excluded: (1) heart failure (ejection fraction <50%), (2) severe valvular diseases, (3) renal insufficiency (serum creatinine >1.0 mg per 100 ml), (4) peripheral artery diseases and (5) history of cardiovascular events. All subjects were completely free from any kind of drugs. The study was approved by the ethics committee of the institute, and all participants gave written informed consent.

### Preparation of human AM

Chemically synthesized human AM was purchased from the Peptide Institute, Osaka, Japan. The homogeneity of human AM was confirmed by reverse-phase high-performance liquid chromatography and amino acid analysis. AM was dissolved in distilled water with 3.75% D-mannitol and 0.05% aminoacetic acid, then sterilized by passage through a 0.22-μm filter (Millipore, Bedford, MA, USA). The chemical nature and content of the human AM in vials were verified by reverse-phase high-performance liquid chromatography.

### Study protocol

All experiments began at 0900 hours with subjects in a fasted state. Experiments were conducted in our outpatient office, which provided a quiet environment with a constant temperature. A 20-gauge cannula was inserted into the forearm vein for infusion of 0.9% saline. Saline was infused at a rate of 100 ml $h^{-1}$ throughout the experiments (Figure 1). Baseline measurements were obtained after a 30-min equilibration period. Then AM (5 pmol $min^{-1}$ $kg^{-1}$) was intravenously administered at a rate of 5 ml $h^{-1}$ for 1.5 h followed by saline infusion for 1.5 h. One week after AM infusion, the same subjects were infused with nicardipine (1–1.5 μg $min^{-1}$ $kg^{-1}$) as a reference drug. Blood pressure and pulse rate were monitored every 10 min by an automated hemodynamometer on a brachial cuff. Every 15 min, blood pressure, heart rate and PWV were measured using an automatic waveform analyzer (form PWV/ABI, BP-203RPE; Omron Colin, Komaki, Japan), as reported in our previous study.[7] Carotid artery pulsation was measured using echo equipment at three time points, as indicated in Figure 1, and the elastic property[16] was calculated. Using the Doppler echo method, blood flow in the common carotid artery and segmental renal artery were measured, as was cardiac output. In addition, blood samples were taken at three time points, namely, before, during and after AM infusion (Figure 1). Plasma total and mature AM were measured by specific immunoradiometric assay kits (Shionogi, Osaka, Japan). Plasma concentrations of other hormones were measured using a commercially available laboratory testing service (SRL, Hachioji, Japan).

### Statistical analyses

All data were expressed as the mean ± s.e.m. Comparisons of parameters between the two groups (AM *vs.* nicardipine) were carried out using paired Student's *t*-tests. Comparisons of the time course of parameters between the two groups were carried out by two-way repeated measures analysis of variances followed by Bonferroni/Dunn's multiple comparison tests. A value of $P<0.05$ was the criterion for statistical significance.

## RESULTS

Table 1 presents the baseline characteristics of the participants. AM and nicardipine achieved the same levels of systolic blood pressure reduction in all subjects, as shown in Figure 2. However, AM produced a stronger diastolic blood pressure reduction and heart rate increase when compared with nicardipine (Figures 2a and b). Most interestingly, AM caused a significantly larger reduction of PWV and elastic property of the carotid artery when compared with nicardipine (Figures 2c and d). These changes rapidly recovered after termination of AM or nicardipine administration, except for the prolonged decrease in systolic blood pressure induced by nicardipine. Table 2

**Table 1 Baseline characteristics of participants**

| | *Normotensive* | *Hypertensive* | *Diabetic* |
|---|---|---|---|
| *N* | 11 | 9 | 8 |
| Age (years) | 40.8±2.5 | 50.0±2.7* | 48.5±3.3 |
| BMI (kg $m^{-2}$) | 24.3±0.5 | 24.1±0.8 | 25.2±0.9 |
| SBP (mm Hg) | 118.9±1.8 | 155.4±4.8** | 131.3±3.2** |
| DBP (mm Hg) | 74.9±2.6 | 95.0±3.6** | 81.0±2.2 |
| Heart rate (b.p.m.) | 59.5±2.4 | 65.8±3.6 | 63.1±2.5 |
| PWV (cm $s^{-1}$) | 1263±52 | 1598±73** | 1435±35* |
| Elastic property (kPa) | 97.7±8.5 | 154.2±9.7** | 107.1±7.6 |
| Peak CAF (cm $s^{-1}$) | 87.1±2.7 | 76.8±3.0* | 87.9±6.9 |
| Mean CAF (cm $s^{-1}$) | 37.5±1.7 | 36.1±2.7 | 38.1±2.0 |
| Peak RAF (cm $s^{-1}$) | 49.1±4.1 | 40.0±2.6 | 48.1±5.2 |
| Mean RAF (cm $s^{-1}$) | 28.9±1.8 | 25.1±1.7 | 28.5±3.3 |
| Cardiac output (l $min^{-1}$) | 4.52±0.28 | 4.55±0.28 | 4.73±0.25 |
| IRI (μU $ml^{-1}$) | 7.7±1.7 | 8.2±2.0 | 6.3±1.5 |
| Blood sugar (mg per 100 ml) | 99.4±2.2 | 104.9±3.3 | 165.0±11.5** |
| Total AM (fmol $ml^{-1}$) | 12.9±0.7 | 13.5±1.0 | 15.4±2.3 |
| Mature AM (fmol $ml^{-1}$) | 2.0±0.2 | 1.9±0.2 | 1.7±0.2 |
| Adrenaline (pg $ml^{-1}$) | 20,4±2.9 | 32.8±7.6 | 25.3±2.6 |
| Noradrenaline (pg $ml^{-1}$) | 255±39 | 282±32 | 188±24 |
| Renin activity (ng $ml^{-1}$ $h^{-1}$) | 0.76±0.28 | 0.73±0.15 | 1.24±0.26 |
| Aldosterone (pg $ml^{-1}$) | 70.2±11.0 | 76.0±7.5 | 77.6±8.9 |
| ANP (pg $ml^{-1}$) | 17.2±2.8 | 29.1±8.2 | 19.1±5.5 |
| BNP (pg $ml^{-1}$) | 12.1±2.9 | 36.7±22.0 | 10.2±3.9 |
| cAMP (pmol $ml^{-1}$) | 11.7±0.6 | 11.9±0.5 | 11.6±0.7 |
| cGMP (pmol $ml^{-1}$) | 2.9±0.3 | 4.4±1.0 | 2.9±0.5 |

Abbreviations: AM, adrenomedullin; ANP, atrial natriuretic peptide; BMI, body mass index; BNP, brain natriuretic peptide; CAF, common carotid artery flow; DBP, diastolic blood pressure; IRI, immunoreactive insulin; PWV, pulse wave velocity; RAF, renal segmental artery flow; SBP, systolic blood pressure.
*$P<0.05$, **$P<0.01$ *vs.* normotensive.

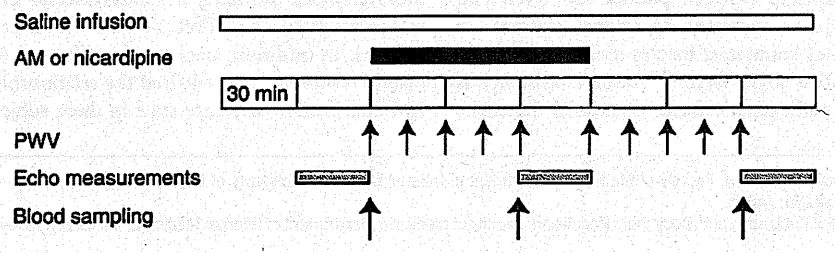


Figure 1 Experimental protocol. After a 60-min baseline period, AM (5 pmol $min^{-1}$ $kg^{-1}$) or nicardipine (1–1.5 μg $min^{-1}$ $kg^{-1}$) was intravenously administered for 90 min followed by a 90 min post-infusion period.

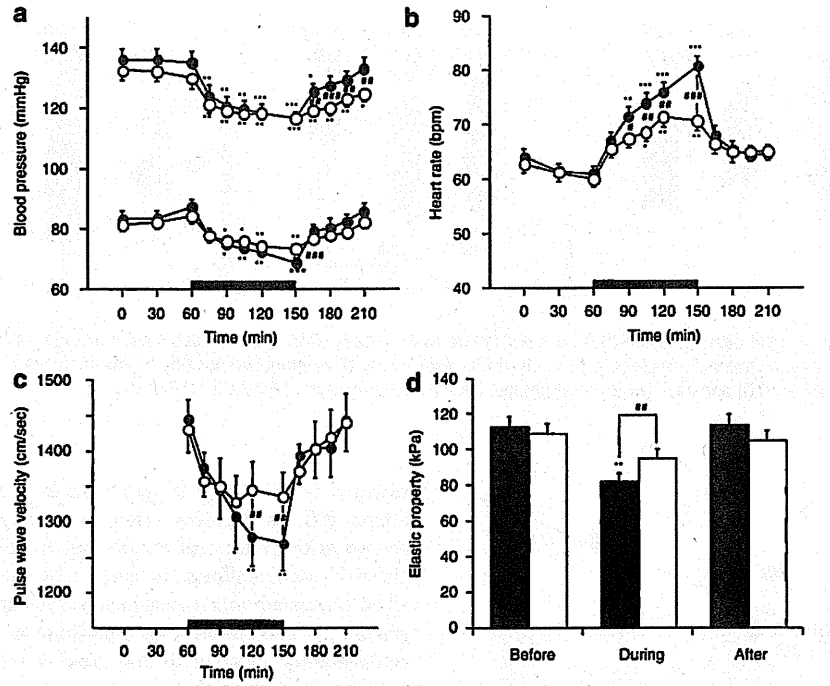


**Figure 2** Changes in blood pressure (a), heart rate (b), pulse wave velocity (c) and elastic property of the carotid artery (d) during infusion of AM (closed symbols) or nicardipine (open symbols). Data are mean ± s.e.m. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.0001$ *vs.* each baseline; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.0001$ *vs.* nicardipine.

