20112003A

厚生労働科学研究費補助金研究報告書表紙

厚生労働科学研究費補助金

循環器疾患・糖尿病等生活習慣病対策総合研究事業

肥満を伴う高血圧症に対する防風通聖散の併用投与による，24時間自由行動下血圧及び糖脂質代謝・酸化ストレスの改善効果についての研究

（ H２２ － 循環器等（生習）－ 一般 － ００９ ）

平成23年度　総括研究報告書

研究代表者　田村　功一

平成２４（２０１２）年　５月

厚生労働科学研究費補助金研究報告書目次

目　　次

厚生労働科学研究費補助金（循環器疾患・糖尿病等生活習慣病対策総合研究事業）
（総括）研究報告書

肥満を伴う高血圧症に対する防風通聖散の併用投与による，24時間自由行動下血圧及び糖脂質代謝・酸化ストレスの改善効果についての研究

研究代表者　田村　功一　横浜市立大学医学部循環器・腎臓内科学　准教授

研究要旨

肥満高血圧は動脈硬化を促進し心血管病・腎不全の根源となり，高齢化を迎えつつある国民の健康レベルのさらなる向上のために，肥満合併高血圧への集学的治療による効率的な医療が必要である．

本研究では，肥満合併高血圧患者に対して漢方薬を用いた東洋医学的治療介入の併用による治療効果の向上を臨床的に検証するとともに，肥満合併高血圧マウスに対して漢方薬を用いた東洋医学的治療介入の効果も検証し，臨床研究と基礎研究の両面から肥満高血圧に対する漢方薬を用いた東洋医学的治療介入の効果を総合的に検証する．

臨床的検討では，横浜市立大学附属病院と地域協力病院，開業医院との多施設共同研究として，試験対象選択基準を満たし文書同意が得られた肥満高血圧患者(目標症例数合計200症例)を無作為に2群に割り付け，防風通聖散併用投与群では，従来の西洋医学的治療介入に加えて東洋医学的治療介入手段の防風通聖散を併用投与する．通常治療継続群では西洋医学的治療介入のみを行う．両群ともにJSH2009記載の目標血圧値までの降圧を目指す．防風通聖散の併用投与による治療効果の改善度は，診察室血圧，24時間自由行動下血圧測定(ABPM)での降圧効果，糖脂質代謝・酸化ストレス，動脈硬化指標を評価する．平成23年度は患者の登録及び評価項目測定を行った．

基礎的検討では，無介入で肥満高血圧を呈するKKAyマウス，および研究代表者らが開発した高脂肪食負荷によりメタボリック症候群（MetS）を呈する遺伝子改変マウス（ヒトMetS型マウス）に防風通聖散を投与し，血圧，脂肪組織，糖脂質代謝，および研究代表者らが単離同定したMetS増悪因子受容体結合機能制御分子（ATRAP）の組織発現を検討する．平成23年度はマウスでの降圧効果の解析を行った．

臨床的検討での防風通聖散投与による肥満高血圧に対する降圧効果の評価では，ABPM施行により臓器合併症予後を正確に反映する24時間血圧・血圧日内変動の解析が可能である．また基礎的検討での防風通聖散投与による肥満高血圧マウスに対する降圧効果の評価では，新規性の高い分子および独自に開発したヒトMetS型マウスも含めて検討することにより，防風通聖散投与による肥満高血圧に対する治療改善効果について総合的に独創性の高い研究成果が得られると予想される．

研究分担者氏名・所属研究機関名及び所属研究機関における職名

梅村　敏・横浜市立大学・教授

戸谷　義幸・横浜市立大学・准教授

A. 研究目的

本研究では，肥満高血圧患者に対して漢方薬を用いた東洋医学的治療介入の併用による治療効果の向上を臨床的に検証するとともに，肥満合併高血圧マウスに対して漢方薬を用いた東洋医学的治療介入の効果も検証し，臨床研究と基礎研究の両面から肥満高血圧に対する漢方薬を用いた東洋医学的治療介入の効果を総合的に検証する．

【研究の必要性及び特色・独創的な点】

肥満と高血圧は相互に密接な関係があり，内臓脂肪型肥満の増悪に伴って臓器合併症が増加する．よって肥満合併高血圧の治療の目的は，食事・運動療法とともに降圧目標値までの確実な降圧と内臓脂肪型肥満の効率的な改善により，心血管病や腎不全を抑制することである．またRASの生理活性物質アンジオテンシンII(Ang II)は主要な受容体の1型Ang II受容体に作用

して情報伝達系を活性化し，メタボリック症候群や心血管病の発症・進展を促進するため，メタボリック症候群合併高血圧患者に対しては高血圧治療ガイドライン(JSH2009)では RAS 阻害薬が第 1 選択薬である．しかし RAS 阻害薬単独投与では目標血圧までの確実な降圧が困難な場合が多く併用療法が必要となる．

一方漢方薬は多種生薬の混合製剤でありその薬効や作用機序は不確定な面が残り臨床試験成績も不十分なため，JSH2009 においても治療薬として推奨されるに至っていない．よって，肥満合併高血圧に対する西洋医学と東洋医学を融合させたさらなる効率的な治療を目指すために漢方薬を併用する根拠となる臨床効果の検討が必要である．

現在までに研究代表者らは 24 時間自由行動下血圧測定(ABPM)での平均血圧や血圧日内変動に加えて新規指標の基底血圧や血圧短期変動性と心血管病の合併及び降圧薬の臓器保護作用との関連性について報告している(Clin Exp Hypertens 2005,2007,2008,2009; Nephron Clin Pract 2009; Hypertens Res 2009; Atherosclerosis 2009)．本研究では防風通聖散の併用降圧効果について，全例に ABPM を施行し，従来の 24 時間血圧・血圧日内変動データに加えて基底血圧・短期血圧変動性などの臓器合併症予後と関連した ABPM 上の新規指標を含めた多面的な検討が可能である．

また肥満高血圧では組織レニン-アンジテンシン系活性化の関与も指摘されており，研究代表者らはこの系の MetS 増悪因子受容体に直接結合して機能を制御する分子（ATRAP）の単離同定に成功し肥満高血圧との関連性を発見している．そこで，本研究では，無介入で肥満高血圧を呈する KKAy マウス，および研究代表者らが開発に成功した高脂肪食負荷により MetS を呈する遺伝子改変マウス（ヒト MetS 型マウス）に対する防風通聖散の作用を基礎医学的にも検討することにより，防風通聖散投与による肥満高血圧に対する臨床的治療効果の基礎医学的な根拠を得ることができ，かつ新規性の高い分子的作用機序も同時に明らかにできると考えられる．これらの総合的な検討の結果，肥満高血圧に対する防風通聖散の治療改善効果について臨床的および基礎的に独創性の高い研究成果が得られると期待される．

B．研究方法

1．臨床的検討

本研究の臨床的検討では，多施設共同研究として，研究代表者の田村及び研究分担者の梅村が所属する横浜市立大学附属病院が中心となって，横浜市立大学附属市民総合医療センター病院，医療連携関連の 4 つの地域中核病院，および横浜内科医会会長を務め ABPM を用いた研究実績のある開業医が参加して行われる．

対象は，上記の各参加施設に通院中であり，試験開始前 4 週間以上の観察期間中に食事・運動療法とともに RAS 阻害薬などによる降圧治療が行われている本態性高血圧患者(男女を問わず)とし，以下の条件を満たした者とする．

対象選択基準は，1．試験開始時の年齢が 20 歳以上，80 歳未満，2．肥満度(BMI) 25 kg/m2 以上，3．本研究の参加への文書同意，である．

除外基準は，1．20 歳未満，あるいは 80 歳以上の患者，2．妊娠あるいは授乳している患者，妊娠している可能性のある患者，3．二次性高血圧，悪性高血圧，あるいは重症(III 度)高血圧(収縮期血圧 180mmHg 以上，あるいは拡張期血圧 110mmHg 以上）の患者，4．その他，担当医師が研究対象として不適当と判断した患者，である．

本研究の参加に同意し，対象選択基準に合致した患者について，観察期間中に無作為に防風通聖散併用治療群あるいは通常治療継続群の 2 群への割り付けをおこなう．防風通聖散併用治療群では防風通聖散(2.5 g/日から開始し，食前又は食間に経口服用する．なお，症状により適宜増減し，最高用量は 7.5 g/日)を 6 ヶ月間(24 週間)投与し，防風通聖散による治療効果について通常降圧治療継続群を対照とした比較調査により検討する．両群ともに同様に JSH2009 記載の降圧目標までの降圧を図る．

本研究は 3 年計画であり，平成 23 年度は平成 22 年度に引き続いて主に対象患者の登録及び評価項目測定を行った．平成 22-24 年度の全研究実施期間における目標症例数は横浜市立大学附属病院 80 例，各参加病院・医院 20 例の合計 200 症例であり，主要評価項目は 24 時間自由行動下血圧測定(ABPM；試験開始時，3，6 ヶ月後の計 3 回施行)による降圧効果(平均血圧，血圧日内変動，基底血圧，短期血圧変動性)，副次的評価項目は，BMI，腹囲，診察室血圧，糖代謝(空腹時血糖，HbA1c，HOMA-IR，血中インスリン，アディポネクチン，レジスチン濃度)，脂質代謝(TCHO，LDLC，HDLC，TG)，酸化ストレス(血中 MDA-LDL，血中ペントシジン，尿中 L-FABP 濃度)，腎内 RAS 活性(尿中アンジオテンシノーゲン濃度)，腎機能(尿中アルブミン排泄量，尿蛋白量，推算 GFR)，動脈硬化検査(血管脈波検査)，心血管病合併の有無，及び有害事象である．

本研究は，研究代表者及び研究分担者が所属しまた ABPM を用いた臨床研究について実績がある横浜市立大学附属病院腎臓高血圧内科を中心とした多施設研究として遂行されているものである．

研究実施にあたっては，ABPM 機器を各施設に設置することにより対象患者の登録及び ABPM を含む血圧測定や検体収集は，各参加施設において行っている．しかし保険適応外の評価項目については，測定精度を上げるために各参加施設からの検体を横浜市立大学の研究室に集めて一括して（株）エスアールエルの検査室に測定を委託するとともに横浜市立大学の研究室において測定を行う．また，研究分担者の横浜市立大学附属病院長兼務の梅村が中心となって測定結果の解析を行い，同じく研究分担者の腎臓高血圧内科部長戸谷が中心となって各参加施設の腎臓高血圧内科部長との間で定期的に本研究の状況について協議するなど連絡を密にして効率的に研究を遂行している．さらに本研究実施にあたり横浜市立大学先端医科学研究センターの臨床研究支援部門の臨床・疫学研究推進室と随時連絡・協議をおこなっている．

