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正常腎機能患者におけるアンジオテンシンII受容体拮抗薬の血清尿酸値への影響

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Uricosuric Effects of Angiotensin II Receptor Antagonists in the Patients With Normal Renal Function

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The effects of angiotensin II type 1 receptor antagonists on serum uric acid (UA) levels were retrospectively examined between December, 1998 and December, 2001 in patients who received 50 mg losartan potassium (LOS) or 8 mg candesartan cilexetil (CAN) per day for 12 months without any change in the prescription over 12 months after initiation of administration. Serum creatinine level at baseline was below 1.2 mg/dL.

Patients having a pretreatment level of serum UA within the normal range (UA≦7.0 mg/dL) and receiving LOS were arbitrarily assigned as NLOS (n=34) and those exceeding the normal range, as HLOS (n=24). Similarly, NCAN (n=35) and HCAN (n=30) were assigned, respectively.

In all NLOS, HLOS, NCAN and HCAN groups, no change was observed in urea nitrogen or serum creatinine level at 12 months after administration of the drugs. The serum UA level was decreased significantly after administration in HLOS group, whereas no significant change was noted in NLOS group. Although the serum UA level was increased significantly at 12 months as compared with the pretreatment value in NCAN group, the levels remained within the normal range. The serum UA levels stayed within the normal range in HCAN group.

Among those who were treated with LOS at a dose of 50 mg per day, serum uric acid levels 12 months after administration showed no change as compared with the pretreatment values in patients with normal renal function and UA≦7.0 mg/dL. Further, the serum uric acid levels after 12 months significantly decreased to 7.0 mg/dL or below as compared with the pretreatment values in patients with UA>7.0 mg/dL. These results might be attributed to the lowering effect of LOS.

This retrospective study suggested that LOS has a lowering effect on serum uric acid levels in patients with hyperuricacidemia but CAN has no such effect.

Key words: angiotensin II receptor antagonist, losartan potassium, candesartan cilexetil, uricosuric effect

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Table 1 患者背景

	gender	age		before medication	12 months after	p value
NLOS	m=14, f=20	62.5±11.6	BUN	16.4±4.7	18.0±5.6	N.S.
			Scr	0.77±0.18	0.81±0.23	N.S.
HLOS	m=12, f=12	62.2±11.0	BUN	15.9±5.5	17.7±6.6	N.S.
			Scr	0.77±0.14	0.80±0.17	N.S.
NCAN	m=12, f=23	65.4±11.0	BUN	15.0±3.4	15.9±4.2	N.S.
			Scr	0.75±0.14	0.79±0.19	N.S.
HCAN	m=16, f=14	65.2±9.9	BUN	15.5±4.1	16.7±5.0	N.S.
			Scr	0.81±0.19	0.90±0.25	N.S.

mg/dL, Mean±S.D., Paired t-test, N.S.: not significant, $p<0.05$, m: male, f: female
NLOS群（投与前の血清尿酸値が7.0 mg/dL以下でlosartanの投与を受けている患者）
HLOS群（投与前の血清尿酸値が7.0 mg/dLを超えlosartanの投与を受けている患者）
NCAN群（投与前の血清尿酸値が7.0 mg/dL以下でcandesartanの投与を受けている患者）
HCAN群（投与前の血清尿酸値が7.0 mg/dLを超えcandesartanの投与を受けている患者）

緒　言

高尿酸血症は高血圧症，高脂血症，耐糖能異常など生活習慣病を高率かつ同時に複数合併することが知られており[1]，高尿酸血症をmultiple risk factor syndromeに加えるかどうかが論議されている．とくに高血圧症と高尿酸血症の合併は，心血管障害の発症する危険性を高めるばかりでなく，腎機能にも悪影響を及ぼすことが推定される．アンジオテンシンII（AII）受容体拮抗薬は，その薬理作用から降圧目的だけでなく，心，腎などの各臓器保護を目的として繁用されるようになった．AII受容体拮抗薬の中では，losartanが尿酸排泄促進作用を有することが報告されている[2~4]．我々は正常腎機能患者に，AII受容体拮抗薬を投与した場合の血清尿酸値の変化をretrospectiveに検討したので報告する．

対　象

国立大阪病院を受診中の患者で，1998年12月から2001年12月までの期間中にlosartan potassium（ニューロタン®，以下LOS）1日50 mgまたはcandesartan cilexetil（ブロプレス®，以下CAN）1日8 mgの投与を受け，投与開始から12カ月間処方変更がなく，投与開始時の血清クレアチニン（Scr）値が1.2 mg/dL未満の症例を解析対象とした．ただし，allopurinol，benzbromaroneなどの高尿酸血症治療薬またはサイアザイド系やループ利尿剤など，明らかに血清尿酸値に影響を及ぼす薬剤の投与を受けている患者は除外した．