**Table 2 Changes of blood flow velocity and cardiac output**

| | *Before* | *During* | *After* | P *in trend* |
|---|---|---|---|---|
| *Peak CAF ($cm\,s^{-1}$)* | | | | |
| AM | 84.0±2.5 | 130.2±3.0$^{**,\#\#}$ | 80.3±2.5 | <0.0001 |
| Nicardipine | 83.9±2.6 | 107.2±2.5$^{**}$ | 84.2±2.0 | <0.0001 |
| *Mean CAF ($cm\,s^{-1}$)* | | | | |
| AM | 37.2±1.2 | 53.8±1.4$^{**,\#\#}$ | 34.3±1.1 | <0.0001 |
| Nicardipine | 36.4±1.1 | 42.7±1.2$^{**}$ | 34.7±1.0 | <0.0001 |
| *Peak RAF ($cm\,s^{-1}$)* | | | | |
| AM | 45.9±2.4 | 61.2±3.3$^{**}$ | 40.9±2.1$^{\#}$ | <0.0001 |
| Nicardipine | 46.1±2.5 | 56.9±2.8$^{**}$ | 45.0±2.2 | 0.0017 |
| *Mean RAF ($cm\,s^{-1}$)* | | | | |
| AM | 27.6±1.3 | 34.0±1.6$^{**,\#}$ | 24.8±1.1$^{\#\#}$ | <0.0001 |
| Nicardipine | 27.7±1.4 | 31.6±1.6 | 27.3±1.3 | 0.066 |
| *Cardiac output ($l\,min^{-1}$)* | | | | |
| AM | 4.59±0.15 | 7.63±0.25$^{**,\#\#}$ | 4.77±0.14 | <0.0001 |
| Nicardipine | 4.58±0.14 | 6.18±0.30$^{**}$ | 4.90±0.27 | <0.0001 |

Abbreviations: AM, adrenomedullin; CAF, common carotid artery flow; RAF, renal segmental artery flow.
Date are mean ± s.e.m.
$^{**}P<0.01$ *vs.* before.
$^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs.* nicardipine.

summarizes the increase in blood flow and cardiac output after AM or nicardipine administration. Both reagents clearly increased blood flow, but AM was more potent than nicardipine. AM induced significantly larger increases in cardiac output when compared with nicardipine.

The responses of these parameters were similar among normotensive, hypertensive and diabetic groups of subjects (data not shown). Next, subjects were divided into three groups according to the homeostasis model assessment of insulin resistance (HOMA-IR) index, according to which the highest tertile (HOMA-IR$\geqslant$2.0) corresponds to an insulin-resistant state in Japan. HOMA-IR values for each group were as follows: low = 0.31–1.39 ($n=9$), middle = 1.43–1.78 ($n=10$) and high = 2.00–6.96 ($n=9$). As shown in Figure 3, only the reduction in PWV induced by AM was blunted in subjects with the highest HOMA-IR, despite the nearly identical reduction in systolic blood pressure. In this group, the reductions in PWV were similar for AM and nicardipine treatments. This phenomenon was also confirmed by the significant correlation between maximum changes in PWV induced by AM and HOMA-IR (Figure 4). The difference in elastic property reduction between AM and nicardipine treatment was not significant in the high HOMA-IR group (Figure 3c).

Table 3 summarizes the changes in humoral factors. AM administration produced significant increases in total AM (approximately 2.5-fold) and mature AM (approximately 7-fold) as well as an approximately 40% increase in the second messenger cAMP. AM and nicardipine produced the same degree of increase in noradrenaline and renin activity. There was no significant difference among the HOMA-IR groups for all humoral factor alterations (data not shown). Finally, AM and nicardipine had no effect on insulin and glucose levels.

## DISCUSSION

In this study, we confirmed the hemodynamic effects of AM as a vasodilative agent in humans. AM increased heart rate and cardiac output and decreased blood pressure. In addition, AM increased blood flow in carotid and renal arteries. These effects were similar

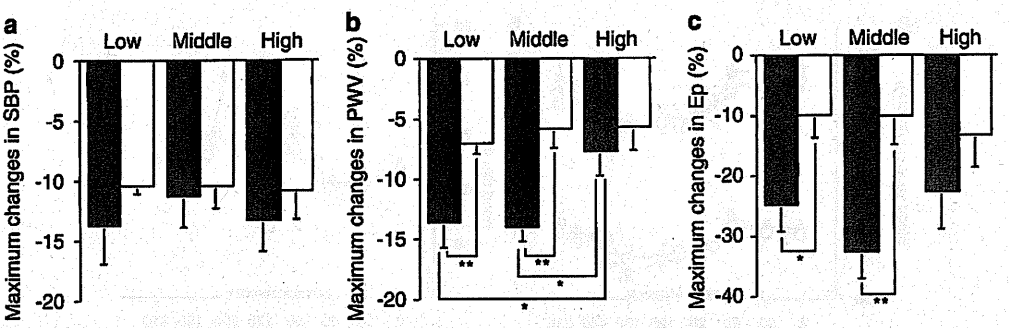


**Figure 3** Maximum changes in systolic blood pressure (SBP, panel a), pulse wave velocity (PWV, panel b) and elastic property of the carotid artery (Ep, panel c) during infusion of AM (closed column) or nicardipine (open column) in each of the three groups divided by HOMA-IR index. Groups divided by HOMA-IR index include low ($n$=9), middle ($n$=10) and high ($n$=9) index groups. Data are mean ± s.e.m. $^*P<0.05$, $^{**}P<0.01$.

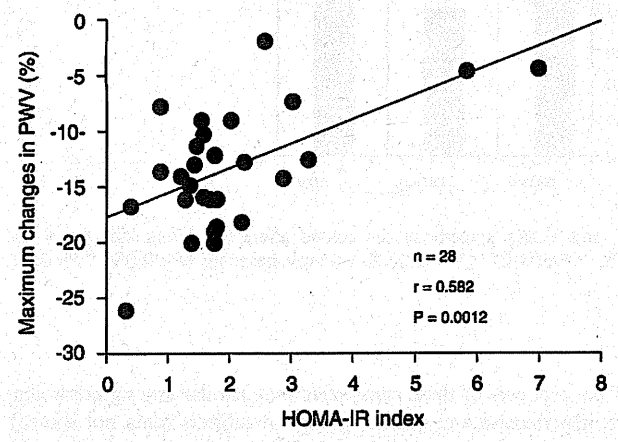


**Figure 4** Relationship between maximum changes in pulse wave velocity (PWV) induced by AM administration and the HOMA-IR index.

to those of the common vasodilator nicardipine. However, AM produced greater increases in cardiac output and heart rate when compared with nicardipine (Table 2). The ventricular myocardium has abundant AM-binding sites; therefore, AM increases cardiac cAMP.[17,18] This cAMP-dependent mechanism mediates the positive inotropic action of β-adrenergic stimulants. AM also enhances angiotensin-II-induced improvement of systolic function, resulting in a further increase in left ventricular ejection fraction.[19] In addition, a cAMP-independent mechanism for the positive inotropic action of AM has been reported.[20] These data suggest that increased cardiac output, and probably heart rate, may be attributable not only to the decrease in cardiac afterload but also to the direct positive inotropic action of AM.

PWV is a convenient indicator of arterial stiffness and is applicable in the casual and prognostic estimation of risk for cardiovascular events. We used brachial ankle PWV (baPWV) in this study. As shown for authentic PWV, baPWV correlates well with blood pressure and aging,[21] and increased baPWV is associated with cardiovascular diseases and risk factors.[22] More importantly, high baPWV values predict poor prognosis in subjects.[23] Conversely, improvement of PWV by antihypertensive therapy may reduce the incidence of cardiovascular events.[15] Strong expression of AM is found ubiquitously in blood vessels where AM functions as a vasodilator, coordinating with other vasodilators, such as nitric oxide, to regulate vascular tonus.[1] AM is suggested to be a significant modulator of arterial stiffness, decreasing blood pressure and vascular wall tension and preventing future wall remodeling. In this study, AM had a greater effect on aortic stiffness, as assessed by baPWV or elastic property, when compared with a common vasodilator $Ca^{2+}$ channel blocker (Figure 2). This feature may contribute to a vascular protective or compensatory function of AM against vascular deterioration and resulting vascular events.

baPWV is mainly altered by blood pressure, but other factors, such as increased heart rate, cardiac output and sympathetic nerve activity, may also increase baPWV. Indeed, the effect of nifedipine on baPWV was reduced by increased sympathetic activity.[24] Although decreases in systolic blood pressure were well matched in nicardipine and AM treatments, AM showed greater baPWV reduction despite larger increments in heart rate and cardiac output when compared with nicardipine (Figure 2 and Table 2). The increases in catecholamines and renin activity were equivalent in both treatments (Table 3). These alterations probably reduced or inhibited the decrease in baPWV in nicardipine treatment. Alternatively, it is conceivable that AM has greater potency against negative alterations to increase baPWV when compared with nicardipine.