（倫理面への配慮）

本研究は臨床研究に関する倫理指針を遵守して行われ，本研究への参加に先立ち，研究対象者である患者に対して，用いる治療薬に起こりうる副作用などを含めて十分な説明を行い，患者の自由意思による文書同意を取得する．また，患者の名前や病名等プラーバシーに関する秘密は固く守られるように細心の注意を払う．

試験薬はいずれも高血圧あるいは肥満症に対する治

療薬として承認を取得しており，その承認用量範囲で使用し，また，高血圧に対する併用薬の投与は可能となっている．さらに用いる治療薬の投与禁忌や慎重投与条件を正確に把握するとともに，副作用等が認められた場合は医師が適切な治療を行う．

主要検査項目としては，2008年4月に保険適応となった携帯型自由行動下自動血圧計を用いた24時間自由行動下血圧測定(ABPM)を試験開始時，3ヶ月後，及び6ヶ月後の計3回行うことにより，非侵襲的に24時間血圧，血圧日内変動，基底血圧，及び血圧短期変動性を評価する．また，尿・血液検査及び動脈硬化検査の回数は，一般診療における検査頻度に基づき，3ヶ月(12週)間隔で合計3回行うこととしている．同様に3ヶ月(12週)間隔で合計3回行われる保険適応外の糖代謝関連指標検査(血中アディポネクチン，レジスチン濃度測定)，腎内RAS活性検査(尿中アンジオテンシノーゲン濃度測定)および酸化ストレス関連検査(血中MDA-LDL，血中ペントシジン，尿中L-FABP濃度)についての検査費用は研究費により行われている．

なお本研究計画については，横浜市立大学および各参加施設の倫理委員会に研究計画を申請して承認を得た．

2. 基礎的検討

本研究の基礎的検討では，無介入で肥満高血圧を呈するKKAyマウス，および研究代表者らが開発した高脂肪食負荷によりメタボリック症候群（MetS）を呈する遺伝子改変マウス（ヒトMetS型マウス）に防風通聖散（1.5～4.5%，4-8週間）を投与し，血圧，脂肪組織（重量，組織所見），糖脂質代謝，および研究代表者らが単離同定したMetS増悪因子受容体結合機能制御分子（ATRAP）の組織発現に与える改善効果について検討する．平成23年度は主に血圧の評価測定を行う．これまでの予備的研究結果からはATRAPにはMetS増悪因子受容体internalizationを促進することにより，同受容体情報伝達系ネットワークのうち一部の情報伝達系の病的な過剰活性化を選択的に抑制する可能性が示されている．

本基礎研究の実施に際しての研究環境であるが，各種遺伝子発現解析用実験装置，ラット/マウス用テレメトリーや心エコーを含めた実験動物用循環動態解析装置，免疫組織学的検査器具，細胞染色実験装置，画像解析装置，動物飼育実験設備（代謝ケージを含む），実験動物センター設備，および質量分析装置関連機器は完備されており，効率的に研究を遂行できる状況にある．また，研究代表者の研究室では長年心血管内分泌制御機構の病態生理学的意義に関する研究を行っており，研究施設・設備・研究資料などに加えて他の所属研究員に助言を受けることも容易であり，研究を効率的に遂行するにあたって理想的な環境である．

（倫理面への配慮）

本研究計画の基礎的検討に含まれる組み換えDNA実験については，研究計画が遺伝子組換え生物等の第二種使用等をする間に執る拡散防止措置の確認を受けるために，遺伝子組換え生物等の使用等の規制による生物の多様性の確保に関する法律第１３条第１項の規定により，横浜市立大学に対して研究年度毎に研究計画を申請して承認を得ている．また，実験動物を用いた解析においては，横浜市立大学動物実験センターにおいて飼育をおこなうが，研究年度毎に学内の審査委員会の承認を受け，そのガイドラインに従って動物実験を行っている．

C. 研究結果

肥満高血圧は動脈硬化を促進し心血管病・腎不全の根源となり，国民健康レベルのさらなる向上のために，肥満合併高血圧への集学的治療による効率的な医療が重要である．

本研究では，肥満合併高血圧患者に対して漢方薬を用いた東洋医学的治療介入の併用による治療効果の向上を臨床的に検証するとともに，肥満合併高血圧マウスに対して漢方薬を用いた東洋医学的治療介入の効果も検証し，臨床研究と基礎研究の両面から肥満高血圧に対する漢方薬を用いた東洋医学的治療介入の効果を総合的に検討する．

1. 臨床的検討

本研究の臨床的検討では，横浜市立大学附属病院と地域協力病院，開業医院との多施設共同研究として，試験対象選択基準を満たし文書同意が得られた肥満合併高血圧患者(目標症例数合計200症例)を無作為に2群に割り付け，防風通聖散併用投与群では，食事・運動療法及びレニン-アンジオテンシン系(RAS)阻害薬などを用いた西洋医学的治療介入に加えて東洋医学的治療介入手段である抗肥満漢方薬の防風通聖散を6ヶ月間併用投与する．また，通常治療継続群では，西洋医学的治療介入を同期間行う．

本研究は3年計画であり，本年度は前年度に引き続いて，臨床的検討では，東洋学的治療介入として防風通聖散を用い，肥満合併高血圧患者を漢方薬投与群および非投与群に無作為割付した後に両群ともに食事・運動療法を含めた西洋学的治療介入を行い，研究開始時，3ヶ月後，6ヶ月後に診察室血圧，24時間自由行動下血圧測定(ABPM)および糖脂質代謝，酸化ストレス，血管脈波速度を指標として測定し両群の治療効果の比較検討を行っている（図１）．本研究は多施設共同研究であり，横浜市立大学附属病院を中心とした病診連携を行っている地域中核病院や開業医などの協力を得て遂行されている．現在までに横浜市立大学附属病院にて42症例，他施設共同研究病院(大森赤十字病院，藤沢市民病院，大和市立病院，他)にて31症例の合計73症例の登録がなされており，来年度も他施設共同研究病院の協力も得て登録症例数を増加させる予定である．そして中間解析結果について第35回日本高血圧学会において発表予定である．

また，本研究のパイロット試験として，高血圧合併顕性腎症2型糖尿病患者に対して西洋医学的介入による血圧，糖・脂質代謝における集約的治療を12ヶ月間にわたって行い，血圧日内変動および腎機能に与える影響について検討した．その結果，西洋医学的集約的治療介入開始12ヶ月後には開始前に比べて診察室血圧と糖・脂質代謝の有意な改善がみられた（収縮期血圧，130±2 vs 150±1 mmHg；拡張期血圧，76±1 vs 86±1 mmHg；空腹時血糖，117±5 vs 153±7 mg/dl；LDL-C，116±8 vs 162±5 mg/dl，P<0.0001）．また，ABPMでは，介入開始12ヶ月後において昼間・夜間の平均血圧および血圧短期変動性の有意な改善が認められた．さらに，腎機能では，介入開始12ヶ月後では開始前に比較して，推算糸球体濾過量（eGFR）は不変であったが（43.1±6.5 vs 44.3±5.1 ml/min/1.73 m2，NS），尿中アルブ

ミン排泄率（UACR）に関しては有意な減少が認められた(1228±355 vs 2340±381 mg/g-cr, P<0.05). 以上の研究成果について第33回日本高血圧学会総会(福岡), 第23回国際高血圧学会（バンクーバー）などの国内外の学会において発表し好評を得た. さらに研究成果の一部については, 国際英文学術雑誌(Kanaoka T, Tamura K, Moriya T, et al. Clin Exp Hypertens, 33: 255-263, 2011）において論文が掲載された.

2. 基礎的検討

基礎的検討では, 無介入で肥満高血圧を呈するKKAyマウス, および研究代表者らが開発した高脂肪食負荷によりメタボリック症候群（MetS）を呈する遺伝子改変マウス(ヒトMetS型マウス)に防風通聖散を投与し, 血圧, 脂肪組織, 糖脂質代謝, 及び研究代表者らが単離同定したMetS増悪因子受容体結合機能制御分子（ATRAP）の組織発現を検討している.

防風通聖散は含有生薬である麻黄に含まれるエフェドリンが交感神経終末からのノルアドレナリン放出を促し, 白色脂肪細胞および褐色脂肪細胞のβアドレナリン受容体を刺激し白色脂肪細胞の分解と褐色脂肪細胞の熱産生を惹起するとともに, 荊芥, 連翹, 甘草のフォスフォジエステラーゼ阻害作用がその効果を増強することにより, 抗肥満作用を発揮することが報告されている. また, 臨床的には防風通聖散は肥満症に加えて肥満をともなう高血圧の随伴症状に対しても保険適応があるが, 防風通聖散の血圧に対する効果は未解明の部分が大きい. よって本年度の基礎的検討では肥満高血圧モデルマウスを用いて防風通聖散の血圧に与える影響を検討した. 最初の検討では9週齢の雄KKAyマウスをControl群（通常食）と防風通聖散群（4.7%防風通聖散食）の2群に分け, 9週間飼育した. 血圧は2週間毎にテールカフを用いて非観血的に測定した. その結果, 収縮期血圧は11週齢から17週齢まで防風通聖散群で有意に低下した. また両群間で体重増加に差はないものの, 防風通聖散群で総摂餌量および総飲水量が有意に低下した.以上の結果から, 食事制限のない環境下では肥満高血圧モデルKKAyマウスにおいて防風通聖散投与により降圧効果が認められた. 今後分子機序を含めた更なる検討を行う予定である. これら中間解析結果については第55回日本腎臓学会において発表予定である.