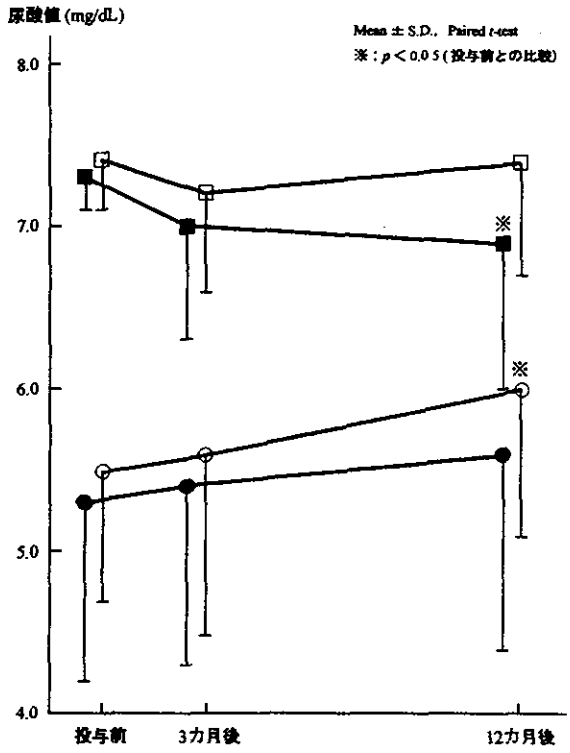
方　法

LOSまたはCANの投与前，投与3カ月後，投与12カ月後の血清尿酸値および対象患者の合併症を調査した．投与前の血清尿酸値が正常域（UA≦7.0 mg/dL）でLOSの投与を受けている患者をNLOS群，正常域の上限を超えている患者をHLOS群，同様にCANの場合はNCAN群，HCAN群とした．糖尿病を合併している患者をNLOS（DM），HLOS（DM），NCAN（DM），HCAN（DM）とした．NLOS，HLOS，NCAN，HCANおよびNLOS（DM），HLOS（DM），NCAN（DM），HCAN（DM）の各群の投与前と投与3カ月後および投与12カ月後の尿素窒素（BUN），Scrの比較，群内における各指標の有意性は対応のあるt検定で検討し，有意水準は5%とした．数値は平均値±標準偏差で表記した．

結　果

解析対象患者は123名で，その内訳はLOS投与患者は58名（男性26名，女性32名，平均年齢62.3±11.3，30～88歳），CAN投与患者は65名（男性28名，女性37名，平均年齢65.3±9.6，41～80歳）であった．NLOS，HLOS，NCAN，HCANのすべての群において，投与前と投与12カ月後のBUN，Scrに変化はなかった．患者背景をTable 1に示す．

NLOS群の投与12カ月後の血清尿酸値は，投与前に比べ有意な変化は認められなかったが，HLOS群では7.0 mg/dL以下まで有意に低下した．NCAN群の投与12カ月後の血清尿酸値は，投与前に比べ有意に上昇したが，正常範囲内であった．HCAN群では



●：NLOS 群（n＝34，投与前の血清尿酸値が 7.0 mg/dL 以下で losartan の投与を受けている患者）
■：HLOS 群（n＝24，投与前の血清尿酸値が 7.0 mg/dL を超え losartan の投与を受けている患者）
○：NCAN 群（n＝35，投与前の血清尿酸値が 7.0 mg/dL 以下で candesartan の投与を受けている患者）
□：HCAN 群（n＝30，投与前の血清尿酸値が 7.0 mg/dL を超え candesartan の投与を受けている患者）