AM and nicardipine produced a larger blood pressure reduction in hypertensive participants when compared with normotensive or diabetic participants, which is a common feature of hypotensive reagents (data not shown). However, other hemodynamic effects of AM and nicardipine evaluated within each treatment were essentially the same for each subgroup of participants. We evaluated potentially influential factors in the effects of AM, and we found that only the insulin resistance interfered with the effects of AM. Insulin resistance is an aggravating factor in vascular function and is an underlying cause of cardiovascular diseases. Insulin resistance also influences the sensitivity or efficacy of many drugs and bioactive substances. More importantly, increased arterial stiffness is commonly found in representative insulin resistance states, namely, metabolic syndrome and diabetes.[25–27] As shown in Figures 3 and 4, favorable effects of AM on arterial stiffness were blunted in a state of insulin resistance. AM is thought to function as a suppressive factor against insulin resistance.[8,9] As such, plasma concentration of AM was progressively increased in patients with impaired glucose tolerance, diabetes and diabetes with nephropathy.[28] In addition, an increase in AM was related to multiple metabolic factors.[28] AM and insulin resistance may conflict with each other. Specifically, decreased reactivity of AM may contribute to increased arterial stiffness during insulin resistance, and this alteration may accelerate the progression of arterial sclerosis in insulin resistance.

**Table 3 Hormonal responses to adrenomedullin or nicardipine**

| | *Before* | *During* | *After* | *P in trend* |
|---|---|---|---|---|
| *Total AM, fmol ml$^{-1}$* | | | | |
| AM | 13.9±0.8 | 32.8±1.8** | 15.9±0.6 | <0.0001 |
| Nicardipine | 13.1±0.4 | 12.4±0.4## | 12.8±0.5## | NS |
| *Mature AM, fmol ml$^{-1}$* | | | | |
| AM | 1.9±0.1 | 12.2±0.8** | 2.6±0.1 | <0.0001 |
| Nicardipine | 1.6±0.2 | 1.8±0.2## | 1.7±0.2## | NS |
| *cAMP, pmol ml$^{-1}$* | | | | |
| AM | 11.8±0.3 | 16.5±0.5** | 12.9±0.4 | <0.0001 |
| Nicardipine | 11.1±0.4 | 11.0±0.4## | 11.1±0.4## | NS |
| *cGMP, pmol ml$^{-1}$* | | | | |
| AM | 3.5±0.4 | 3.7±0.3 | 3.4±0.4 | NS |
| Nicardipine | 3.6±0.3 | 3.7±0.3 | 3.0±0.2 | NS |
| *Renin activity, ng ml$^{-1}$ h$^{-1}$* | | | | |
| AM | 0.9±0.1 | 1.7±0.3** | 1.0±0.1 | 0.006 |
| Nicardipine | 0.9±0.1 | 1.7±0.3** | 1.1±0.1 | 0.016 |
| *Aldosterone, pg ml$^{-1}$* | | | | |
| AM | 74.4±5.2 | 69.7±5.3 | 65.3±3.7 | NS |
| Nicardipine | 78.2±4.7 | 77.2±5.3# | 71.0±4.5 | NS |
| *Noradrenaline, pg ml$^{-1}$* | | | | |
| AM | 245±20 | 429±33** | 267±21 | <0.0001 |
| Nicardipine | 247±19 | 423±33** | 346±25**,## | <0.0001 |
| *Adrenaline, pg ml$^{-1}$* | | | | |
| AM | 27.6±3.3 | 32.1±3.8 | 25.8±2.2 | NS |
| Nicardipine | 21.8±2.1# | 24.4±3.2## | 31.8±3.2*,# | 0.04 |
| *ANP, pg ml$^{-1}$* | | | | |
| AM | 21.8±3.2 | 25.4±4.1 | 22.7±3.7 | NS |
| Nicardipine | 17.0±1.8 | 19.0±2.0# | 14.0±1.2## | NS |
| *BNP, pg ml$^{-1}$* | | | | |
| AM | 12.4±2.0 | 11.4±1.8 | 14.0±2.2 | NS |
| Nicardipine | 9.4±1.6 | 10.0±1.7 | 10.1±1.6 | NS |
| *IRI, μIU ml$^{-1}$* | | | | |
| AM | 7.2±1.0 | 7.1±0.9 | 6.0±0.7 | NS |
| Nicardipine | 8.0±0.8 | 7.1±0.7 | 6.4±0.6 | NS |
| *Glucose, mg per 100 ml* | | | | |
| AM | 119±6 | 116±6 | 110±5 | NS |
| Nicardipine | 124±8 | 121±8 | 116±7 | NS |

Abbreviations: AM, adrenomedullin; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; IRI, immunoreactive insulin; NS, not significant.
Date are mean±s.e.m.
*$P$<0.05, **$P$<0.01 *vs.* before.
#$P$<0.05, ##$P$<0.01 *vs.* AM.

However, this intervention is only a temporary treatment, so further studies are required to clarify the relationship between AM and insulin resistance.

Plasma concentration of total AM was increased approximately 2.4-fold above the control value after AM administration (Table 3). This level of AM concentration has been found in renal failure or heart failure patients,[29] so the level of AM used was pathophysiological, not pharmacological. AM administration also increased cAMP, which is a second messenger of AM, approximately 1.4-fold above the control value. Similar changes in AM and cAMP have been reported in previous studies.[10–12] AM and nicardipine also produced similar hormonal alterations, namely, stimulated sympathetic activity and renin release, which was also observed in another study.[10,11] The only difference between the effects of AM and nicardipine in our study was on aldosterone release. AM tended to inhibit aldosterone release despite increased renin activity, although this difference was not significant ($P$=0.051, Table 3). AM did not change aldosterone levels in healthy volunteers or patients with essential hypertension,[10,11] but AM suppressed increased aldosterone levels in patients with heart failure.[12] Furthermore, AM may have renin-independent suppressive potency for aldosterone release, and this feature should be elucidated in future studies.

In conclusion, exogenous AM and $Ca^{2+}$ channel blocker nicardipine caused similar vasodilations in humans, accompanied with resemble interactions with the renin–angiotensin and sympathetic nervous systems. However, AM had a greater potency in its cardiac inotropic action when compared with nicardipine. AM also more effectively decreased arterial stiffness, but the effect was weakened to a similar level as for nicardipine in a state of insulin resistance. Our results support the hypothesis that AM may modulate vasoactive substances and vascular tonus and also have a role in pathophysiological conditions, such as an insulin resistance state.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

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1 Eto T, Kato J, Kitamura K. Regulation of production and secretion of adrenomedullin in the cardiovascular system. *Regl Pept* 2002; **112**: 61–69.
2 Elsasser TH, Kahl S. Adrenomedullin has multiple roles in disease stress: development and remission of the inflammatory response. *Microsc Res Tech* 2002; **57**: 120–129.
3 Wong LY, Cheung BM, Li Y-Y, Tang F. Adrenomedullin is both proinflammatory and anti-inflammatory: its effects on gene expression and secretion of cytokines and macrophage migration inhibitory factor in NR8383 macrophage cell line. *Endocrinology* 2005; **146**: 1321–1327.
4 Shimosawa T, Shibagaki Y, Ishibashi K, Kitamura K, Kangawa K, Kato S, Ando K, Fujita T. Adrenomedullin, an endogenous peptide, counteracts cardiovascular damage. *Circulation* 2002; **105**: 106–111.
5 Marutsuka K, Hatakeyama K, Sato Y, Yamashita A, Sumiyoshi A, Asada Y. Immunohistological localization and possible functions of adrenomedullin. *Hypertens Res* 2003; **26** (Suppl): S33–S40.
6 Shinomiya K, Ohmori K, Ohyama H, Hosomi N, Takahashi T, Osaka K, Kohno M. Association of plasma adrenomedullin with carotid atherosclerosis in chronic ischemic stroke. *Peptides* 2001; **22**: 1873–1880.
7 Kita T, Kitamura K, Hashida S, Morishita K, Eto T. Plasma adrenomedullin is closely correlated with pulse wave velocity in middle-aged and elderly patients. *Hypertens Res* 2003; **26**: 887–893.
8 Shimosawa T, Ogihara T, Matsui H, Asano T, Ando K, Fujita T. Deficiency of adrenomedullin induces insulin resistance by increasing oxidative stress. *Hypertension* 2003; **41**: 1080–1085.
9 Xing G, Shimosawa T, Ogihara T, Matsui H, Itakura K, Qingyou X, Asano T, Ando K, Fujita T. Angiotensin II-induced insulin resistance is enhanced in adrenomedullin-deficient mice. *Endocrinology* 2004; **145**: 3647–3651.
10 Lainchbury JG, Troughton RW, Lewis LK, Yandle TG, Richards AM, Nicholls MG. Hemodynamic, hormonal, and renal effects of short-term adrenomedullin infusion in healthy volunteers. *J Clin Endocrinol Metab* 2000; **85**: 1016–1020.
11 Troughton RW, Levis LK, Yandle TG, Richards AM, Nicholls MG. Hemodynamic, hormone, and urinary effects of adrenomedullin infusion in essential hypertension. *Hypertension* 2000; **36**: 588–593.