D. 考察

国内外からの報告では, 肥満合併高血圧患者数は増加傾向にあり, 合併する生活習慣病管理の面からも大きな問題となっている. また, 本邦での大規模コホート研究, あるいは海外での複数のコホート研究を対象とした大規模メタ解析において, 肥満による心血管病発症の促進による死亡率の増加が報告されている. したがって, 肥満合併高血圧に対する包括的で効率的な治療は, 心血管病の発症予防に結びつくと考えられる. 高血圧における血圧管理では, 診察室血圧測定のみならず家庭血圧測定や24時間自由行動下血圧測定(ABPM)を行うことがガイドラインにおいて推奨される. 特にABPMでは血圧日内変動の詳細な評価が可能である. ABPMで評価可能な血圧日内変動関連指標のうち長時間周期の変動指標としては夜間血圧下降度（dipper, non-dipper）や早朝の血圧上昇の程度（morning surge）等があり, 短時間周期の変動指標としては血圧短期変動性が挙げられる. 合併症のある高血圧の病態では長時間周期の血圧変動関連指標の意義に関する研究が先行しており, 例えば慢性腎臓病患者では血圧日内変動上の特徴として, 夜間降圧がみられないnon-dipper型となることが多く, 夜間血圧は微量アルブミン尿～蛋白尿の程度と相関し, non-dipper型高血圧は慢性腎臓病および心腎連関を進展させることが明らかにされている. そのため慢性腎臓病患者の血圧管理においては, 末期腎不全・心腎連関の抑制のために, 早朝血圧や夜間血圧も評価すべきであり, 診察室血圧に加えて家庭血圧やABPMにより血圧日内変動を評価することが望ましいとされている.

一方, ABPMで評価可能な短時間周期の血圧変動関連指標である血圧短期変動性の高血圧治療における意義については未解明な部分も多く今後の詳細な検討が必要な状況である. 我々は, 血圧短期変動性に着目して, ABPMで測定される血圧の標準偏差(SD)あるいは変動係数(CV)を血圧短期変動性の指標とし, 合併症をもつ高血圧患者における病態生理学的意義について, 横断的研究, 前向き研究, および介入研究による検討を行っている. 例えば, 慢性腎臓病合併高血圧患者を対象とした横断的研究では, 慢性腎臓病合併高血圧患者において血圧短期変動性が増加していること, 血中コレステロール値やノルエピネフリン濃度が血圧短期変動性と関連すること, あるいは冠動脈疾患を合併した慢性腎臓病合併高血圧患者では血圧短期変動性のさらなる増加がみられることを報告している. また, 慢性腎臓病合併高血圧患者を対象とした介入研究では, 降圧薬による降圧作用や長時間周期の血圧日内変動指標への作用に加えて血圧短期変動性への作用が臓器保護作用に影響を与える可能性があることを明らかにしている. これらの結果は, ABPMでの血圧短期変動性が合併症をもつ高血圧における動脈硬化・臓器障害の病態を反映し合併症をもつ高血圧での血圧管理における治療標的のひとつとなりうる可能性を提唱していると考えられる.

肥満高血圧に対する防風通聖散の改善効果に関する臨床的検討の結果, 防風通聖散による併用治療が肥満高血圧患者に対して24時間自由行動下血圧(平均血圧, 血圧日内変動, 基底血圧, 短期血圧変動性), 糖脂質代謝, 及び酸化ストレスにどのような影響を与えるかが西洋医学的にも明らかになると予想される. また, 肥満高血圧に対する防風通聖散の作用機序に関する基礎的検討の結果, 臨床的作用の基礎医学的な根拠を得ることができるとともに, MetS増悪因子受容体結合性機能制御因子（ATRAP）への作用を含めた新規性の高い分子的作用機序も同時に明らかにでき, 科学的なインパクトもある成果が得られると予想される.

そして, 肥満高血圧に対する防風通聖散を用いた東洋医学的治療介入の西洋医学的意義が明らかになり, 肥満高血圧に対する集学的治療法における選択肢のひとつとして防風通聖散による併用療法が今後の高血圧治療ガイドラインなどにも採用される可能性がある.

E. 結論

本研究の結果, 防風通聖散による併用治療が肥満合併高血圧患者に対して24時間自由行動下血圧(平均血圧, 血圧日内変動, 基底血圧, 短期血圧変動性)改善作用, 糖脂質代謝改善作用, 及び酸化ストレス抑制作用を発揮することが西洋医学的に明らかになると予想さ

れる．その結果，肥満合併高血圧に対する防風通聖散を用いた東洋医学的治療介入の西洋医学的意義が確立され，肥満合併高血圧に対する集学的治療法における有力な選択肢として防風通聖散による併用療法が今後の高血圧治療ガイドラインにも採用されると期待される．さらに，現在我が国においても増加しつつある肥満合併高血圧患者に対する防風通聖散による併用治療の普及とともに，肥満高血圧患者における重篤な心血管病(脳卒中，冠動脈疾患)および腎障害(腎不全)の抑制による国民全体の健康・医療水準のさらなる向上，および国全体の医療費の減少が期待される．

F. 健康危険情報

特になし．

G. 研究発表

1. 論文発表

1 Tamura K, Kanaoka T, Ohsawa M, Haku S, Azushima K, Maeda A, Dejima T, Wakui H, Ozawa M, Shigenaga A, Umemura S. Emerging concept of anti-hypertensive therapy based on ambulatory blood pressure profile in chronic kidney disease. **Am J Cardiovasc Dis.** 1 (3): 236-243, 2011.

2 Dejima T, Tamura K, Wakui H, Maeda A, Ohsawa M, Kanaoka T, Haku S, Azushima K, Masuda S, Shigenaga A, Azuma K, Matsuda M, Yabana M, Hirose T, Uchino K, Kimura K, Nagashima Y, Umemura S. Prepubertal angiotensin blockade exerts long-term therapeutic effect through sustained ATRAP activation in salt-sensitive hypertensive rats. **J Hypertens.** 29 (10): 1919-1929, 2011.

3 Yasuda N, Akazawa H, Ito K, Shimizu I, Kudo-Sakamoto Y, Yabumoto C, Yano M, Yamamoto R, Ozasa Y, Minamino T, Naito A, Oka T, Shiojima I, Tamura K, Umemura S, Paradis P, Nemer M, Komuro I. Agonist-independent constitutive activity of angiotensin II receptor promotes cardiac remodeling in mice. **Hypertension.** 59 (3): 627-633, 2012.

4 Kanaoka T, Tamura K, Moriya T, Tanaka K, Koono Y, Kondoh S, Toyoda M, Umezono T, Fujikawa T, Ohsawa M, Dejima T, Maeda A, Wakui H, Haku S, Yanagi M, Mitsuhashi H, Ozawa M, Okano Y, Ogawa N, Yamakawa T, Mizushima S, Suzuki D, Umemura S. Effects of multiple factorial intervention on ambulatory BP profile and renal function in hypertensive type 2 diabetic patients with overt nephropathy - A pilot study. **Clin Exp Hypertens.** 33 (4): 255-263, 2011.

5 Matsuda M, Tamura K, Wakui H, Dejima T, Maeda A, Ohsawa M, Kanaoka T, Haku S, Azushima K, Yamasaki H, Saito D, Hirose T, Maeshima Y, Nagashima Y, Umemura S. Involvement of Runx3 in the basal transcriptional activation of the mouse angiotensin II type 1 receptor-associated protein gene. **Physiol Genomics.** 43 (14): 884-894, 2011.

6 Tamura K, Azushima K, Umemura S. Day-by-day home-measured blood pressure variability: another important factor in hypertension with diabetic nephropathy? **Hypertens Res.** 34 (12): 1249-1250, 2011.

2. 学会発表

1 田村功一，涌井広道，出島　徹，前田晃延，大澤正人，梅村　敏．高血圧，腎硬化症：高血圧・腎障害における新規アンジオテンシン受容体結合因子の病態生理学的意義の検討．シンポジウム5；CKD進展における炎症：進展メカニズムから治療標的まで．第54回日本腎臓学会学術総会．横浜，2011年6月15-17日．

2 Tamura K, Wakui H, Tsurumi-Ikeya Y, Dejima T, Maeda A, Ohsawa M, Kanaoka T, Oshikawa J, Kobayashi Y, Minegishi S, Yoshida S, Masuda S, Azuma K, Ishigami T, Umemura S. Renal enhancement of AT1 receptor-associated protein prevents salt-induced hypertension. The 21st Annual Scientific Meeting of the European Society of Hypertension (ESH) (Milan, Italy), June 17-20, 2011.

3 田村功一，涌井広道，出島　徹，前田晃延，金岡知彦，大澤正人，白　善雅，小豆島健護，戸谷義幸，堀内正嗣，梅村　敏．Angiotensin II type 1 receptor-associated protein, ATRAP, functions as a selective inhibitory molecule of receptor signaling at local tissue sites. SY11 (シンポジウム11)：RAA 系のニューパラダイム. The 75thAnnual Scientific Meeting of the Japanese Circulation Society. 横浜，2011年8月3-4日．

4 田村功一．ABPMでの血圧短期変動性の慢性腎臓病合併高血圧における新たな治療標的としての可能性-Ambulatory short-term blood pressure variability as a new potential therapeutic target in cardiorenal syndrome. Panel Discussion 4: New Perspectives of Blood Pressure Variability. The 34th Annual Scientific Meeting of the Japanese Society of Hypertension. 宇都宮，2011年10月20-22日．

H. 知的財産権の出願・登録状況

(予定を含む。)

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
田村功一，涌井広道，梅村　敏.	アンジオテンシノーゲン遺伝子	熊谷裕生，小室一成，堀内正嗣，森下竜一	高血圧ナビゲーター，第3版	メディカルレビュー社	東京	2011	p.56-p.57

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tamura K, Kanaoka T, Ohsawa M, Haku S, Azushima K, Maeda A, Dejima T, Wakui H, Ozawa M, Shigenaga A, Umemura S.	Emerging concept of anti-hypertensive therapy based on ambulatory blood pressure profile in chronic kidney disease.	Am J Cardiovasc Dis.	1 (3)	236-243	2011
Dejima T, Tamura K, Wakui H, Maeda A, Ohsawa M, Kanaoka T, Haku S, Azushima K, Masuda S, Shigenaga A, Azuma K, Matsuda M, Yabana M, Hirose T, Uchino K, Kimura K, Nagashima Y, Umemura S.	Prepubertal angiotensin blockade exerts long-term therapeutic effect through sustained ATRAP activation in salt-sensitive hypertensive rats.	J Hypertens.	29 (10)	1919-1929	2011