Fig. 1 Losartan potassium, candesartan cilexetil 投与前，3 カ月後，12 カ月後の尿酸値の変化

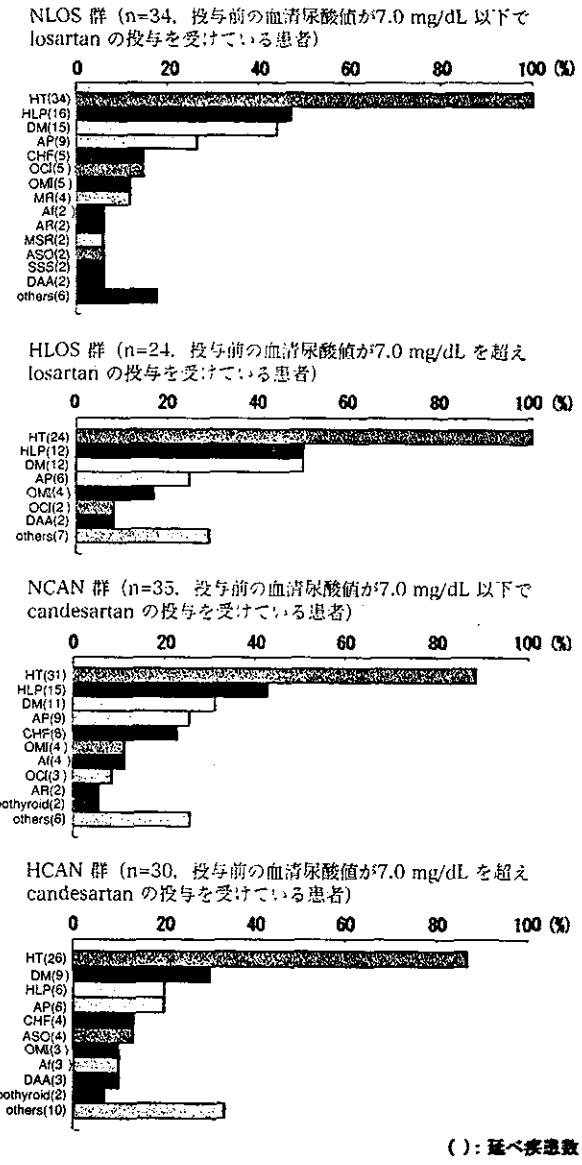


Fig. 2 合併症の割合

Af：心房細動，AP：狭心症，AR：大動脈弁閉鎖不全，ASO：閉塞性動脈硬化症，CHF：慢性心不全，DAA：解離性大動脈瘤，DM：糖尿病，HLP：高脂血症，HT：高血圧症，hypothyroid：甲状腺機能低下症，MR：僧帽弁閉鎖不全症，MSR：僧帽弁狭窄症兼閉鎖不全症，OCI：陳旧性脳梗塞，OMI：陳旧性心筋梗塞，SSS：洞機能不全症候群

有意な変化は認められなかった（Fig. 1）．

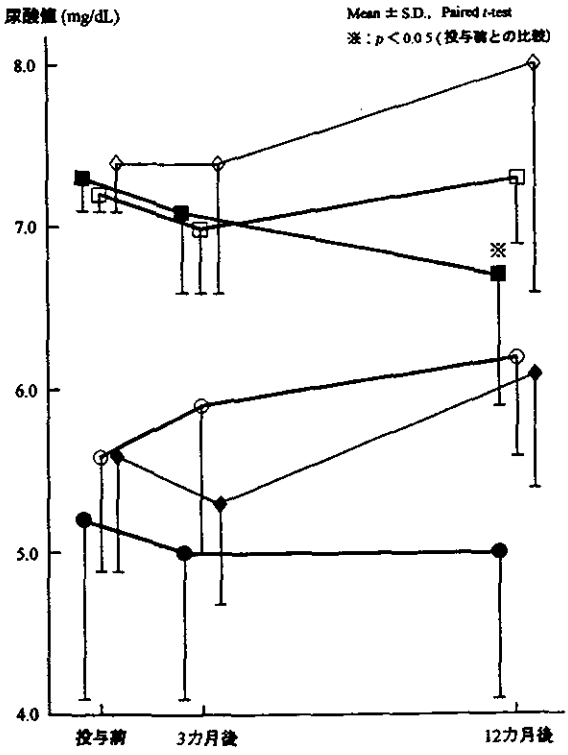
NLOS 群，HLOS 群はすべて高血圧を有していたが，NCAN 4 名，HCAN 4 名は高血圧症ではなかった（Fig. 2）．高血圧症ではない NCAN 4 名，HCAN 4 名の各血清尿酸値の平均値は，12 カ月後で上昇傾向を示した（Fig. 3）．

糖尿病を合併している患者は，NLOS（DM）15 名，HLOS（DM）12 名，NCAN（DM）11 名，HCAN（DM）9 名で，各群の投与前と投与 12 カ月後の BUN，Scr に有意な変化はなかった．患者背景を Table 2 に示す．

NLOS（DM）群の投与 12 カ月後の血清尿酸値は，投与前に比べ有意な変化は認められなかったが，HLOS（DM）群では 7.0 mg/dL 以下まで有意に低下した．NCAN 群（DM）の投与 12 カ月後の血清尿酸値は，投与前に比べ上昇傾向を示したが，正常範囲内であった．HCAN(DM)群では有意な変化は認められなかった（Fig. 3）．

考　察

高尿酸血症は心血管系イベントの危険因子の 1 つと考えられ，Worksite study によれば高血圧症患者において血清尿酸値が 1 mg/dL 上昇するリスクは，収縮期血圧が 10 mmHg，あるいは血清コレステロール値が 20 mg/dL 上昇するリスクと同等であると報告している[5]．本態性高血圧症に合併する高尿酸血症の原



●：NLOS (DM) 群 (n=15，投与前の血清尿酸値が 7.0 mg/dL 以下で losartan の投与を受け，糖尿病を合併している患者)
■：HLOS (DM) 群 (n=12，投与前の血清尿酸値が 7.0 mg/dL を超え losartan の投与を受け，糖尿病を合併している患者)
○：NCAN (DM) 群 (n=11，投与前の血清尿酸値が 7.0 mg/dL 以下で candesartan の投与を受け，糖尿病を合併している患者)
□：HCAN (DM) 群 (n=9，投与前の血清尿酸値が 7.0 mg/dL を超え candesartan の投与を受け，糖尿病を合併している患者)
◆：NCAN 群の中で高血圧症を合併していない 4 例
◇：HCAN 群の中で高血圧症を合併していない 4 例

Fig. 3 糖尿病を合併した患者の losartan potassium，candesartan cilexetil 投与前，3 カ月後，12 カ月後の血清尿酸値の変化および高血圧症を合併していない 8 例の血清尿酸値の変化