12 Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, Oya H, Kyotani S, Nakanishi N, Goto Y, Masuda Y, Miyatake K, Kangawa K. Hemodynamic, renal, and hormonal effects of adrenomedullin infusion in patients with congestive heart failure. *Circulation* 2000; **101**: 498–503.
13 Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999; **32**: 570–574.
14 Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; **37**: 1236–1241.
15 Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation* 2001; **103**: 987–992.
16 Blankenhorn DH, Chin HP, Conover DJ, Nessim SA. Ultrasound observation on pulsation in human carotid artery lesions. *Ultrasound Med Biol* 1988; **14**: 583–587.
17 Owji AA, Smith DM, Coppock HA, Morgan DG, Bhogal R, Ghatei MA, Bloom SR. An abundant and specific binding site for the novel vasodilator adrenomedullin in the rat. *Endocrinology* 1995; **136**: 2127–2134.
18 Ihara T, Ikeda U, Tate Y, Ishibashi S, Shimada K. Positive inotropic effects of adrenomedullin on rat papillary muscle. *Eur J Pharmacol* 2000; **390**: 167–172.
19 Luodonpää M, Leskinen H, Ilves M, Vuolteenaho O, Ruskoaho H. Adrenomedullin modulates hemodynamic and cardiac effects of angiotensin II in conscious rats. *Am J Physiol* 2004; **286**: R1085–R1092.
20 Szokodi I, Kinnunen P, Tavi P, Weckstrom M, Toth M, Ruskoaho H. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. *Circulation* 1998; **97**: 1062–1070.
21 Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, Hori S, Yamamoto Y, Doba N, Hinohara S. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement—a survey of 12517 subjects. *Atherosclerosis* 2003; **166**: 303–309.
22 Yamashina A, Tomiyama H, Arai T, Hirose H, Koji Y, Hirayama Y, Yamamoto Y, Hori S. Brachial-ankle pulse wave velocity as a marker of atherosclerotic vascular damage and cardiovascular risk. *Hypertens Res* 2003; **26**: 615–622.
23 Kitahara T, Ono K, Tsuchida A, Kawai H, Shinohara M, Ishii Y, Koyanagi H, Noguchi T, Matsumoto T, Sekihara T, Watanabe Y, Kanai H, Ishida H, Nojima Y. Impact of brachial-ankle pulse wave velocity and ankle-brachial blood pressure index on mortality in hemodialysis patients. *Am J Kidney Dis* 2005; **46**: 688–696.
24 Munakata M, Nagasaki A, Nunokawa T, Sakuma T, Kato H, Yoshinaga K, Toyota T. Effects of valsartan and nifedipine coat-core on systemic arterial stiffness in hypertensive patients. *Am J Hypertens* 2004; **17**: 1050–1055.
25 Ghiadoni L, Penno G, Giannarelli C, Plantinga Y, Bernardini M, Pucci L, Miccoli R, Taddei S, Salvetti A, Del Prato S. Metabolic syndrome and vascular alterations in normotensive subjects at risk of diabetes mellitus. *Hypertension* 2008; **51**: 440–445.
26 Kovaite M, Petrulioniene Z, Ryliskyte L, Badariene J, Dzenkeviciute V, Cypiene A, Laucevicius A, Polena S, Gintautas J. Systemic assessment of arterial wall structure and function in metabolic syndrome. *Proc West Pharmacol Soc* 2007; **50**: 123–130.
27 El Feghali R, Topouchian J, Pannier B, Asmar R. Ageing and blood pressure modulate the relationship between metabolic syndrome and aortic stiffness in never-treated essential hypertensive patients. A comparative study. *Diabetes Metab* 2007; **33**: 183–188.
28 Lim SC, Morgenthaler NG, Subramanian T, Wu YS, Goh SK, Sum CF. The relationship between adrenomedullin, metabolic factors, and vascular function in individuals with type 2 diabetes. *Diabetes Care* 2007; **30**: 1513–1519.
29 Nishikimi T. Adrenomedullin in cardiovascular disease. *Adv Pharmacol* 1998; **42**: 599–603.

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ORIGINAL ARTICLE

# One-year effectiveness and safety of open-label losartan/hydrochlorothiazide combination therapy in Japanese patients with hypertension uncontrolled with ARBs or ACE inhibitors

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**The long-term antihypertensive efficacy and safety of losartan/hydrochlorothiazide (HCTZ) combinations have not been appropriately evaluated in Japan. In this study, treated hypertensive patients taking angiotensin-receptor blocker (ARB) or angiotensin-converting enzyme inhibitor (ACEI) regimens not at blood pressure (BP) goals proposed by the Japanese Society of Hypertension (JSH) were switched to losartan/HCTZ combinations and followed for 1 year. Data analysis included 244 patients aged 64.5 ± 10.7 years, 56% male, 27% with diabetes mellitus and 36% with dyslipidemia. Pre-switching BP 157 ± 16/88 ± 10 mm Hg promptly decreased and maintained a steady state, reaching 132 ± 15/77 ± 9 mm Hg ($P<0.001$) 1 year later. After 1 year of treatment, 50% of patients cleared the goals of the JSH guideline for systolic BP and 79% for diastolic BP. Patients with maximal doses of ARBs tended to show larger decreases in BP (159 ± 11/90 ± 10 to 128 ± 10/75 ± 8 mm Hg, $P<0.001$, $n=32$). Clinical and laboratory adverse events were reported for 29 patients (11%), but serious abnormalities were not observed. In particular, plasma levels of uric acid (UA) were well-maintained for 1 year, and significant decreases in UA were observed in patients with higher levels of UA ($\geqslant 7.0\ mg\ dl^{-1}$). Losartan/HCTZ combinations showed strong and steady hypotensive abilities and acceptable safety and tolerability in patients currently not at BP goals with regimens including ARBs or ACEIs in Japan.**
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**Keywords:** angiotensin-receptor blocker (ARB); Japanese; losartan/hydrochlorothiazide; uric acid

## INTRODUCTION

Guidelines for hypertension treatment, including those of the Japanese Society of Hypertension (JSH), have recommended strict blood pressure (BP) control, with the aim of improving protection against cardiovascular and renal accidents.[1,2] However, considerable numbers of hypertensive patients have not achieved the recommended goals of BP in Japan.[3] The JSH guideline recommends angiotensin-receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACEIs), $Ca^{2+}$ channel blockers (CCB), β-blockers and diuretics as first-line drugs for hypertensive treatment.[1] The guideline also recommends appropriate combinations of the drugs, in particular low-dose (quarter to half dose) diuretics are recommended as an important candidate for satisfactory BP control.[1] However, the prescribing rate of diuretics was quite low (under 10%) in cases of monotherapy or combination therapy for hypertension in Japan.[4] The principal reason for reluctance to prescribe thiazide diuretics is the metabolic side effects of the drugs. However, low-dose thiazide diuretics retain their hypotensive abilities with minimal side effects.[5] Therefore, proper application of low-dose diuretics, particularly in combination therapies, is desirable in Japan to improve BP control.

A fixed dose combination of losartan (50 mg)/hydrochlorothiazide (HCTZ, 12.5 mg) (Preminent; Banyu/Merck, Tokyo, Japan) is the first combination of an ARB and a diuretic for hypertensive treatment in Japan, and is expected to be effective and safe from the pharmacological properties of both drugs. However, limited data were available on the combination drug in Japan, especially with regard to long-term treatment, large numbers of patients and its use in a clinical setting.[6–8] We organized a study group mainly consisting of clinical physicians in Miyazaki Prefecture in Japan (Preminent Assigned League in Miyazaki by Primary care physicians: PALM-1 study group), and evaluated the

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efficacy and safety of the fixed combination of losartan/HCTZ for patients with essential hypertension for 1 year.

## METHODS

### Study subjects

This study was conducted at 43 centers for the PALM-1 study group (Appendix). Patients with essential hypertension (20–79 years old) were considered for screening and potential recruitment into the trial. They had visited the attending clinics from February 2007 to March 2008 and had not reached BP goals with antihypertensive therapy regimens, including ARBs or ACEIs, but not diuretics, over 1 month. Patients were excluded from the study if there was any evidence of secondary hypertension, renal failure (serum creatinine $\geqslant 2.0\,mg\,dl^{-1}$), severe liver dysfunction and symptomatic heart failure (New York Heart Association functional class-III or IV for dyspnea at exertion). Patients with concomitant use of two or more ARBs and/or ACEIs and any type of diuretics were also excluded.