Yasuda N, Akazawa H, Ito K, Shimizu I, Kudo-Sakamoto Y, Yabumoto C, Yano M, Yamamoto R, Ozasa Y, Minamino T, Naito A, Oka T, Shiojima I, Tamura K, Umemura S, Paradis P, Nemer M, Komuro I.	Agonist-independent constitutive activity of angiotensin II receptor promotes cardiac remodeling in mice.	**Hypertension.**	59 (3)	627-663	2012
Kanaoka T, Tamura K, Moriya T, Tanaka K, Koono Y, Kondoh S, Toyoda M, Umezono T, Fujikawa T, Ohsawa M, Dejima T, Maeda A, Wakui H, Haku S, Yanagi M, Mitsuhashi H, Ozawa M, Okano Y, Ogawa N, Yamakawa T, Mizushima S, Suzuki D, Umemura S.	Effects of multiple factorial intervention on ambulatory BP profile and renal function in hypertensive type 2 diabetic patients with overt nephropathy – A pilot study.	**Clin Exp Hypertens.**	33 (4)	255-263	2011
Matsuda M, Tamura K, Wakui H, Dejima T, Maeda A, Ohsawa M, Kanaoka T, Haku S, Azushima K, Yamasaki H, Saito D, Hirose T, Maeshima Y, Nagashima Y, Umemura S.	Involvement of Runx3 in the basal transcriptional activation of the mouse angiotensin II type 1 receptor-associated protein gene.	**Physiol Genomics.**	43 (14)	884-894	2011
Tamura K, Azushima K, Umemura S.	Day-by-day home-measured blood pressure variability: another important factor in hypertension with diabetic nephropathy?	**Hypertens Res.**	34 (12)	1249-1250	2011

Am J Cardiovasc Dis 2011;1(3):236-243
www.AJCD.us /ISSN: 2160-200X/AJCD1107006

Review Article

Emerging concept of anti-hypertensive therapy based on ambulatory blood pressure profile in chronic kidney disease

Kouichi Tamura, Tomohiko Kanaoka, Masato Ohsawa, Sona Haku, Kengo Azushima, Akinobu Maeda, Toru Dejima, Hiromichi Wakui, Motoko Ozawa, Atsu-ichiro Shigenaga, Yoshiyuki Toya, Satoshi Umemura

Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

Received July 21, 2011; accepted August 12, 2011; Epub September 8, 2011; published September 30, 2011

Abstract: Presently hypertensive patients with chronic kidney disease (CKD) particularly diabetic nephropathy are increasing in number, and cardiovascular and renal complications are the most common cause of death in these patients. The control of blood pressure (BP) is an important issue in cardiovascular and renal protection in hypertensive patients with CKD. Although hypertension is usually diagnosed based on measurements of BP recorded during a visit to a physician, that is, office BP, several studies have shown that target organ damage and prognosis are more closely associated with ambulatory BP than with office BP. It should be important to achieve the target absolute BP levels in hypertensive patients obtained either by office or home measurements or by ambulatory recordings for the cardiovascular and renal protection. Noninvasive techniques for measuring ambulatory BP have allowed BP to be monitored during both day and night. Additionally, ambulatory BP monitoring can provide information on circadian BP variation and short-term BP variability, which is suggested to be associated with cardiovascular and renal morbidity and mortality. This review will briefly summarize the emerging concept of anti-hypertensive therapy based on ambulatory BP profile in hypertensive patients with CKD.

Keywords: Blood pressure variability, diabetic nephropathy, hypertension, chronic kidney disease, renin-angiotensin system.

Introduction

Presently hypertensive patients with CKD and diabetes are increasing in number, and cardiovascular complications are the most common cause of death in these hypertensive patients. Thus, it would be of considerable value to identify the mechanisms involved in the cardiovascular events associated with hypertension complicated by CKD and diabetes. Ambulatory blood pressure (BP) monitoring has allowed an easier and more accurate determination of the circadian rhythm of BP under different pathophysiological conditions. The circadian pattern of BP in hypertensive patients with CKD and diabetes has been found to exhibit a blunted nocturnal decrease in BP, which is associated with autonomic neuropathy and nephropathy in these hypertensive patients [1]. The loss of nocturnal BP dipping has been considered to be a risk factor for the progression of nephropathy and to be of prognostic value with respect to target organ damage and cardiovascular morbidity in these CKD patients [2-4].

Estimation of ambulatory short-term BP variability

Ambulatory BP monitoring allows the acquisition of valuable information on not only the average 24-h BP, but also the variations in the BP values that happen during the course of daily life. Among the information obtained by ambulatory BP monitoring, previous studies have shown that BP variability is a complex phenomenon that involves both short- and long-lasting changes [5]. Thus the 24-h BP varies not only because of a reduction in BP during nighttime sleep and increase in the morning, but also because of sudden, quick, and short-lasting

changes that occur both during the daytime and, to a lesser extent, at nighttime. This phenomenon, short-term BP variability, has been shown to depend on sympathetic vascular modulation and on atherosclerotic vascular changes [6,7]. Several previous animal studies showed that exaggerated short-term BP variability without significant changes in mean BP induced chronic cardiovascular inflammation and remodeling [8,9]. Short-term BP variability is also suggested to be clinically relevant by the fact that hypertensive patients with similar 24-h mean BP values exhibit more severe organ damage when the short-term BP variability is greater [7,10-16].

Home-measured BP variability and CKD

On the other hand, several clinical studies have provided epidemiological basis for supporting the greater accuracy of home BP monitoring compared with clinic pressures for prognosis of fatal and nonfatal cardiovascular disease in long-term follow-up surveys and in cross-sectional studies. There is a general consensus that home BP monitoring is more convenient, available, and less costly than ambulatory BP monitoring, but the superiority of ambulatory BP monitoring for special clinical problems (i.e., 1) detection of non-dippers or need for sleep pressures in chronic renal disease, autonomic neuropathies, and sleep apnea; 2) estimation of short-term BP variability) is also clearly recognized [17]. Surveys of both physicians and patients suggest that home BP monitoring is both appreciated and recognized as a valuable strategy. Several experts in the field of hypertension research and care have published appeals to expand the use of home BP monitoring for routine care and to have it supported by health care systems.

Concerning home-measured BP variability, a previous study showed that high day-by-day BP variability is associated with increases in total, cardiovascular, and stroke mortality, independently of BP value and other cardiovascular risk factors in the general population of Ohasama study [18]. In the state of type 2 diabetes, while high short-term BP variability on ambulatory BP monitoring is reported to be associated with atherosclerosis and proteinuria in hypertensive patients with type 2 diabetes [11,19,20], the recent study by Ushigome et al. adds further information on the clinical relevance of home-measured BP variability in the pathophysiology of diabetic nephropathy [21]. Although the hypothesis that home-measured BP variability favors the development of nephropathy in type 2 diabetes is appealing, the cross-sectional nature of this study makes it impossible to evaluate the causal relationships between day-by-day BP variability and diabetic nephropathy. Further studies, such as outcome studies focusing on whether a therapeutic intervention reducing day-by-day BP variability also carries additional prognostic benefit by a concomitant suppression of the development of diabetic nephropathy, are warranted to confirm the prognostic value of home-measured BP variability.

Effects of Ang II type 1 receptor-specific blockers (ARB) on ambulatory short-term BP variability in diabetic nephropathy patients

Presently, inhibitors of renin-angiotensin system (RAS), such as ARB and angiotensin-converting enzyme inhibitors (ACEI) are recommended as the first-line anti-hypertensive medication to treat hypertensive patients with CKD, particularly those with albuminuria. Inhibitors of RAS exerts the BP lowering effects through the suppression of circulating and tissue RAS and the additional anti-proteinuric effect through the inhibition of intra-glomerular hypertension. With respect to effects of RAS inhibitors on ambulatory BP profile in hypertensive patients with CKD, we performed a series of clinical studies by administrating ARB to hypertensive CKD patients including those on dialysis therapy.

We examined whether ARB would improve ambulatory short-term BP variability in hypertensive patients with diabetic nephropathy. A total of 30 patients with type 2 diabetes along with hypertension and overt nephropathy were enrolled in this randomized, two-period, crossover trial of 12 weeks of treatment with losartan and telmisartan [11]. After 12 weeks of treatment, 24-h, daytime, and nighttime short-term BP variability, assessed on the basis of the coefficient of variation of ambulatory BP, was significantly decreased by telmisartan. Both of losartan and telmisartan reduced urinary protein excretion and baPWV. However, compared with losartan, telmisartan significantly decreased urinary protein excretion, baPWV, and low frequency (LF)-to-high frequency (HF) ratio, an index of sympathovagal balance. Multiple regression analysis showed significant correlations between

urinary protein excretion and baPWV, 24-h LF-to-HF ratio, nighttime systolic BP, and 24-h short-term systolic BP variability. Although the results of AMADEO study showed that telmisartan was more effective than losartan in reducing proteinuria in hypertensive patients with diabetic nephropathy at levels of office BP that were not different between the telmisartan and losartan treatment groups, the possible mechanisms involved in this difference in antiproteinuric effect were not elucidated [22]. The results of this study suggest that ARB, particularly telmisartan, is effective in reducing proteinuria in hypertensive patients with overt diabetic nephropathy, partly through inhibitory effects on ambulatory short-term BP variability and sympathetic nerve activity, in addition to its longer duration of action on nighttime BP reduction.

Accumulating evidence has shown that CKD patients with diabetes are increasing in number, and renal and cardiovascular complications are the most common cause of death in these patients. Thus, it is important to identify the mechanisms involved in the progression of renal impairment and cardiovascular injury associated with diabetic nephropathy. Recent evidence also indicated that multifactorial intervention is able to reduce the risk of cardiovascular disease and death among patients with diabetes and microalbuninuria [23]. Thus, in another study we examined the effects of intensified multifactorial intervention, with tight glucose regulation and the use of valsartan and fluvastatin, on ambulatory BP profile, estimated glomerular filtration rate (eGFR), and urinary albumin to creatinine ratio (UACR), in hypertensive patients with type 2 diabetes mellitus and overt nephropathy [20]. In this study we showed that the intensified multifactorial intervention including the use of valsartan and fluvastatin is able to improve ambulatory BP profile, preserve renal function, and reduce urinary albumin excretion in type 2 diabetic hypertensive patients with overt nephropathy.