Table 2 糖尿病を合併している患者背景

	gender	age		before medication	12 months after	*p* value
NLOS (DM)	m=6, f=9	60.2±8.2	BUN	14.7±3.3	15.9±4.3	N.S.
			Scr	0.70±0.16	0.73±0.18	N.S.
HLOS (DM)	m=6, f=6	62.6±8.2	BUN	17.3±4.7	19.8±7.7	N.S.
			Scr	0.77±0.12	0.81±0.16	N.S.
NCAN (DM)	m=4, f=7	62.7±7.6	BUN	16.0±3.4	15.1±4.2	N.S.
			Scr	0.73±0.20	0.85±0.25	N.S.
HCAN (DM)	m=5, f=4	67.2±8.8	BUN	14.3±1.9	16.3±4.6	N.S.
			Scr	0.80±0.19	0.86±0.26	N.S.

mg/dL, Mean±S.D., Paired *t*-test, N.S.: not significant, $p<0.05$, m: male, f: female
NLOS (DM) 群 (投与前の血清尿酸値が 7.0 mg/dL 以下で losartan の投与を受け，糖尿病を合併している患者)
HLOS (DM) 群 (投与前の血清尿酸値が 7.0 mg/dL を超え losartan の投与を受け，糖尿病を合併している患者)
NCAN (DM) 群 (投与前の血清尿酸値が 7.0 mg/dL 以下で candesartan の投与を受け，糖尿病を合併している患者)
HCAN (DM) 群 (投与前の血清尿酸値が 7.0 mg/dL を超え candesartan の投与を受け，糖尿病を合併している患者)

因として尿酸排泄低下が重要であると考えられてきたが[6]，近年になり，近位尿細管における尿酸の分泌低下は乳酸や β-ヒドロキシ酪酸塩，アセト酪酸塩などの有機酸により，陰イオン交換輸送システムが阻害されるためであることがわかってきた．さらに，高血圧によって引き起こされた微少血管障害による虚血は，アデノシントリフォスフェート (ATP) の破壊によって増加したアデノシンやヒポキサンチンなどの基質を増加させ，キサンチンオキシダーゼの作用を介して尿酸生成の増加をもたらすことが解明されつつある[7]．

LOS は AII 受容体に特異的な拮抗薬であり[8]，AII 受容体拮抗作用による持続的な降圧作用を示し，AII 刺激による昇圧反応やアルドステロン分泌・産生亢進の抑制作用[9]を有することが確認されている．LOS は生体内でチトクローム P450 によって酸化を受け，未変化体以上に AII 受容体拮抗作用が強いカルボン酸代

謝物であるEXP-3174となる．本薬の強力かつ持続的な降圧効果は，LOS未変化体とEXP-3174の双方が寄与していると考えられている[10]．また，LOSは強力な尿酸排泄作用を有していることが確認されており[2]，その作用機序はヒト腎臓の近位尿細管刷子縁膜小包において尿酸陰イオン交換輸送系を抑制し[3]，腎近位尿細管における尿酸の再吸収を抑制するためであることが推定されている[4]．吉永ら[11]は1.2≦Scr≦4.0 mg/dLを示す本態性高血圧症または腎生検，尿検査などにより確定された腎実質性高血圧症で，Scr≦4.0 mg/dLの患者に対しLOSを1日最大100 mgを最大10週間投与した結果，24名の血清尿酸値は観察期6.3±1.6 mg/dL，治療期6.1±1.5 mg/dLと有意な変化は認められなかったと報告しているが，血清尿酸値による層別結果は不明である．浜田ら[12]はLOS（50 mg）を19例の患者に投与し，投与前と投与1～2カ月後の血清尿酸値を測定した結果，血清尿酸値は6.5±1.3 mg/dLから5.8±1.3 mg/dLへ有意に低下し，約半数例において投与前比1.0 mg/dL以上の低下を認めたことは，心血管系イベントの危険因子の軽減に十分に寄与するものであると報告している．我々の調査では，3カ月後のNLOS群の血清尿酸値は上昇傾向を示したものの，有意な変化がなかったという点において吉永らの報告と一致する．HLOS群の投与3カ月後の血清尿酸値は，浜田らの報告と異なり低下しなかったが，投与12カ月後の血清尿酸値は有意に低下したことから，LOSの血清尿酸値低下作用が確認できた．本検討で血清尿酸値低下作用が認められたことは，anionである尿酸がurate/anion exchangerによって尿細管細胞内に取り込まれ，側底膜に存在するpotential-driven pathway（膜電位による輸送系）によって間質に輸送されるとの考え[13]に基づき，LOSがurate/anion exchangerを阻害する機序により尿酸排泄作用を有するというEdwardsら[14]の報告と合致する．しかし，今回我々が調査したNLOS群の血清尿酸値が有意に低下しなかった理由についてはいまだ解明されておらず，高値の血清尿酸値は低下させるが正常範囲内の血清尿酸値には影響を及ぼさないのかについて，引き続きNLOS群の血清尿酸値の投与12カ月以降の変化を調査する必要があると考える．