### Study protocol

The study was conducted in accordance with the principles of the declaration of Helsinki. The investigational protocol was approved by the ethics committee for human studies at the University of Miyazaki. Informed consent was obtained from all patients prior to recruitment.

This was an open-label, multicenter study consisting of a 3-month screening/baseline period and 1-year treatment period. Under antihypertensive treatment with regimens including ARBs or ACEIs, at least two BP measurements were conducted within 3 months of the baseline period to confirm baseline BP measurements were over the recommended BP goals of the JSH. The BP goals were 130/85 mm Hg for patients aged less than 65 years, 140/90 mm Hg for those aged 65 years or more, 130/80 mm Hg for patients with diabetes and/or chronic kidney disease and/or history of myocardial infarction, and 140/90 mm Hg for patients with a history of stroke.[1] After screening 311 patients, 266 entered the trial. Then only ARBs or ACEIs were switched to the fixed dose combination of losartan/HCTZ and patients were followed for 1 year. Changed prescriptions were kept for the initial 3 months and then, if needed, adjustments of antihypertensive drugs were allowed except for ARBs, ACEIs and diuretics. Symptoms, sitting BP, pulse rate and blood tests, including potassium, uric acid (UA), lipid profile, creatinine, glucose, hemoglobin-A1c (HbA1c, diabetic patients only), were evaluated every 3 months. Major complications were also evaluated. The criteria for diabetes and dyslipidemia were as follows: diabetes, using antiglycemic drugs or fasting blood glucose $\geqslant 126\,mg\,dl^{-1}$; dyslipidemia, using lipid-lowering drugs or total cholesterol $\geqslant 220\,mg\,dl^{-1}$ and/or high-density lipoprotein-cholesterol $<40\,mg\,dl^{-1}$, and/or triglyceride $\geqslant 150\,mg\,dl^{-1}$.

### Statistical analysis

All data are expressed as mean ± s.d. The significance of differences was evaluated by one-factor analysis of variance with repeated measures on the time course of variables followed by Bonferroni/Dunn *post hoc* comparison tests. Comparisons of parameters among subgroups were made by unpaired Dunnet's C-test or analysis of variance followed by Scheffe's *post hoc* comparison test. *P*-value $<0.05$ was the criterion for statistical significance.

## RESULTS

As indicated in Figure 1, 22 of the 266 enrolled patients dropped out within the first 3 months. The remaining 244 patients were considered as full analytical objects. Finally, 222 patients completed the entire trial and were used for evaluation of efficacy.

The baseline characteristics of the study population are summarized in Table 1. Patients' age was 64.5 ± 10.7 years, 56% were male and major complications included 27% of patients with diabetes, 36% with dyslipidemia and 18% with mild heart failure. Pre-prescribed ARBs or ACEIs were well distributed from among drugs on the market and, noteworthy, the average doses per day of the drugs were very close to the usual dosage of each drug (Table 1). ARBs or ACEIs were used as monotherapy for 93 patients (38%) and as combined therapy, mainly with CCB, for 151 patients (62%). Other pre-prescribed drugs were as follows and these drugs were not altered after introduction of the losartan/HCTZ combination: antiglycemic drugs for 38 of 266 patients (37 of 222), lipid-lowering drugs for 58 of 266 (53 of 222) and UA-lowering drugs for 14 of 266 (14 of 222).

The time course of BP in all patients is illustrated in Figure 2. Baseline BP 157 ± 16/88 ± 10 mm Hg significantly decreased to 134 ± 14/77 ± 9 mm Hg at 3 months ($P<0.001$) (fixed prescription period), and then steady levels were maintained throughout the remaining treatment period. The respective goals of BP were cleared by 50% of the patients for systolic BP and 79% of the patients for diastolic BP in the final assessment 1 year later. Interestingly, 32 of 222 patients who were switched from the maximum dose of ARBs showed a similar to larger decrease in BP

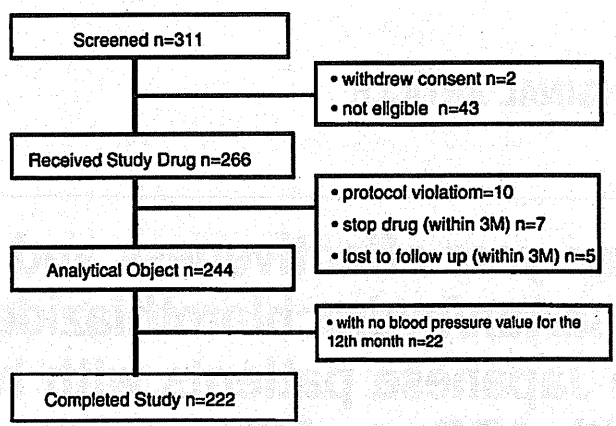



**Figure 1** Patient disposition and reasons for exclusion.

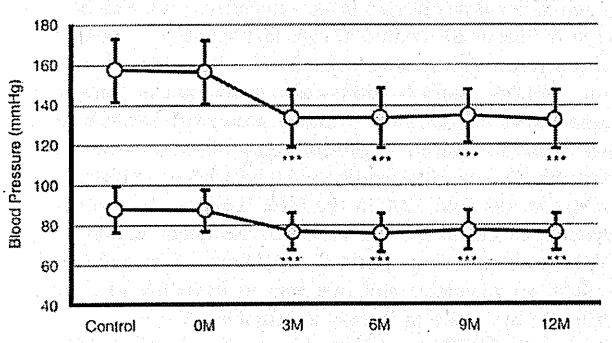
**Table 1 Baseline characteristics (*n*=244)**

| *Variable* | *Value* | *Average doses (mg day$^{-1}$)* |
|---|---|---|
| Age (years) | 64.5 ± 10.7 | |
| Male (*n*) | 136 (56%) | |
| Body mass index (kg m$^{-2}$) | 25.2 ± 5.6 | |
| Waist circumference (cm) | 85.9 ± 8.6 | |
| Obesity (*n*) | 110 (48%) | |
| Diabetes (*n*) | 66 (27%) | |
| Dyslipidemia (*n*) | 88 (36%) | |
| Heart diseases (*n*) | 43 (18%) | |
| Renal insufficiency (*n*) | 8 (3%) | |
| *Antihypertensives (n)* | | |
| One drug | 93 (38%) | |
| Over two drugs | 151 (62%) | |
| *Pre-prescribed drugs (n)* | | |
| Valsartan | 68 (28%) | 88.8 ± 40.1 |
| Candesartan | 54 (22%) | 8.4 ± 2.2 |
| Losartan | 34 (14%) | 51.5 ± 8.6 |
| Telmisartan | 32 (13%) | 40.3 ± 10.0 |
| Olmesartan | 31 (13%) | 22.6 ± 8.6 |
| ACE inhibitors | 25 (10%) | |

Abbreviation: ACE, angiotensin-converting enzyme.

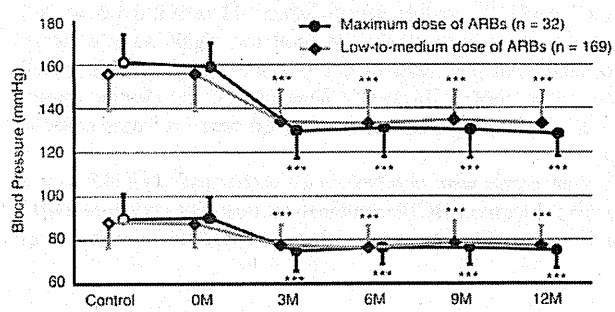
as compared with patients with low-to-medium dose of ARBs (Figure 3). There was a significant difference in the changes of BP from 3 months to 1 year between patients switched from low-to-medium dose of ARBs and maximum dose of ARBs (at 1 year: systolic BP, 23 ± 19 *vs.* 31 ± 13 mm Hg, $P$=0.005; diastolic BP, 10 ± 11 *vs.* 15 ± 10 mm Hg, $P$=0.027). As shown in Figure 4, similar and significant decreases in systolic and diastolic BP were achieved in all patients grouped based on pre-prescribed drugs at 1 year. Also there was no difference in BP changes among all ARBs and ACEI-receiving patients. The systolic and diastolic BPs at 0 and 12 month (changes of the BPs) for each drug were as follows: losartan, 154 ± 17 to 135 ± 10 mm Hg (−19 ± 17 mm Hg, $P$<0.001) and 87 ± 11 to 78 ± 8 mm Hg (−9 ± 10 mm Hg, $P$<0.001); candesartan, 156 ± 14 to 131 ± 14 mm Hg (−24 ± 17 mm Hg, $P$<0.001) and 87 ± 9 to 76 ± 9 mm Hg (−11 ± 10 mm Hg, $P$<0.001); valsartan, 160 ± 16 to 134 ± 13 mm Hg (−26 ± 18 mm Hg, $P$<0.001) and 89 ± 9 to 77 ± 8 mm Hg (−12 ± 10 mm Hg, $P$<0.001); telmisartan, 156 ± 17 to 132 ± 20 mm Hg (−24 ± 15 mm Hg, $P$<0.001) and 85 ± 12 to 75 ± 11 mm Hg (−10 ± 8 mm Hg, $P$<0.001); olmesartan, 153 ± 18 to 129 ± 14 mm Hg (−24 ± 24 mm Hg, $P$<0.001) and 88 ± 15 to 77 ± 10 mm Hg (−11 ± 15 mm Hg, $P$<0.001); and ACEIs, 159 ± 16 to 133 ± 19 mm Hg (−26 ± 20 mm Hg, $P$<0.001) and 87 ± 9 to 76 ± 12 mm Hg (−11 ± 12 mm Hg, $P$=0.001). There were very limited number of alterations in antihypertensive drugs after 3 months (8 of 222): two terminations of CCBs, one decrease of CCB, four introductions of low doses of CCBs for patients receiving low-to-medium dose of ARBs and one introduction of atenolol (12.5 mg) for a patient with maximum dose of ARBs.