Effects of ARB on ambulatory short-term BP variability in hemodialysis patients

Although cardiovascular disease is the leading cause of mortality in CKD patients on dialysis therapy, ARB is reported be effective in reducing cardiovascular events in patients undergoing hemodialysis [24,25]. Thus, we examined whether ARB would improve ambulatory short-term BP variability in hypertensive patients on hemodialysis [12]. In this study hypertensive patients on hemodialysis therapy were randomly assigned to the losartan treatment group or the control treatment group. After 6- and 12-months of treatment, nighttime short-term BP variability, assessed on the basis of the coefficient of variation of ambulatory BP, was significantly decreased in the losartan group, but remained unchanged in the control group. Compared with the control group, losartan significantly decreased left ventricular mass index (LVMI), baPWV, and the plasma levels of brain natriuretic peptide and advanced glycation end products (AGE). Furthermore, multiple regression analysis showed significant correlations between changes in LVMI and changes in nighttime short-term BP variability, as well as between changes in LVMI and changes in the plasma levels of AGE. These results suggest that ARB is beneficial for the suppression of pathological cardiovascular remodeling though its inhibitory effect on ambulatory short-term BP variability during nighttime. A recent study also shows that a direct renin inhibitor aliskiren was effective for BP control and may have cardiovascular protective effects in hypertensive CKD patients on hemodialysis [26].

Effects of ARB on ambulatory short-term BP variability in peritoneal dialysis patients

Among CKD patients on peritoneal dialysis, we examined whether addition of ARB, including high-dose ARB, to conventional antihypertensive treatment could improve BP variability in hypertensive patients [15]. Hypertensive patients on chronic peritoneal dialysis therapy were randomly assigned to the ARB treatment groups either by candesartan or valsartan, or the control group. After the 6-months treatment, 24-h ambulatory BP values were similarly decreased in both the control group and ARB groups. However, short-term BP variability assessed on the basis of the standard deviation of 24-h ambulatory BP was significantly decreased in the ARB groups, but remained unchanged in the control group. Furthermore, parameters of cardiovascular remodeling assessed by natriuretic peptides, echocardiography, and baPWV were significantly improved in the ARB groups but not in the control group. These results indicate that ARB treatment is beneficial for the suppression of pathological cardiovascular remodeling with a decrease in BP variability in

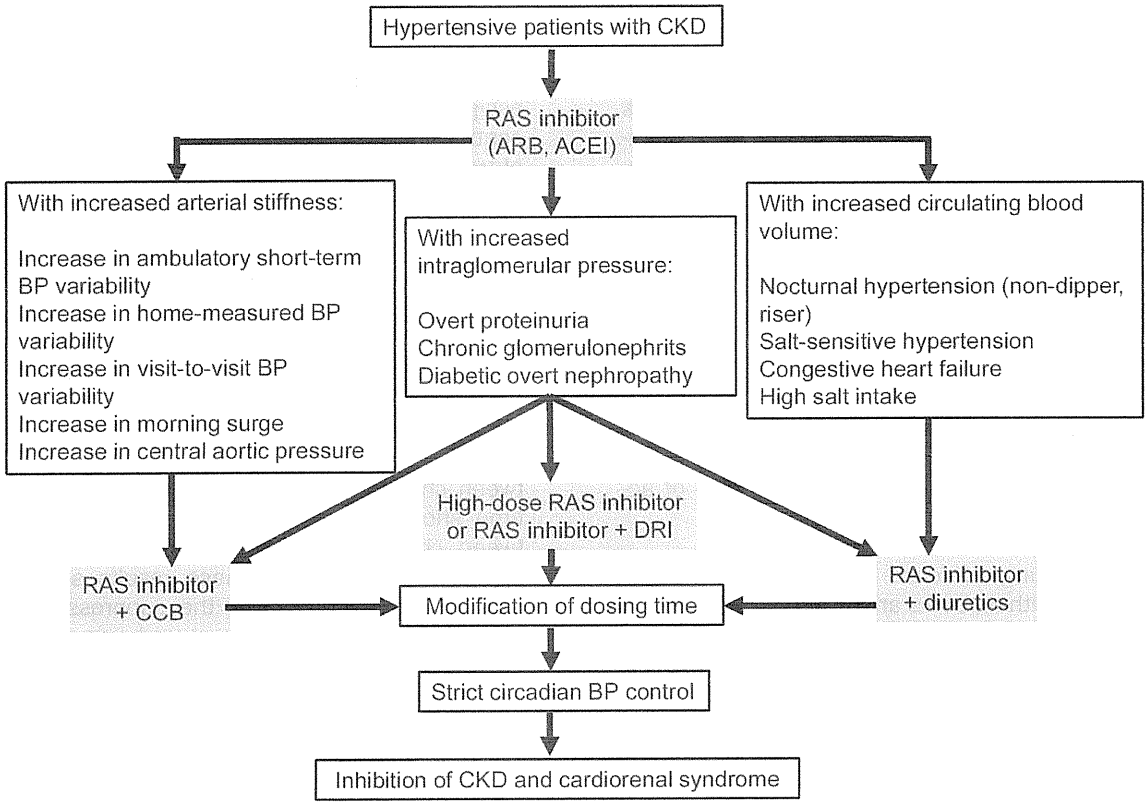


Figure 1. Schema showing the proposed strategy of RAS inhibitor-based combination therapy for hypertensive patients with CKD. ACEI, angiotensin-converting enzyme inhibitor; ARB, Ang II type 1 receptor-specific blocker; BP, blood pressure; CKD, chronic kidney disease; CCB, calcium channel blocker; DRI, direct renin inhibitor; RAS, renin-angiotensin system.

hypertensive patients on peritoneal dialysis

.

RAS inhibitor-based combination therapy in CKD

Although clinical guidelines specify that inhibitors of RAS are the drugs of choice for the treatment of hypertension in patients with CKD, the results of previous meta-analysis indicate that the benefits of RAS inhibitors on renal outcomes in clinical trials mainly result from a BP-lowering effect [27]. Thus, present guidelines also recommend RAS inhibitors-based combination therapy to achieve the target office BP level. The results of GUARD study showed that combination therapy with a RAS inhibitor and thiazide diuretic resulted in a greater reduction in albuminuria compared to that with a RAS inhibitor and calcium channel blocker (CCB) [28]. Previous studies showed that in CKD patients who have a sodium-sensitive type of hypertension, BP failed to fall during the night, thereby exhibiting non-dipper or riser types of ambulatory BP profile which correspond to abnormality of circadian BP rhythm. Although the sodium sensitivity of BP with non-dipper or riser types of ambulatory BP profile contributes as an independent risk factor for cardiovascular morbidity, both sodium restriction and thiazide diuretics are able to shift circadian BP rhythm from riser or non-dipper to dipper. Thus, RAS inhibitors-based combination therapy with thiazide diuretics may have an additional therapeutic advantage to relieve the renal and cardiovascular risks by different ways: systemic BP reduction and normalization of circadian BP rhythm.

On the other hand, the results of ACCOMPLISH study showed that combination therapy with a RAS inhibitor and CCB slows progression of nephropathy and inhibits cardiovascular death to a greater extent with a better preservation of eGFR, compared to combination therapy with a

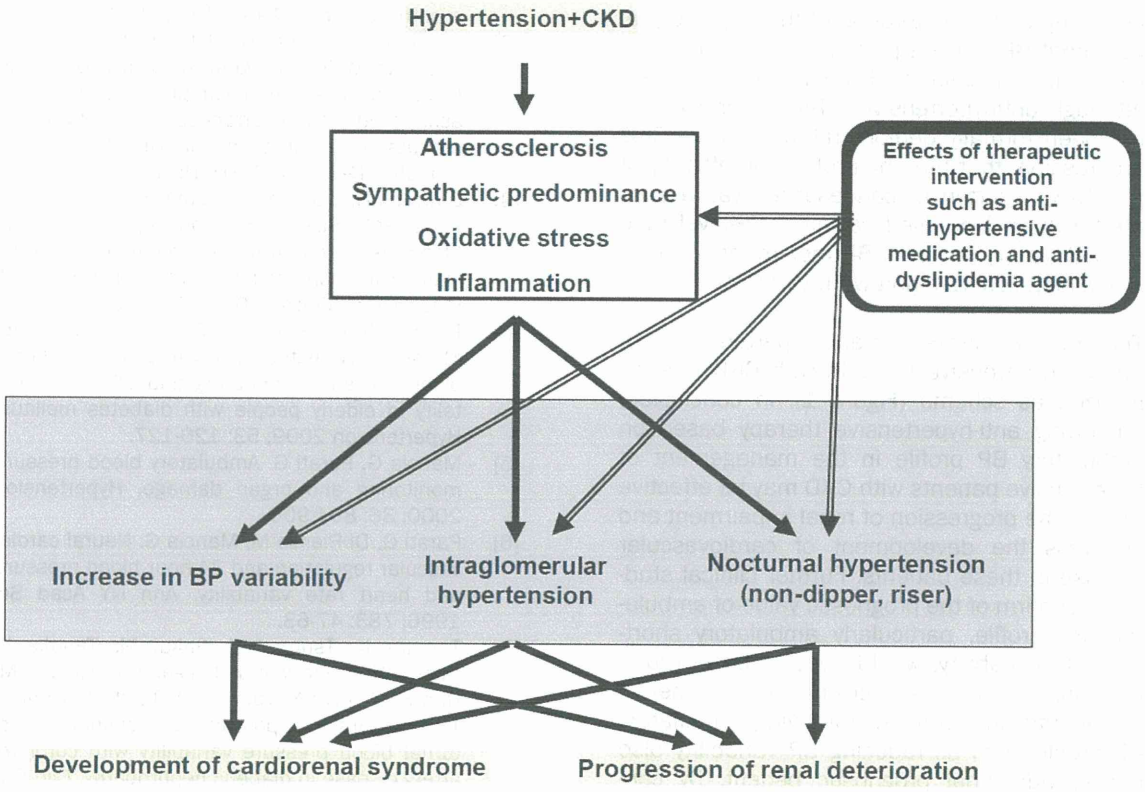


Figure 2. Increasing importance of clinical studies examining effects of various therapeutic intervention such as anti-hypertensive medication and anti-dyslipidemia agent on altered ambulatory BP profile in hypertensive patients with CKD. BP, blood pressure; CKD, chronic kidney disease.