次に，CANはベンズイミダゾール骨格を持ち，7位にカルボキシル基を有する経口投与可能な非ペプチド性のエステル型プロドラッグであり，経口投与後は生体内で活性の高いcandesartanに変換される[15]．McClellanら[16]，Kawabataら[17]，Iseら[18]の報告によれば，CANは尿酸の排泄に影響を及ぼすことはなく，CANを1日8 mgまたは16 mgを内服した試験結果[19]では，低カリウム血症および高尿酸血症は認められなかったと述べている．Malmqvistら[20]はCANとenalaprilまたはhydrochlorothiazide（HCTZ）の無作為割り付け二重盲検比較試験において，enalapril 10 mg群，HCTZ 12.5 mg群，CAN 8 mgを6週間投与し，拡張期血圧が90 mmHg以上の場合，CAN 16 mgをさらに6週間投与した結果，HCTZ群の血清尿酸値はCAN群と比較して有意に上昇し，CAN群の血清尿酸値に変化は認められなかったと報告している．伊勢ら[21]は1日最大8 mgを6カ月間投与した症例において，投与2週間後の血清尿酸値は低下傾向を示したものの，明らかな尿酸排泄促進作用はみられなかったと報告している．このように，ヒトでのCANの血清尿酸値低下作用が明らかに認められたとする報告はなく，我々の調査でも投与3カ月後のNCAN群，HCAN群は両群とも投与前と比較して有意な変化はなく，Malmqvistら，伊勢らの報告と一致する．投与12カ月後の血清尿酸値はNCAN群で有意に上昇し，HCAN群では有意な変化は認められなかったことから，CANに血清尿酸値低下作用はないと考えられる．

一方，2型糖尿病患者を対象としたRENAAL[22]大規模試験では，ARBによってprimary end pointであるScrが2倍になるリスクおよび末期腎不全に至るリスクに加え，心不全による初回入院のリスクの低下が報告されている．我々の調査でも，NLOS，HLOS，NCAN，HCANの各群，糖尿病を合併するNLOS（DM），HLOS（DM），NCAN（DM），HCAN（DM）の各群とも，投与12カ月後のScrが投与前の2倍になることはなかった．NLOS（DM）群の投与12カ月後の血清尿酸値は，投与前に比べ有意な変化は認められなかったが，HLOS（DM）群の投与12カ月後の血清尿酸値は投与前に比べ7.0 mg/dL以下まで有意に低下したことは，LOSの血清尿酸値低下作用は糖尿病患者に対しても有効であると考えられる．NCAN（DM）群およびHCAN（DM）群の投与12カ月後の血清尿酸値は，投与前に比べ有意な変化は認められず，糖尿病患者に対して血清尿酸値低下作用は期待できなかった．NCAN（DM）群の血清尿酸値は，投与12カ月後で上昇傾向が認められるが，正常範囲内であり，今後の動向を引き続き経過観察する必要がある．

高血圧症を合併していない8例（NCAN群4例，HCAN群4例）は，高脂血症または糖尿病を合併していたが，今回の調査では症例数が少ないため，各合併症と血清尿酸値の変化について明らかにすることはできなかった．8例の血清尿酸値の変化を追跡するとともに，合併症と血清尿酸値との因果関係についても検討していきたい．

腎機能が正常でかつ血清尿酸値が正常域の上限を超えている患者にLOS 1日50 mg投与した結果，12カ月後の血清尿酸値が有意に低下したことはLOSの血清尿酸値低下作用によるものと考えられ，高尿酸血症と高血圧症を合併する患者には合理的な薬剤である．CANの尿酸排泄作用は明らかではないが，我々の調査ではCANの血清尿酸値低下作用は認められなかった．今回の調査は，retrospectiveに行ったため十分なデータ収集が行えず，life styleの改善，体重，アルコール摂取量の変化，高脂血症または糖尿病などの合併症と血清尿酸値との関係をprospectiveに調査する必要があると考える．

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Original Article

Lack of Association between Angiotensin II Type 1 Receptor Gene Polymorphism and Hypertension in Japanese

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Angiotensin II type 1 (AT_1) receptor mediates the vasoconstriction and growth-promoting effect of angiotensin II in humans. It has been reported that a polymorphism of the AT_1 receptor gene (an A/C transversion at position 1166; A1166C) may be associated with essential hypertension (HT). However, several conflicting results have also been reported. Therefore, we conducted an association study between A1166C variants of the AT_1 receptor gene and hypertension in the Japanese population. We genotyped this variant in 3,918 subjects (1,492 hypertensive subjects and 2,426 normotensive subjects) recruited from the Suita study. In subjects not receiving antihypertensive medication, the influence of the genotype on blood pressure values adjusted for clinical covariates was analyzed. The genotype distribution did not differ between hypertensive and normotensive subjects in either men (frequency of the C allele: 8.1% *vs.* 7.8%, $p=0.74$) or women (8.1% *vs.* 7.7%, $p=0.60$). There were no significant differences in systolic blood pressure, diastolic blood pressure, or pulse pressure among the three genotypes in either men or women who had not received hypertensive medication. Our data suggest that the A1166C polymorphism of AT_1 receptor is unlikely to influence blood pressure status in the Japanese population. (*Hypertens Res* 2003; 26: 131–134)