To determine the difference in receptivity to losartan/HCTZ between specific backgrounds of the patients, we compared BP




| | Control | 0M | 3M | 6M | 9M | 12M |
|---|---|---|---|---|---|---|
| SBP : | 157 ± 16 | 157 ± 16 | 134 ± 14 | 133 ± 15 | 134 ± 14 | 132 ± 15 |
| DBP : | 88 ± 12 | 88 ± 10 | 77 ± 9 | 76 ± 9 | 78 ± 10 | 77 ± 9 |
| HR : | 74 ± 11 | 74 ± 12 | 74 ± 13 | 74 ± 11 | 74 ± 12 | 73 ± 11 |

**Figure 2** The time course of BP in all patients ($n$=222). ***$P$<0.001 compared with month 0. BP, blood pressure.




Maximum dose of ARBs

| | Control | 0M | 3M | 6M | 9M | 12M |
|---|---|---|---|---|---|---|
| SBP : | 161 ± 13 | 159 ± 11 | 130 ± 13 | 131 ± 13 | 130 ± 13 | 128 ± 10 |
| DBP : | 89 ± 12 | 90 ± 10 | 75 ± 9 | 76 ± 8 | 76 ± 8 | 75 ± 8 |
| HR : | 71 ± 11 | 70 ± 12 | 71 ± 12 | 72 ± 11 | 72 ± 11 | 72 ± 12 |

Low-to-medium dose of ARBs

| | Control | 0M | 3M | 6M | 9M | 12M |
|---|---|---|---|---|---|---|
| SBP : | 156 ± 16 | 156 ± 17 | 134 ± 15 | 133 ± 15 | 135 ± 13 | 133 ± 15 |
| DBP : | 88 ± 11 | 87 ± 11 | 77 ± 9 | 76 ± 10 | 78 ± 10 | 77 ± 9 |
| HR : | 75 ± 11 | 74 ± 11 | 74 ± 13 | 74 ± 11 | 74 ± 12 | 74 ± 10 |

**Figure 3** The time course of BP in patients switched from maximum dose ($n$=32) and low-to-medium dose ($n$=169) of ARBs. ***$P$<0.001 compared with month 0. ARB, angiotensin-receptor blocker; BP, blood pressure.

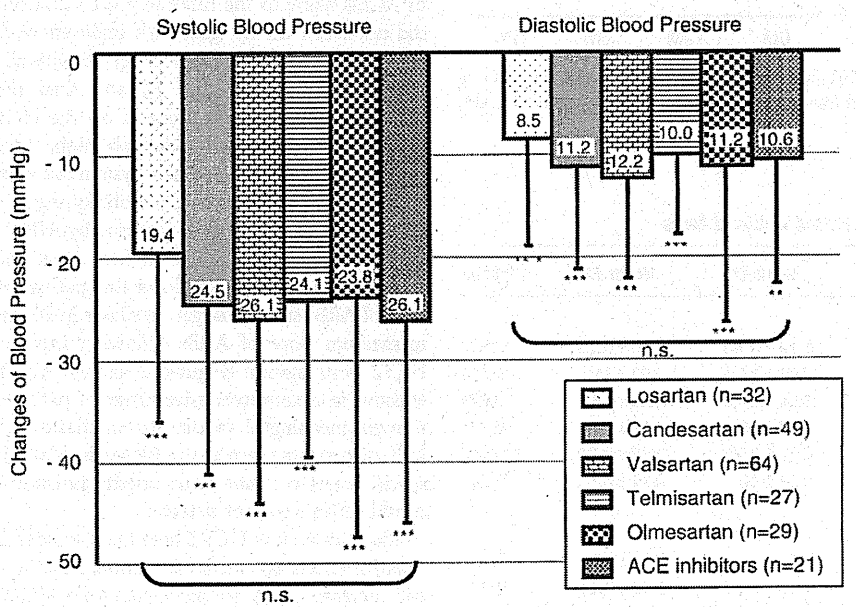



**Figure 4** Decreases in BP after 12 months for each pre-prescribed drug. **$P$<0.01, ***$P$<0.001 compared with month 0. BP, blood pressure.

changes at 1 year for various subgroups. However, there was no difference among the subgroups and specific factors contributing to resistance against losartan/HCTZ were not detected. For example, if patients are grouped according presence (+) or absence (−) of diabetes (D) and obesity (O) (body mass index, $\geqslant 25\,kg\,m^{-2}$), decreases in systolic BP were $24 \pm 18$ mm Hg (D+/O+, $n=35$), $23 \pm 17$ mm Hg (D+/O−, $n=27$), $24 \pm 15$ mm Hg (D−/O+, $n=63$) and $25 \pm 21$ mm Hg (D−/O−, $n=97$). This indicates that the losartan/HCTZ combination is effective even for patients with diabetes and obesity.

Remarkable changes were not observed in metabolic parameters after 1 year of treatment with losartan/HCTZ. Figure 5 shows changes in UA levels in all patients ($5.46 \pm 1.43$ to $5.62 \pm 1.43\,mg\,dl^{-1}$) and subgroups with high levels of UA at baseline and others. UA level was slightly increased in patients with relatively low levels of UA (UA $< 7.0\,mg\,dl^{-1}$, middle panel): $5.02 \pm 1.11$ to $5.37 \pm 1.34\,mg\,dl^{-1}$ ($P<0.001$). But, interestingly, UA level was significantly decreased in patients with high level of UA (UA $\geqslant 7.0\,mg\,dl^{-1}$, right panel): $7.66 \pm 0.57$ to $6.88 \pm 1.16\,mg\,dl^{-1}$ ($P=0.004$). Other changes (month 0 to 12) concerning parameters in blood tests are summarized in Table 2.

Adverse events were observed in 29 of 266 patients (10.9%) who received the losartan/HCTZ combination, including accidental events, and 16 (5.4%) discontinued the losartan/HCTZ combination, while the remaining 13 patients continued receiving the drug. Among the 16 patients who discontinued, 13 events (4.9%) were considered possibly, probably or definitely drug-related. Laboratory abnormalities were observed for 13 patients. The 13 drug-related adverse events included three cases of hypokalemia, two patients who complained of skin rash, one patient who suffered photosensitive dermatoses, worsening of diabetes in one patient and excessive BP depression in six patients. Four patients of 266 discontinued the losartan/HCTZ combination because of patient circumstances or requests, without adverse events. No death occurred during the study.

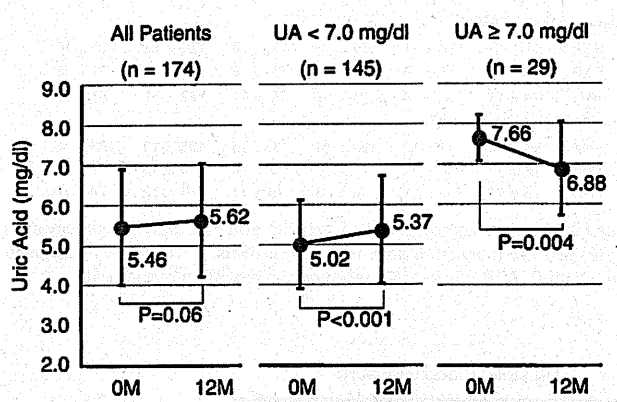



**Figure 5** Changes in serum UA levels in all patients (left panel) and in those with high (middle panel) and low-to-medium levels (right panel) of UA. UA, uric acid.

**Table 2 Changes of parameters in blood tests**

| | *Month 0* | *Month 12* | P-*value* |
|---|---|---|---|
| *All patients* | | | |
| Potassium ($mEq\,l^{-1}$) | 4.13±0.48 | 4.15±0.52 | 0.67 |
| Total cholesterol ($mg\,dl^{-1}$) | 199±34 | 191±31 | 0.001 |
| HDL-cholesterol ($mg\,dl^{-1}$) | 56.4±14.6 | 55.1±13.6 | 0.075 |
| Triglyceride ($mg\,dl^{-1}$) | 147±96 | 149±96 | 0.74 |
| Creatinine ($mg\,dl^{-1}$) | 0.83±0.29 | 0.88±0.30 | <0.001 |
| Glucose ($mg\,dl^{-1}$) | 118±46 | 121±52 | 0.24 |
| *Diabetic patients only (n=52)* | | | |
| Glucose ($mg\,dl^{-1}$) | 154±62 | 155±73 | 0.83 |
| HbA1c (%) | 6.45±1.22 | 6.46±1.15 | 0.91 |

Abbreviations: ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein.