RAS inhibitor and thiazide diuretic in high-risk hypertensive patients with CKD [29]. Although the detailed mechanistic basis for this difference in cardiorenal protection, in spite of similar mean 24-hour systolic and diastolic BP patterns by combination therapy [30], should be resolved by future studies, combination therapy with a RAS inhibitor and CCB is reported to effectively decrease central aortic pressure and ambulatory short-term BP variability with a preventive effect on the progression of arterial stiffness [31,32].

The direct renin inhibitor aliskiren is available as alternative or complementary approaches to pharmacological RAS blockade. Direct renin inhibitors constitute a novel class of RAS antagonists that block the conversion of angiotensinogen to angiotensin I. Aliskiren, the first approved compound of this class, reduces BP levels with similar potency as ACE inhibitor and ARB. Aliskiren as add-on treatment to standard therapy including the optimal dose of losartan, in the AVOID study, reduced albuminuria and slowed development of renal dysfunction more than placebo across different levels of eGFR in patients with type 2 diabetes, hypertension, and nephropathy [33-35]. The long-term nephroprotective potential of aliskiren-based therapy and its superiority over existing therapies as a possible first-line regimen remains to be elucidated.

Chronotherapy as a possible another therapeutic option in CKD

Finally, given that nocturnal BP non-dipping is a potential independent risk factor for CKD progression and development of cardiorenal syndrome, the timing of administration of antihypertensive drugs may be of relevance. Even compounds with recommended once-daily administration based on their pharmacokinetic properties may reduce nocturnal BP level more efficiently when applied in the evening, thereby

achieving partial restoration of the physiological nocturnal BP dipping pattern and exerting efficient cardiovascular and renal protection [36]. Although antihypertensive "chronotherapy" has not been formally demonstrated to affect CKD progression, that the anti-proteinuric efficacy of the ARB valsartan or candesartan was associated with an increased day:night BP-level ratio on ambulatory or home BP monitoring induced by evening dosing is noteworthy [37,38].

The proposed strategy of anti-hypertensive therapy in hypertensive patients with CKD is summarized as schema (**Figure 1**). In conclusion, employing anti-hypertensive therapy based on ambulatory BP profile in the management of hypertensive patients with CKD may be effective to slow the progression of renal impairment and suppress the development of cardiovascular disease in these patients. Further clinical studies to confirm of the prognostic value of ambulatory BP profile, particularly ambulatory short-term BP variability, would need to be provided by outcome studies focusing on whether a therapeutic intervention improving ambulatory BP profile such as reducing BP variability also carries additional prognostic benefit, by concomitantly reducing also the rate of renal deterioration and cardiovascular events (**Figure 2**).

Acknowledgments

This review was supported in part by grants from the Japanese Ministry of Education, Science, Sports and Culture, by Health and Labor Sciences Research grant; and by grants from Salt Science Research Foundation (No. 1033, 1134), the Kidney Foundation, Japan (JKFB11-25) and Strategic Research Project of Yokohama City University. There are no conflicts of interest.

Please address correspondence to: Kouichi Tamura, MD, PhD, Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, JAPAN. Tel: +81-45-787-2635; Fax: +81-45-701-3738. E-mail: tamukou@med.yokohama-cu.ac.jp

References

[1] Spallone V, Bernardi L, Ricordi L, Solda P, Maiello MR, Calciati A, Gambardella S, Fratino P, Menzinger G. Relationship between the circadian rhythms of blood pressure and sympathovagal balance in diabetic autonomic neuropathy. Diabetes 1993; 42: 1745-1752.

[2] Nakano S, Fukuda M, Hotta F, Ito T, Ishii T, Kitazawa M, Nishizawa M, Kigoshi T, Uchida K. Reversed circadian blood pressure rhythm is associated with occurrences of both fatal and nonfatal vascular events in NIDDM subjects. Diabetes 1998; 47: 1501-1506.

[3] Sturrock ND, George E, Pound N, Stevenson J, Peck GM, Sowter H. Non-dipping circadian blood pressure and renal impairment are associated with increased mortality in diabetes mellitus. Diabet Med 2000; 17: 360-364.

[4] Palmas W, Pickering TG, Teresi J, Schwartz JE, Moran A, Weinstock RS, Shea S. Ambulatory blood pressure monitoring and all-cause mortality in elderly people with diabetes mellitus. Hypertension 2009; 53: 120-127.

[5] Mancia G, Parati G. Ambulatory blood pressure monitoring and organ damage. Hypertension 2000; 36: 894-900.

[6] Parati G, Di Rienzo M, Mancia G. Neural cardiovascular regulation and 24-hour blood pressure and heart rate variability. Ann NY Acad Sci 1996; 783: 47-63.

[7] Tamura K, Tsurumi Y, Sakai M, Tanaka Y, Okano Y, Yamauchi J, Ishigami T, Kihara M, Hirawa N, Toya Y, Yabana M, Tokita Y, Ohnishi T, Umemura S. A possible relationship of nocturnal blood pressure variability with coronary artery disease in diabetic nephropathy. Clin Exp Hypertens 2007; 29: 31-42.

[8] Eto M, Toba K, Akishita M, Kozaki K, Watanabe T, Kim S, Hashimoto M, Ako J, Iijima K, Sudoh N, Yoshizumi M, Ouchi Y. Impact of blood pressure variability on cardiovascular events in elderly patients with hypertension. Hypertens Res 2005; 28: 1-7.

[9] Kudo H, Kai H, Kajimoto H, Koga M, Takayama N, Mori T, Ikeda A, Yasuoka S, Anegawa T, Mifune H, Kato S, Hirooka Y, Imaizumi T. Exaggerated blood pressure variability superimposed on hypertension aggravates cardiac remodeling in rats via angiotensin II system-mediated chronic inflammation. Hypertension 2009; 54: 832-838.

[10] Eguchi K, Ishikawa J, Hoshide S, Pickering TG, Schwartz JE, Shimada K, Kario K. Night time blood pressure variability is a strong predictor for cardiovascular events in patients with type 2 diabetes. Am J Hypertens 2009; 22: 46-51.

[11] Masuda S, Tamura K, Wakui H, Kanaoka T, Ohsawa M, Maeda A, Dejima T, Yanagi M, Azuma K, Umemura S. Effects of angiotensin II type 1 receptor blocker on ambulatory blood pressure variability in hypertensive patients with overt diabetic nephropathy. Hypertens Res 2009; 32: 950-955.

[12] Mitsuhashi H, Tamura K, Yamauchi J, Ozawa M, Yanagi M, Dejima T, Wakui H, Masuda S, Azuma K, Kanaoka T, Ohsawa M, Maeda A, Tsurumi-Ikeya Y, Okano Y, Ishigami T, Toya Y, Tokita Y, Ohnishi T, Umemura S. Effect of losar-

tan on ambulatory short-term blood pressure variability and cardiovascular remodeling in hypertensive patients on hemodialysis. Atherosclerosis 2009; 207: 186-190.
[13] Ozawa M, Tamura K, Okano Y, Matsushita K, Ikeya Y, Masuda S, Wakui H, Dejima T, Shigenaga A, Azuma K, Ishigami T, Toya Y, Ishikawa T, Umemura S. Blood pressure variability as well as blood pressure level is important for left ventricular hypertrophy and brachial-ankle pulse wave velocity in hypertensives. Clin Exp Hypertens 2009; 31: 669-679.
[14] Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. . Lancet 2010; 375: 938-948.
[15] Shigenaga A, Tamura K, Dejima T, Ozawa M, Wakui H, Masuda S, Azuma K, Tsurumi-Ikeya Y, Mitsuhashi H, Okano Y, Kokuho T, Sugano T, Ishigami T, Toya Y, Uchino K, Tokita Y, Umemura S. Effects of angiotensin II type 1 receptor blocker on blood pressure variability and cardiovascular remodeling in hypertensive patients on chronic peritoneal dialysis. Nephron Clin Pract 2009; 112: c31-40.
[16] Shintani Y, Kikuya M, Hara A, Ohkubo T, Metoki H, Asayama K, Inoue R, Obara T, Aono Y, Hashimoto T, Hashimoto J, Totsune K, Hoshi H, Satoh H, Imai Y. Ambulatory blood pressure, blood pressure variability and the prevalence of carotid artery alteration: the Ohasama study. J Hypertens 2007; 25: 1704-1710.
[17] Bilo G, Parati G. Rate of blood pressure changes assessed by 24 h ambulatory blood pressure monitoring: another meaningful index of blood pressure variability? J Hypertens 2011; 29: 1054-1058.
[18] Kikuya M, Ohkubo T, Metoki H, Asayama K, Hara A, Obara T, Inoue R, Hoshi H, Hashimoto J, Totsune K, Satoh H, Imai Y. Day-by-day variability of blood pressure and heart rate at home as a novel predictor of prognosis: the Ohasama study. Hypertension 2008; 52: 1045-1050.
[19] Ozawa M, Tamura K, Okano Y, Matsushita K, Yanagi M, Tsurumi-Ikeya Y, Oshikawa J, Hashimoto T, Masuda S, Wakui H, Shigenaga A, Azuma K, Ishigami T, Toya Y, Ishikawa T, Umemura S. Identification of an increased short-term blood pressure variability on ambulatory blood pressure monitoring as a coronary risk factor in diabetic hypertensives. Clin Exp Hypertens 2009; 31: 259-270.
[20] Kanaoka T, Tamura K, Moriya T, Tanaka K, Konno Y, Kondoh S, Toyoda M, Umezono T, Fujikawa T, Ohsawa M, Dejima T, Maeda A, Wakui H, Haku S, Yanagi M, Mitsuhashi H, Ozawa M, Okano Y, Ogawa N, Yamakawa T, Mizushima S, Suzuki D, Umemura S. Effects of Multiple Factorial Intervention on Ambulatory BP Profile and Renal Function in Hypertensive Type 2 Diabetic Patients with Overt Nephropathy - A Pilot Study. Clin Exp Hypertens 2011; 33: 255-263.
[21] Ushigome E, Fukui M, Hamaguchi M, Senmaru T, Sakabe K, Tanaka M, Yamazaki M, Hasegawa G, Nakamura N. The coefficient variation of home blood pressure is a novel factor associated with macroalbuminuria in type 2 diabetes mellitus. Hypertens Res 2011 (e-pub ahead of print 4 August 2011; doi:10.1038/hr.2011.128).
[22] Bakris G, Burgess E, Weir M, Davidai G, Koval S. Telmisartan is more effective than losartan in reducing proteinuria in patients with diabetic nephropathy. Kidney Int 2008; 74: 364-369.
[23] Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. N Engl J Med 2008; 358: 580-591.
[24] Suzuki H, Kanno Y, Sugahara S, Ikeda N, Shoda J, Takenaka T, Inoue T, Araki R. Effect of angiotensin receptor blockers on cardiovascular events in patients undergoing hemodialysis: an open-label randomized controlled trial. Am J Kidney Dis 2008; 52: 501-506.
[25] Cice G, Di Benedetto A, D'Isa S, D'Andrea A, Marcelli D, Gatti E, Calabro R. Effects of telmisartan added to Angiotensin-converting enzyme inhibitors on mortality and morbidity in hemodialysis patients with chronic heart failure a double-blind, placebo-controlled trial. J Am Coll Cardiol 2010; 56: 1701-1708.
[26] Morishita Y, Hanawa S, Chinda J, Iimura O, Tsunematsu S, Kusano E. Effects of aliskiren on blood pressure and the predictive biomarkers for cardiovascular disease in hemodialysis-dependent chronic kidney disease patients with hypertension. Hypertens Res 2011; 34: 308-313.
[27] Casas JP, Chua W, Loukogeorgakis S, Vallance P, Smeeth L, Hingorani AD, MacAllister RJ. Effect of inhibitors of the renin-angiotensin system and other antihypertensive drugs on renal outcomes: systematic review and meta-analysis. Lancet 2005; 366: 2026-2033.
[28] Bakris GL, Toto RD, McCullough PA, Rocha R, Purkayastha D, Davis P. Effects of different ACE inhibitor combinations on albuminuria: results of the GUARD study. Kidney Int 2008; 73: 1303-1309.
[29] Bakris GL, Sarafidis PA, Weir MR, Dahlof B, Pitt B, Jamerson K, Velazquez EJ, Staikos-Byrne L, Kelly RY, Shi V, Chiang YT, Weber MA. Renal outcomes with different fixed-dose combination therapies in patients with hypertension at high risk for cardiovascular events (ACCOMPLISH): a prespecified secondary analysis of a randomised controlled trial. Lancet 2010; 375: 1173-1181.
[30] Jamerson KA, Devereux R, Bakris GL, Dahlof B, Pitt B, Velazquez EJ, Weir M, Kelly RY, Hua TA, Hester A, Weber MA. Efficacy and duration of benazepril plus amlodipine or hydrochlorothiazide on 24-hour ambulatory systolic blood