***Key Words*: epidemiology, genetics, blood pressure**

Introduction

Angiotensin II is an important effecter controlling blood pressure and volume in the cardiovascular system. Its importance is reflected by the efficacy of angiotensin-converting enzyme inhibitors in the treatment of hypertension and congestive heart failure. Angiotensin II interacts with two pharmacologically distinct subtypes of cell surface receptors, types 1 and 2. Angiotensin II type 1 (AT_1) receptors seem to play a key role in mediating the vasoconstrictor and growth-promoting effects of angiotensin II (*1*). It has been reported that a polymorphism of the AT_1 receptor gene (an A/C transversion at position 1166; A1166C) occurs more frequently in hypertensive subjects with a positive family history of hypertension than in control subjects (*2*). On the other hand, it has also been reported that this locus is not linked with this disease, and recent studies have demonstrated that the distribution of the genotypes did not differ between normotensive and hypertensive subjects (*3*, *4*). Moreover, a recent population-based survey of Caucasian hypertensives reported lower blood pressure values in CC homozygotes than in heterozygotes and AA homozygotes (*5*). In response to these controversial results, we performed an association study in a large epidemiological cohort to examine whether A1166C genetic variants influence blood pressure in the Japanese population.

Methods

Subjects

The selection criteria and design of the Suita study have

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Table 1. Characteristics of Men and Women Subjects by AT_1 Receptor Genotype

Characteristics	AT_1 receptor genotype		
	AA	AC	CC
Men (n=1,854)			
n	1,575	267	12
Age (years)	60.7±12.1	61.4±12.3	59.4±12.3
Body mass index (kg/m^2)	23.1±2.8	22.7±2.7	22.3±2.60
Waist-to-hip circumference ratio	0.91±0.06	0.90±0.06	0.94±0.04
Alcohol consumption (ml/day)	25.2±26.2	26.4±29.7	23.5±22.5
Smoking habit (%)	38.9	39.7	75
Ischemic heart disease (%)	4.75	6.37	0
Diabetes mellitus (%)	22.2	21.7	33.3
Proteinuria (%)	7.6	6.4	16.7
Use of antihypertensive drugs (%)	18.4	17.2	16.7
Women (n=2,062)			
n	1,755	290	17
Age (years)	58.8±11.8	59.1±11.6	57.2±13.7
Body mass index (kg/m^2)	22.3±3.2	22.5±2.9	21.1±2.7
Waist-to-hip circumference ratio	0.89±0.08	0.89±0.07	0.86±0.07
Alcohol consumption (ml/day)	5.1±11.2	4.6±10.0	6.1±13.3
Smoking habit (%)	8.1	8.6	11.8
Ischemic heart disease (%)	2.9	4.8	0
Diabetes mellitus (%)	14.2	13.1	5.9
Proteinuria (%)	3.9	4.5	5.9
Use of antihypertensive drugs (%)	16.1	16.2	17.6

Values are the mean±SEM. AT_1, angiotensin II type 1.

been described previously (*6*, *7*). The present study was approved by the Ethics Committee of the National Cardiovascular Center and by the Committee on Genetic Analysis and Genetic Therapy of the National Cardiovascular Center. Informed consent on genetic analysis was obtained from about 4,000 subjects, and the genotype of A1166C was determined in 3,918 consecutive subjects. Subjects were categorized as hypertensives when they had a systolic blood pressure of ≧140 mmHg or a diastolic blood pressure of ≧90 mmHg. Subjects who were taking hypertensive medication were also categorized as hypertensives.

DNA Studies

DNA was isolated from peripheral leukocytes according to standard procedures. Polymorphisms were determined by the TaqMan system. The primers and probes for genotype determination were as follows: Sense 5′-CATTCCTCTGCAGCA CTTCACT-3′, Antisense 5′-CGGTTCAGTCCACATAATG CAT-3′, Probe for A(1166) 5′-Fam-AAATGAGCATTAGCT ACT-MGB-3′, Probe for C(1166) 5′-Fam-AAATGAGCCTT AGCTACT-MGB-3′. Each reaction included 20 ng of genomic DNA, 30 pmol of each primer, 12.5 pmol of each TaqMan probe and 1X TaqMan Universal Master Mix (PE Biosystems, Foster City, USA) in a volume of 50 μl. Polymerase chain reaction (PCR) cycling conditions were 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 10 min, and 40 cycles at 95°C for 15 s and 60°C for 1 min. The results were analyzed using an ABI PRISM 7700 Sequence Detection System (PE Biosystems) using allelic discrimination software supplied by the manufacture.

Statistical Analyses

Values are expressed as the means±SEM. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc., Cary, USA). Multiple regression and multiple logistic analyses were performed with other covariates. Differences in numerical data among the groups were calculated by one-way analysis of variance (ANOVA) and unpaired Student's t-test. Values of $p<0.05$ were considered to indicate statistical significance. Differences in frequencies were tested by contingency table analysis.