## DISCUSSION

Only 42% of hypertensive patients reached the guideline BP goals in the J-HOME (Japan Home versus Office Blood Pressure Measurement Evaluation) study.[3] Mori *et al.*[4] reported that hypertensive patients attaining BP under 140/90 mm Hg by monotherapy were limited to 34.0% with ARBs and 40.3% with CCBs. Additionally, strict BP goals (130/80 mm Hg) are recommended for hypertensive patients with diabetes, chronic kidney disease and old myocardial infarction.[1] Addition of low-dose diuretics is recommended as a key combination therapy for better BP control in the JSH guideline.[1] However, the prescription rate of diuretics remains low in Japan, for example, 9.3% in the J-HOME study.[9] Additionally, combination therapy with diuretics seems to contribute to organ protection. Many large-scale clinical trials have shown organ-protective effects of losartan, and, importantly, the majority of patients in these trials concomitantly used diuretics, for example, 72% in the LIFE (Losartan Intervention For Endpoint) trial and 84% in the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin-II Antagonist Losartan) trial.[10,11] Therefore, an acceptable and safe way to introduce low doses of diuretics for hypertension therapy is desirable in Japan.

The losartan/HCTZ combination is composed of losartan, which displays superior activity under the activated renin–angiotensin system[12] and a thiazide-diuretic that activates renin–angiotensin system through a diuretic effect,[13] so this combination is expected to be efficient in BP lowering by the synergistic effect of both the drugs. In this study, BP was decreased by $23 \pm 17/11 \pm 10$ mm Hg at 3 months and $24 \pm 18/11 \pm 11$ mm Hg at 12 months after switching from ARBs or ACEIs alone to the losartan/HCTZ combination for patients who did not reach the BP goal with regimens including ARBs or ACEIs. Similar decreases in BP were observed with all types of pre-prescribed ARBs and ACEIs (Figure 4), and thus these strong and steady decreases in BP seem to depend on the HCTZ 'add-on' effect. Salt intake of the Japanese is relatively high,[14] and thus excess salt may suppress the renin–angiotensin system and disturb the ability of ARBs or ACEIs. In particular, this possibility seems high for patients whose BP was not satisfactorily suppressed by ARBs or ACEIs. Alternatively, HTCZ probably works well in that situation, and this possibility is indirectly supported by evidence that patients pre-using the maximum dose of ARBs showed larger decreases in BP than those using the low-to-medium dose of ARBs following introduction of the losartan/HCTZ combination (Figures 2 and 3). Also this synergistic effect is effective in a comprehensive range of patients; over 90% of patients showed meaningful reductions in diastolic BP ($\geqslant 10$ mm Hg) and 79% of patients reached the BP goals of the JSH guideline, and thus specific cases of diabetes or obesity resistant against losartan/HCTZ combination were not detected.

Diuretics such as HCTZ have been avoided in Japan for fear of their negative effects on metabolic parameters.[4] In particular, hypokalemia and increase in UA are associated with HCTZ. In combination with losartan, hypokalemia may be canceled by the anti-aldosterone effect

of ARBs and UA elevation may be enfeebled by the UA-decreasing ability of losartan. Losartan has a unique effect of stimulating UA excretion in urine by suppressing UA transporters URAT1 and URATv1, with a resulting decrease in the serum levels of UA.[15,16] In this study, these expectations were well achieved and potassium and UA levels were kept within normal ranges. Additionally, a significant decrease in UA was observed for patients with high levels of UA (Figure 5). Except for losartan, clinical doses of ARBs do not have suppressive properties on the UA transporters.[17] This property of losartan should be profitable in combination with HCTZ.

Another concern with HCTZ is worsening of glucose metabolism. A recent cohort study in Taiwan showed that diuretic or β-blocker monotherapy increased the risk of new-onset diabetes, but combination therapies composed of diuretics or β-blocker with ACEI or ARB did not. Conversely, there was a decrease in the risk of new-onset diabetes.[18] In this study, blood glucose and HbA1c levels were stable in patients with diabetes (Table 2), as was glucose level in all patients, and so the losartan/HCTZ combination appears to be safe for glucose metabolism. However, the sensitivity of glucose metabolism under diuretics use could be changed by gene variation,[19] and thus there may be small numbers of susceptible patients. In fact, one patient dropped out because of worsening of diabetes in this study. Therefore, careful monitoring of glucose metabolism is required.

Fixed dose combination drugs decrease the number of pills taken and may contribute to better adherence. Patients on a fixed-combination regimen showed better persistence after 1 year of antihypertensive treatment, namely 58% for combination therapy with ACEI plus diuretics in two pills, and 70% for one-pill fixed combination.[20] In this study, a limited number of patients, 44 of 266 (16.5%), dropped out despite the clinical setting, so this fixed combination could be beneficial in clinical use.

In summary, a fixed dose combination of losartan/HCTZ for 1 year of treatment in a clinical setting resulted in sufficient and steady BP decrease in a majority of Japanese hypertensive patients who had not been controlled with a regimen including ARBs or ACEIs. Also this combination showed acceptable safety and tolerability. A fixed dose combination of losartan/HCTZ is an available tool to introduce low-dose diuretics for treatment of uncontrolled hypertension in Japan.

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1 Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ito S, Iwao H, Kario K, Kawano Y, Kim-Mitsuyama S, Kimura G, Matsubara H, Matsuura H, Naruse M, Saito I, Shimada K, Shimamoto K, Suzuki H, Takishita S, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Ueshima H, Umemura S, Ishimitsu T, Rakugi H, on behalf of The Japanese Society of Hypertension Committee. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). *Hypertens Res* 2009; **32**: 3–107.

2 Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Erdine S, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Lindholm LH, Viigimaa M, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Waeber B, Williams B, Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; **25**: 1105–1187.

3 Ohkubo T, Obara T, Funahashi J, Kikuya M, Asayama K, Metoki H, Oikawa T, Takahashi H, Hashimoto J, Totsune K, Imai Y, J-HOME Study Group. Control of blood pressure as measured at home and office, and comparison with physicians' assessment of control among treated hypertensive patients in Japan: first report of the Japan Home versus Office Blood Pressure Measurement Evaluation (J-HOME) study. *Hypertens Res* 2004; **27**: 755–763.

4 Mori H, Ukai H, Yamamoto H, Saitou S, Hirao K, Yamauchi M, Umemura S. Current status of antihypertensive prescription and associated blood pressure control in Japan. *Hypertens Res* 2006; **29**: 143–151.

5 ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 2002; **288**: 2981–2997.

6 Shimosawa T, Gohchi K, Yatomi Y, Fujita T. Effectiveness of add-on low-dose diuretics in combination therapy for hypertension: losartan/hydrochlorothiazide vs candesartan/amlodipine. *Hypertens Res* 2007; **30**: 831–837.

7 Saruta T, Ogihara T, Matsuoka H, Suzuki H, Toki M, Hirayama Y, Nonaka K, Takahashi K. Antihypertensive efficacy and safety of fixed-dose combination therapy with losartan plus hydrochlorothiazide in Japanese patients with essential hypertension. *Hypertens Res* 2007; **30**: 729–739.

8 Minami J, Abe C, Akashiba A, Takahashi T, Kameda T, Ishimitsu T, Matsuoka H. Long-term efficacy of combination therapy with losartan and low-dose hydrochlorothiazide in patients with uncontrolled hypertension. *Int Heart J* 2007; **48**: 177–186.

9 Murai K, Obara T, Ohkubo T, Metoki H, Oikawa T, Inoue R, Komai R, Horikawa T, Asayama K, Kikuya M, Totsune K, Hashimoto J, Imai Y, J-Home Study Group. Current usage of diuretics among hypertensive patients in Japan: the Japan Home versus Office Blood Pressure Measurement Evaluation (J-HOME) study. *Hypertens Res* 2006; **29**: 857–863.

10 Dahlöf B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H, LIFE Study Group. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 2002; **359**: 995–1003.

11 Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S, RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345**: 861–869.

12 Wong PC, Price WA, Chiu AT, Duncia JV, Carini DJ, Wexler RR, Johnson AL, Timmermans PB. Nonpeptide angiotensin II receptor antagonists. VIII. Characterization of functional antagonism displayed by DuP 753, an orally active antihypertensive agent. *J Pharmacol Exp Ther* 1990; **252**: 719–725.

13 Lijnen P, Fagard R, Staessen J, Amery A. Effect of chronic diuretic treatment on the plasma renin–angiotensin–aldosterone system in essential hypertension. *Br J Clin Pharmacol* 1981; **12**: 387–392.

14 Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: implications for public health. *Int J Epidemiol* 2009; **38**: 791–813.