pressure control. Hypertension 2011; 57: 174-179.
[31] Ichihara A, Kaneshiro Y, Sakoda M, Takemitsu T, Itoh H. Add-on amlodipine improves arterial function and structure in hypertensive patients treated with an angiotensin receptor blocker. J Cardiovasc Pharmacol 2007; 49: 161-166.
[32] Matsui Y, Eguchi K, O'Rourke MF, Ishikawa J, Miyashita H, Shimada K, Kario K. Differential effects between a calcium channel blocker and a diuretic when used in combination with angiotensin II receptor blocker on central aortic pressure in hypertensive patients. Hypertension 2009; 54: 716-723.
[33] Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Aliskiren combined with losartan in type 2 diabetes and nephropathy. N Engl J Med 2008; 358: 2433-2446.
[34] Persson F, Lewis JB, Lewis EJ, Rossing P, Hollenberg NK, Parving HH. Impact of baseline renal function on the efficacy and safety of aliskiren added to losartan in patients with type 2 diabetes and nephropathy. Diabetes Care 2010; 33: 2304-2309.
[35] Persson F, Lewis JB, Lewis EJ, Rossing P, Hollenberg NK, Parving HH. Aliskiren in combination with losartan reduces albuminuria independent of baseline blood pressure in patients with type 2 diabetes and nephropathy. Clin J Am Soc Nephrol 2011; 6: 1025-1031.
[36] Hermida RC, Ayala DE, Fernandez JR, Portaluppi F, Fabbian F, Smolensky MH. Circadian rhythms in blood pressure regulation and optimization of hypertension treatment with ACE inhibitor and ARB medications. Am J Hypertens 2011; 24: 383-391.
[37] Hermida RC, Calvo C, Ayala DE, Lopez JE. Decrease in urinary albumin excretion associated with the normalization of nocturnal blood pressure in hypertensive subjects. Hypertension 2005; 46: 960-968.
[38] Kario K, Hoshide S, Shimizu M, Yano Y, Eguchi K, Ishikawa J, Ishikawa S, Shimada K. Effect of dosing time of angiotensin II receptor blockade titrated by self-measured blood pressure recordings on cardiorenal protection in hypertensives: the Japan Morning Surge-Target Organ Protection (J-TOP) study. J Hypertens 2010; 28: 1574-1583.

Prepubertal angiotensin blockade exerts long-term therapeutic effect through sustained ATRAP activation in salt-sensitive hypertensive rats

Toru Dejima[a], Kouichi Tamura[a], Hiromichi Wakui[a], Akinobu Maeda[a], Masato Ohsawa[a], Tomohiko Kanaoka[a], Sona Haku[a], Azushima Kengo[a], Shin-ichiro Masuda[a], Atsu-ichiro Shigenaga[a], Koichi Azuma[a], Miyuki Matsuda[a], Machiko Yabana[a], Tomonori Hirose[b], Kazuaki Uchino[a], Kazuo Kimura[c], Yoji Nagashima[d] and Satoshi Umemura[a]

Objective We previously showed that the molecule interacting with Ang II type 1 receptor (AT1R), ATRAP, promotes AT1R internalization and attenuates AT1R-mediated pathological responses. In this study we examined whether the regulation of renal ATRAP expression is related to the development of salt-induced hypertension and renal injury as well as to the beneficial effects of AT1R blockade.

Methods and results Dahl Iwai salt-sensitive hypertensive and Dahl Iwai salt-resistant rats were divided into six groups for the administration of vehicle or olmesartan either continuously from 3 to 16 weeks, or transiently from weaning to puberty (3–10 weeks), and fed high salt diet from 6 to 16 weeks. In Dahl Iwai salt-sensitive rats, not only continuous, but also prepubertal olmesartan treatment improved hypertension at 15 weeks. Renal ATRAP expression was suppressed in vehicle-treated Dahl Iwai salt-sensitive rats, concomitant with up-regulation of renal oxidative stress, inflammation and fibrosis-related markers such as p22phox, TGF-β, fibronectin, monocyte chemotactic protein-1 and type 1 collagen. However, prepubertal as well as continuous olmesartan treatment recovered the suppressed renal ATRAP expression and inhibited the renal activation of p22phox, TGF-β, fibronectin, MCP-1 and type 1 collagen. In Dahl Iwai salt-resistant rats, such suppression of renal ATRAP expression or induction of renal pathological responses by salt loading was not observed.

Conclusions These results indicate that prepubertal transient blockade of AT1R signaling exerts a long-term therapeutic effect on salt-induced hypertension and renal injury in Dahl Iwai salt-sensitive rats, partly through a sustained enhancement of renal ATRAP expression, thereby suggesting ATRAP a novel molecular target in salt-induced hypertension and renal injury. *J Hypertens* 29:1919–1929

Journal of Hypertension 2011, 29:1919–1929

Keywords: angiotensin receptors, basic science, gene expression/regulation, hypertension (kidney), oxidative stress (kidney), receptors, salt-sensitive

Abbreviations: AQP1, aquaporin 1; ARB, AT1R-specific blocker; AT1R, Ang II type 1 receptor; AT2R, Ang II type 2 receptor; ATRAP, AT1R-assciated protein; HS, high salt loading; MCP-1, monocyte chemotactic protein-1; NS, normal salt loading; SHR, spontaneously hypertensive rats

[a]Department of Medical Science and Cardiorenal Medicine, [b]Department of Molecular Biology, [d]Department of Molecular Pathology, Yokohama City University Graduate School of Medicine and [c]Division of Cardiology, Yokohama City University Medical Center, Yokohama, Japan

Correspondence to Kouichi Tamura, MD, PhD, FACP, FAHA, Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan
Tel: +81 45 787 2635; fax: +81 45 701 3738;
e-mail: tamukou@med.yokohama-cu.ac.jp

Received 12 February 2011 Revised 11 June 2011
Accepted 28 June 2011

See editorial comment on page 1857

Introduction

Previous epidemiological studies showed that dietary salt intake during the prepubertal period affects the blood pressure profile after adolescence [1,2], and animal studies using salt-sensitive hypertension models, including Dahl Iwai salt-sensitive rats, also showed that high salt intake at a young age promotes the development of hypertension and tissue injury later in life [3–5]. On the contrary, activation of the renal renin–angiotensin system plays a critical role in overall renal pathophysiology via the development of salt-sensitive hypertension and diabetic nephropathy. In Dahl Iwai salt-sensitive rats, high salt loading results in the development of severe hypertension as well as extensive cardiovascular and renal damage. In Dahl Iwai salt-sensitive rats, high salt loading decreases the activity of the circulating renin–angiotensin system, but previous studies have shown significant increases in components of the tissue renin–angiotensin system at local tissue sites such as the brain, heart, vessel wall and kidney [6–8].

The pathophysiological effects of the tissue renin–angiotensin system are mainly mediated by the activation of the Ang II type 1 receptor (AT1R). The carboxyl-terminal portion of AT1R is involved in the control of AT1R internalization independent of G-protein-coupling, and

0263-6352 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI:10.1097/HJH.0b013e32834a5a46

plays an important role in linking receptor-mediated signal transduction to specific pathophysiological responses through the promotion of oxidative stress and inflammatory responses at local tissue sites [9–12]. The AT1R-associated protein (ATRAP), which is a molecule specifically interacting with the carboxyl-terminal domain of AT1R, was cloned using a yeast-two hybrid screening system [13,14]. The results of previous in-vitro studies showed that ATRAP suppresses Ang II-mediated pathological responses in cardiovascular cells by promoting AT1R internalization and decreasing the cell surface AT1R number [15,16], thereby suggesting ATRAP is an endogenous inhibitor of AT1R signaling [17,18].