Results

AT_1 Receptor Genotype and Allele Frequencies and Clinical Characteristics

The overall frequencies of the genotypes AA, AC, and CC were as follows: in men, 84.9%, 14.5%, and 0.6%, respectively; in women, 85.1%, 14.1%, and 0.8%; and overall, 85.0%, 14.1%, 0.8%. The allele frequencies for A and C were 92.1% and 7.9%, 92.0% and 8.0%, and 92.0% and 8.0%, respectively, in men, in women, and

Table 2. Genotype and Allele Distribution of AT$_1$ Receptor A1166C Polymorphism in Hypertensive Subjects and Normotensive Subjects

	Men (*n* (%))		Women (*n* (%))	
	Hypertensive	Normotensive	Hypertensive	Normotensive
Genotype				
AA	627 (84.4)	948 (85.2)	632 (84.4)	1,123 (85.5)
AC	112 (15.1)	157 (14.1)	112 (15.0)	178 (13.6)
CC	4 (0.5)	8 (0.7)	5 (0.6)	12 (0.9)
	$\chi^2=0.55, p=0.76$		$\chi^2=1.09, p=0.58$	
Allele				
A	1,366 (91.9)	2,053 (92.2)	1,376 (91.9)	2,424 (92.3)
C	120 (8.1)	173 (7.8)	122 (8.1)	202 (7.7)
	$\chi^2=0.11, p=0.74$		$\chi^2=0.27, p=0.60$	

Table 3. Blood Pressure of Subjects Not Receiving Antihypertensive Medication by AT$_1$ Receptor Genotype

	AT$_1$ receptor genotype			ANOVA
	AA	AC	CC	*p*
Men ($n=1,518$)				
n	1,285	223	10	
SBP (mmHg)				
Unadjusted	125.8±0.5	126.4±1.2	126.2±5.8	0.88
Age-adjusted	125.8±0.5	126.5±1.1	128.0±5.4	0.93
Age and BMI-adjusted	125.7±0.5	127.1±1.1	128.6±5.3	0.73
DBP (mmHg)				
Unadjusted	80.3±0.3	79.5±0.7	77.1±3.3	0.40
Age-adjusted	80.4±0.3	78.8±0.7	74.3±3.3	0.38
Age and BMI-adjusted	80.4±0.3	79.2±0.7	74.7±3.2	0.69
PP (mmHg)				
Unadjusted	45.5±0.4	46.9±0.9	49.1±4.1	0.22
Age-adjusted	45.3±0.3	47.7±0.8	53.8±3.6	0.20
Age and BMI-adjusted	45.3±0.3	47.8±0.8	53.9±3.6	0.17
Women ($n=1,730$)				
n	1,473	243	14	
SBP (mmHg)				
Unadjusted	125.0±0.5	126.1±1.2	123.6±5.2	0.67
Age-adjusted	125.0±0.5	126.9±1.1	124.1±4.7	0.73
Age and BMI-adjusted	125.0±0.5	126.6±1.1	125.5±4.7	0.80
DBP (mmHg)				
Unadjusted	77.5±0.3	79.0±0.7	78.4±2.8	0.12
Age-adjusted	77.3±0.3	80.2±0.7	79.5±2.8	0.12
Age and BMI-adjusted	77.3±0.3	80.0±0.7	80.7±2.7	0.17
PP (mmHg)				
Unadjusted	47.5±0.4	47.2±0.9	45.3±3.6	0.77
Age-adjusted	47.6±0.3	46.8±0.8	44.7±3.2	0.82
Age and BMI-adjusted	47.6±0.3	46.7±0.8	44.9±3.2	0.80

Values are the mean±SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; BMI, body mass index.

overall. The observed genotype frequencies were in agreement with those predicted by Hardy-Weinberg equilibrium.

The clinical characteristics of male and female subjects are summarized in Table 1. There were no significant differences among the three genotypes in any characteristics in either men or women.

Relationship between the AT$_1$ Receptor Genotype and Blood Pressure

The genotype distribution and the allele distribution of the A1166C variants of AT$_1$ receptor gene polymorphisms in hypertensive subjects were compared to those in normotensive

subjects for both men and women (Table 2). No significant differences in the genotype distribution or the allele distribution were observed between hypertensive subjects and normotensive subjects in either men or women.

The influence of the AT_1 receptor genotype on residuals of blood pressure values after adjusting for age and body mass index (BMI) is shown in Table 3. No significant influence of the genotype on blood pressure was observed.

Discussion

Bonnardeaux *et al.* identified a positive association between A1166C variants of AT_1 receptor gene polymorphisms and hypertension in 206 Caucasian patients with essential hypertension (*2*). Wang *et al.* did a case-control study of the A1166C variant in 108 Caucasian hypertensive subjects and found that the A1166C variant was associated with hypertension (*8*). On the other hand, Bonnardeaux *et al.* failed to detect such an association using affected sibling pair analysis, and recent studies have found no difference in the distribution of the genotypes between normotensive and hypertensive subjects (*3*, *4*). Two other studies conducted in Japan also failed to show a significant association between this polymorphism and hypertension (*9*, *10*). Moreover, a recent population based survey of Caucasian hypertensives reported lower blood pressure values in CC homozygotes than in heterozygotes and AA homozygotes (*5*).