15 Enomoto A, Kimura H, Chairoungdua A, Shigeta Y, Jutabha P, Cha SH, Hosoyamada M, Takeda M, Sekine T, Igarashi T, Matsuo H, Kikuchi Y, Oda T, Ichida K, Hosoya T, Shimokata K, Niwa T, Kanai Y, Endou H. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature* 2002; **417**: 447–452.

16 Anzai N, Ichida K, Jutabha P, Kimura T, Babu E, Jin CJ, Srivastava S, Kitamura K, Hisatome I, Endou H, Sakurai H. Plasma urate level is directly regulated by a voltage-driven urate efflux transporter URATv1 (SLC2A9) in humans. *J Biol Chem* 2008; **283**: 26834–26838.

17 Iwanaga T, Sato M, Maeda T, Ogihara T, Tamai I. Concentration-dependent mode of interaction of angiotensin II receptor blockers with uric acid transporter. *J Pharmacol Exp Ther* 2007; **320**: 211–217.

18 Liou YS, Ma T, Tien L, Lin CM, Jong GP. The relationship between antihypertensive combination therapies comprising diuretics and/or beta-blockers and the risk of new-onset diabetes: a retrospective longitudinal cohort study. *Hypertens Res* 2009; **32**: 496–499.

19 Bozkurt O, de Boer A, Grobbee DE, de Leeuw PW, Kroon AA, Schiffers P, Klungel OH. Variation in renin-angiotensin system and salt-sensitivity genes and the risk of diabetes mellitus associated with the use of thiazide diuretics. *Am J Hypertens* 2009; **22**: 545–551.

20 Dezii CM. A retrospective study of persistence with single-pill combination therapy vs concurrent two-pill therapy in patients with hypertension. *Manag Care* 2000; **9**(Suppl): 2–6.

## APPENDIX

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ORIGINAL ARTICLE
Cardiovascular Intervention

# Repeated Sirolimus-Eluting Stent Implantation to Treat Sirolimus-Eluting Stent and Bare-Metal Stent Restenosis

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***Background:*** In-stent restenosis (ISR) remains a persistent, unresolved issue even in the era of percutaneous coronary intervention (PCI) using drug-eluting stents. The present study compares the clinical and angiographic outcomes of using sirolimus-eluting stents (SES) for re-intervention against ISR that was originally treated with sirolimus-eluting or bare-metal (BMS) stents.

***Methods and Results:*** This prospective single-center registry investigated 179 ISR lesions in 158 consecutive patients (53 lesions in 49, and 126 in 109 patients originally treated with SES and BMS, respectively), who had undergone re-intervention with SES. The patients were clinically and angiographically followed up at 8 months after re-PCI. The incidence of re-restenosis (29 vs 12%, P<0.01), ischemia-driven target lesion revascularization (TLR; 21 vs 8%, P<0.05) and major adverse cardiac events (MACE; 21 vs 9%, P<0.05) were significantly greater in ISR lesions originally treated with SES than in those originally treated with BMS at 8 months after re-PCI. Moreover, late luminal loss was significantly greater in the group with post-SES restenosis (P<0.05). Even after adjustment, post-SES restenosis was the only independent predictor of re-restenosis and MACE (P<0.05, each).

***Conclusions:*** Although the re-restenosis rate is acceptable, the incidence rates of late restenosis, ischemia-driven TLR and MACE are higher after repeated SES implantation to treat SES, than BMS restenosis. These results might affect the mid-term clinical outcomes of re-intervention with SES. (*Circ J* 2010; **74:** 2329–2333)

**Key Words:** Cardiovascular diseases; Coronary re-intervention; Ischemia; Revascularization

Although drug-eluting stents (DES) have significantly decreased the incidence of in-stent restenosis (ISR) and the need for repeated revascularization compared with bare-metal stents (BMS),[1–5] DES restenosis still develops and ISR remains an important clinical issue especially for patients with highly complex lesions.[2–7] The increasing use of DES in complex settings coupled with the worldwide implantation of >10 million DES[6] implies that DES restenosis will become a significant global problem. However, an optimal treatment for DES restenosis remains unknown, and some experts propose repeated DES implantation. In contrast, several studies have shown the effectiveness of DES in patients with BMS ISR.[4,5] A recent study suggested different pathological features between intra-DES and intra-BMS restenotic tissue.[8] These 2 types of ISR lesion might have different biological responses and clinical outcomes after DES implantation. We therefore compared the clinical and angiographic outcomes of re-intervention with sirolimus-eluting stents (SES; Cypher, Cordis/Johnson & Johnson, Warren, NJ, USA) for ISR lesions that were originally treated with SES or BMS.

## Methods

### Study Patients

The study population of this prospective single-center registry comprised 158 consecutive patients (179 lesions) who underwent re-intervention with SES for ISR between August 2004 and June 2007. Among them, restenosis developed in 49 patients with SES (53 lesions) and in 109 with BMS (126

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**Table 1. Baseline Clinical Characteristics of Patients**

| Patients (n) | SES (n=49) | BMS (n=109) | P value |
|---|---|---|---|
| Age (years) | 67±8.3 | 66.6±10.8 | 0.88 |
| Male gender, n (%) | 41 (84) | 92 (84) | >0.99 |
| Previous MI, n (%) | 20 (41) | 65 (60) | 0.04 |
| Previous CABG, n (%) | 1 (2) | 5 (5) | 0.75 |
| Risk factor, n (%) | | | |
| Hypertension | 36 (73) | 80 (73) | >0.99 |
| Hyperlipidemia | 28 (57) | 42 (41) | 0.09 |
| Current smoking | 6 (12) | 19 (17) | 0.55 |
| Diabetes mellitus | 28 (57) | 36 (33) | <0.01 |

Data are expressed as mean±SD when appropriate.
SES, sirolimus-eluting stent; BMS, bare-metal stent; MI, myocardial infarction; CABG, coronary artery bypass graft.

**Table 2. Baseline Angiographic and Procedural Characteristics**

| Target lesion (n) | SES (n=53) | BMS (n=126) | P value |
|---|---|---|---|
| **Target vessel, n (%)** | | | 0.32 |
| Left anterior descending | 19 (36) | 57 (45) | |
| Left circumflex | 13 (24) | 20 (16) | |
| Right coronary artery | 21 (40) | 49 (39) | |
| **In-stent restenosis type, n (%)*** | | | <0.01 |
| Focal | | | |
| I | 36 (68) | 25 (20) | |
| Non-focal | | | |
| II | 11 (21) | 65 (52) | |
| III | 5 (9) | 29 (23) | |
| IV | 1 (2) | 7 (5) | |
| **Pre-procedure** | | | |
| Reference diameter (mm) | 2.66±0.54 | 2.66±0.61 | 0.97 |
| MLD (mm) | 0.58±0.37 | 0.74±0.41 | 0.02 |
| Diameter stenosis (%) | 77.9±13.9 | 71±17.6 | 0.01 |
| Lesion length (mm) | 17±6.2 | 21±8.1 | <0.01 |
| **Post-procedure MLD (mm)** | 2.68±0.48 | 2.79±0.51 | 0.18 |

Data are expressed as mean±SD when appropriate.
MLD, minimal lumen diameter. Other abbreviations see in Table 1.
*According to the classification by Mehran et al.[9]

lesions). Patients were eligible for the study if they had initial ISR in a native coronary artery with objective evidence of ischemia and without clinical contraindication against prolonged double antiplatelet therapy. All patients provided written, informed consent to participate in the study, and our institutional ethics committee approved the study protocol. We defined ISR using quantitative coronary angiography (QCA) as luminal stenosis of >50% within the stent or within 5 mm of the stent edges. The type of restenosis was categorized as focal (length <10 mm) and non-focal (diffuse, proliferative and occlusive) according to the classification of Mehran et al.[9]

Procedural anticoagulation therapy included heparin targeted to an activated clotting time of 200 to 300 s. All patients underwent repeated percutaneous coronary intervention (re-PCI) with SES according to current guidelines, and the choice of the implanted PCI devices was left to the operators' discretion. Then, if possible, intravascular ultrasound (IVUS)-guided re-PCI was performed to rule out a possible mechanism of stent failure such as underexpansion and to confirm neointimal growth in ISR lesions. All patients received aspirin (100 or 200 mg/day) before, and indefinitely after the procedure. Patients were also concomitantly treated with ticlopidine (200 mg/day) or clopidogrel (50 mg/day or 75 mg/day)[10] for at least 8 months. The patients were followed up angiographically at 8 months post re-PCI or earlier if non-invasive evaluation or clinical presentation suggested ischemia.

### Quantitative Coronary Angiography

Coronary angiograms were analyzed using a validated edge detection system (CMS, MEDIS, Leiden, The Netherlands) by 2 experienced cardiologists (K.N. and T.N.) who were blinded to the clinical classification of the patients. Minimal lumen diameter (MLD), reference vessel diameter, and %diameter stenosis at baseline, post procedure and at follow up were measured. Angiographic re-restenosis was defined by QCA as stenosis of >50% diameter within a previously stented segment (within the stent or 5 mm of the stent edges) on 8-month follow-up angiograms. Late luminal loss was defined as the difference between MLD at the time of the post stenting procedure and that at follow up.

### Clinical Follow up

Patients were followed up to assess the incidence of major