With respect to the tissue distribution and regulation of ATRAP expression *in vivo*, ATRAP is broadly expressed in many tissues [13]. We showed that there is a tissue-specific regulatory balancing of the expression of ATRAP and AT1R during the development of hypertension in spontaneously hypertensive rats (SHRs) [19]. The activation of ATRAP in transgenic-models in which ATRAP expression was increased beyond baseline promoted Ang II-mediated AT1R internalization and inhibited renal angiotensinogen production and cardiac hypertrophy in response to Ang II stimulation [20].

The development of interventional approaches to preventing the excessive activation of the renin–angiotensin system and attenuating oxidative stress, inflammation and fibrosis at local tissue sites is crucial for achieving the ultimate goal of preventing hypertension and renal injury. The feasibility of transient inhibition of the renin–angiotensin system to prevent the development of hypertension in humans was reported in the Trial of Preventing Hypertension (TROPHY) study [21], and the results of animal studies also showed that transient blockade of the renin–angiotensin system during a prepubertal critical period resulted in an attenuation of hypertension and renal injury in genetically hypertensive rats [22,23]. Thus, in this study, we examined renal ATRAP modulation in salt-induced hypertension as well as in the therapeutic effects of the AT1R-specific blocker (ARB) olmesartan on high blood pressure and renal damage, including oxidative stress, inflammation and fibrosis in Dahl Iwai salt-sensitive rats, a representative animal model of human salt-sensitive forms of hypertension and renal injury.

Methods

The study was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. All of the animal studies were reviewed and approved by the Animal Studies Committee of Yokohama City University.

Materials

Ang II was purchased from Sigma. The ARB olmesartan (RNH6270) was supplied by Daiichi-Sankyo Pharmaceuticals.

Animals and treatments

Male Dahl Iwai salt-sensitive rats (3 weeks of age) were purchased from SLC (Shizuoka), fed a normal salt diet containing 0.3% NaCl (Oriental Yeast Kogyo), and randomly divided into four groups (groups 1–4; $n=6$ per group) for the dietary high salt loading and oral administration of vehicle or olmesartan (RNH6270; Daiichi-Sankyo Pharmaceuticals). Male Dahl Iwai salt-resistant rats (3 weeks of age) were also divided into two groups (groups 5 and 6; $n=6$ per group) for the dietary high salt loading.

Whereas the Dahl Iwai salt-sensitive rats in group 1 and Dahl Iwai salt-resistant rats in group 5 were fed a normal salt diet (0.3% NaCl) and treated with vehicle throughout the experimental period from 3 to 16 weeks of age, Dahl Iwai salt-sensitive rats in groups 2, 3, and 4 and Dahl Iwai salt-resistant rats in group 6 were initially fed a normal salt diet and then fed a high salt diet (8% NaCl) from 6 to 16 weeks. The Dahl Iwai salt-sensitive rats in group 2 were treated with vehicle throughout the experimental period until 16 weeks. The Dahl Iwai salt-sensitive rats in group 3 were transiently treated with olmesartan (8 mg/kg per day) in drinking water during the prepubertal period from 3 to 10 weeks. The Dahl Iwai salt-sensitive rats in group 4 were continuously treated with olmesartan (8 mg/kg per day) in drinking water throughout the experimental period from 3 to 16 weeks. The olmesartan dosage was determined from previous studies [19].

SBP was measured by the tail cuff method (BP-monitor MK-2000; Muromachi Kikai Co) at the age of 3, 7, 11 and 15 weeks [19]. The rats were anesthetized and the tissues were placed into liquid nitrogen at the end of the experimental period (16 weeks of age). Plasma renin activity was measured by radioimmunoassay as described previously [24].

Western blot analysis

The characterization and specificity of the anti-ATRAP antibody and the anti-AT1R antibody (sc-1173; Santa Cruz Biotechnology Inc., Santa Cruz, California, USA) were described previously [19,20,25,26]. The anti-p22phox antibody (sc-20781; Santa Cruz Biotechnology Inc.), anti-p47phox antibody (sc-14015; Santa Cruz Biotechnology, Inc.), anti-Rac1 antibody (DAM1522857; Millipore) and anti-Ang II type 2 receptor (AT2R) antibody (sc-9040; Santa Cruz Biotechnology Inc.) were also used in this study. Western blot analysis was performed essentially as described [19,20,25,26].

Immunohistochemical analysis

The 4-μm thick sections were blocked for endogenous biotin activity using peroxidase blocking reagent (DAKO, Tokyo, Japan) and were incubated with either anti-ATRAP antibody diluted at 1 : 100; anti-AT1R antibody diluted at 1 : 100; anti-p22phox antibody diluted at

1:100; or antiaquaporin 1 (AQP1) antibody (ab9566, Abcam) diluted at 1:100, essentially as described previously [25,27].

Real-time quantitative RT-PCR analysis

Real-time quantitative RT-PCR was performed by incubating the RT product with TaqMan Universal PCR Master Mix and a designed TaqMan probe (Applied Biosystems, Foster City, California, USA), essentially as described previously [20,26,28]. The RNA quantity was expressed relative to the 18S rRNA endogenous control.

Statistical analysis

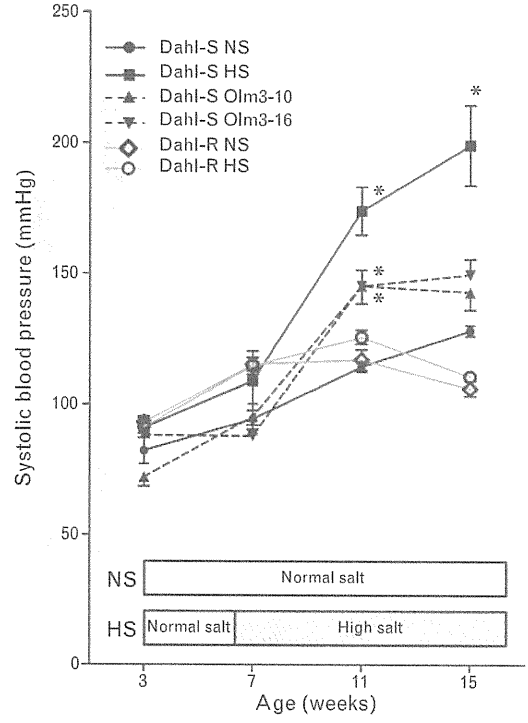
Data are expressed as mean ± SEM. Statistical significance was determined by unpaired Student's *t*-test, with P less than 0.05 being deemed statistically significant.

Results

Effects of prepubertal treatment with olmesartan on blood pressure in Dahl Iwai salt-sensitive rats

SBP did not differ among the six groups of Dahl Iwai salt-sensitive and Dahl Iwai salt-resistant rats at 3 weeks of age (Fig. 1). SBP of the Dahl Iwai salt-resistant rats in group 5 (normal salt loading) and group 6 (high salt loading) did not show any significant changes during the experimental period. Also, SBP of the Dahl Iwai salt-sensitive rats in group 1 (normal salt loading) remained normotensive at 15 weeks (128 ± 2 mmHg). In contrast, the group 2 Dahl Iwai salt-sensitive rats (high salt loading) showed a progressive increase in SBP at 11 weeks (174 ± 9 mmHg) and 15 weeks (199 ± 15 mmHg). On the contrary, the Dahl Iwai salt-sensitive rats in group 4 (Olm3-16) exhibited a significant suppression of the increase in SBP at 15 weeks (150 ± 6 mmHg). Furthermore, the group 3 Dahl Iwai salt-sensitive rats (Olm3-10) also exhibited a (comparably) significant suppression of the increase in SBP at 15 weeks (143 ± 7 mmHg).

Fig. 1



Effects of prepubertal transient olmesartan treatment on salt-induced hypertension in Dahl Iwai salt-sensitive rats. Male DS and DR rats (3 weeks of age) were randomly divided into six groups for dietary high salt loading and oral administration of vehicle or olmesartan. Normal salt loading (NS), DS and DR rats fed a normal salt diet (0.3% NaCl); high salt loading, DS and DR rats fed a high salt diet (8% NaCl) from 6 to 16 weeks and treated with vehicle throughout the experimental period until 16 weeks; Olm 3-10, DS rats fed a high salt diet (8% NaCl) from 6 to 16 weeks and transiently treated with olmesartan (8 mg/kg per day) during the prepubertal period from 3 to 10 weeks; Olm 3-16, DS rats fed a high salt diet (8% NaCl) from 6 to 16 weeks and continuously treated with olmesartan (8 mg/kg per day) throughout the experimental period from 3 to 16 weeks. The values of the SBP measured by the tail cuff method are expressed as the mean ± SE ($n = 6$ in each group). *$P < 0.05$ vs. NS. DR, Dahl Iwai salt-resistant; DS, Dahl Iwai salt-sensitive.

Effects of prepubertal treatment with olmesartan on development of renal oxidative stress, inflammation and fibrosis in Dahl Iwai salt-sensitive rats

Since the salt-induced hypertension was observed only in Dahl Iwai salt-sensitive rats, we next examined effects of high salt loading and olmesartan treatment on the renal pathological responses in Dahl Iwai salt-sensitive rats at 16 weeks. The results of real-time RT-PCR analysis showed that high salt loading increased the renal cortical TGF-β, fibronectin and type 1 and type 3 collagen mRNA, compared with normal salt loading (Fig. 2a–d). Among these upregulated mRNA levels in response to high salt loading, the mRNA expression of TGF-β, fibronectin and type 1 collagen was significantly suppressed by continuous treatment with olmesartan (Olm3-16). Furthermore, prepubertal transient treatment with olmesartan comparably inhibited the up-regulation of TGF-β, fibronectin and type 1 collagen mRNA levels induced by high salt loading (Olm3-10).

In order to investigate the mechanisms involved in the prepubertal olmesartan treatment-mediated suppression of renal damage in response to high salt loading, we analyzed the renal cortical expression of oxidative stress-related proteins, including Rac1, p47phox and p22phox, at 16 weeks (Fig. 2e–g). Although neither high salt loading nor olmesartan treatment affected Rac1 or p47phox expression, high salt loading significantly increased the renal cortical p22phox expression compared with normal salt loading. On the contrary, continuous olmesartan treatment (Olm3-16) completely abolished the high salt loading-mediated increase in