In the original report with Caucasian subjects, the frequencies of the 1166C allele were 0.36 in hypertensives and 0.28 in normotensives (*2*). However, in the present study, the frequencies of the 1166C allele were 0.09 in hypertensives and 0.08 in controls. Since similar results have also been reported in other studies in Japan, it would appear that the C allele is less frequent in Japanese than in whites. This different allele frequency may have affected the different results between Japanese and whites.

Since a population of only several hundred subjects may be too small to draw statistically certain conclusions, and because independent confirmation is of critical value, we genotyped this variant in a large cohort representing the general Japanese population (the Suita study) consisting of 3,918 subjects.

Our results indicate that the genotype distribution did not differ between hypertensive and normotensive subjects, and that the genotype had no significant effects on blood pressure values in either men or women. However, the present observations do not necessarily exclude the possibility that the AT_1 receptor gene is involved in hypertension in Japanese. It is still possible that some other polymorphisms in this gene may influence blood pressure. Takahashi *et al.* identified seven polymorphisms in the 5′-flanking region of the AT_1 receptor gene and found a significantly higher frequency of the (−535)T allele in hypertensive subjects (*11*). However, the sample size was small and further confirmation may still be required.

In future studies, it will be necessary to identify a large number of polymorphisms throughout the AT_1 receptor gene in Japanese and to perform association studies between these polymorphisms and blood pressure.

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Association of a promoter variant of the haeme oxygenase-1 gene with hypertension in women

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Objective **To examine the relationship between the gene for haeme oxygenase (HO)-1 (*HMOX-1*) and human essential hypertension, because both the acute and systemic induction of *HMOX-1* have been suggested to attenuate vascular tone and blood pressure.**

Methods **We screened for sequence variations in *HMOX-1* and conducted an association study, using these polymorphisms, in a large cohort (1998 individuals) representing the general Japanese population.**

Results **We sequenced *HMOX-1* and found a *T(−413)A* polymorphism in the promoter region. The frequency of hypertensive individuals and the use of antihypertensive drugs were significantly greater in the *AA* genotype than in other genotypes among women: 45.5, 34.2, and 35.0% ($P = 0.0099$) and 23.4, 17.5, and 15.0% ($P = 0.038$), respectively, for the *AA*, *AT*, and *TT* genotypes, respectively. However, this association was not observed in men. Multiple logistic analyses indicated that the *T(−413)A* (*AA/TA+TT*) polymorphism, age, and body mass index affected the occurrence of hypertension in women. The odds ratio of the *AA* genotype for hypertension in women was 1.59 ($P = 0.0058$; 95% confidence interval 1.14 to 2.20). A luciferase reporter assay indicated that the *A* allele-promoter had eight-fold greater activity than the *T* allele promoter ($P < 0.01$).**

Conclusions **The *AA* genotype of *HMOX-1* is associated with an increased incidence of hypertension in women. Oestrogen attenuates vasoconstriction by increasing the expression of inducible nitric oxide synthase. As carbon monoxide, which is one of the products of HO-1, can attenuate nitric oxide-induced vasodilatation, a high expression of HO-1 may cause hypertension, especially in women. *J Hypertens* 21:1497–1503 © 2003 Lippincott Williams & Wilkins.**

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Keywords: haeme oxygenase-1, hypertension, epidemiology, carbon monoxide, nitric oxide

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See editorial commentary page 1447

Introduction

Haeme oxygenases, which are essential for haeme degradation, produce bile pigments, ferritin, and carbon monoxide [1]. Haeme oxygenases and carbon monoxide participate in the haemostatic control of cardiovascular functions, including the regulation of blood pressure [2].

Inhibition of haeme oxygenase (HO)-1 with metalloporphyrins is known to lead to increased blood pressure and augmented arterial neointimal development after balloon injury [3,4]. Enhancement of the HO-1 system with haeme oxygenase inducers decreased blood pressure and prevented neointimal development [3,5]. In contrast, transgenic mouse strains that chronically overexpress HO-1 site-specifically in vascular smooth muscle cells exhibited a significant increase in arterial blood pressure and reduced vasodilatory responses [6]. Thus it remains unknown whether chronic and systemic activation of HO-1 could affect vascular tone and blood pressure.

Carbon monoxide is well recognized as a physiologically important vasoactive substance. A previous in-vitro experiment showed that nitric oxide and carbon monoxide activated soluble guanylate cyclase by distinct mechanisms and that carbon monoxide is far less potent than nitric oxide [7]. Therefore, carbon monoxide may suppress the vasodilatory response to nitric oxide by competition, and thereby lead to an increase in arterial pressure when the concentration of carbon monoxide is not excessive. Even in greater concentrations, carbon monoxide begins to inhibit endothelium-dependent nitric oxide synthase (eNOS) activity and nitric oxide generation [8].

Recently, Johnson *et al.* [9] reported that increased concentrations of endogenous carbon monoxide contri-

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bute to arteriolar nitric oxide dysfunction in Dahl salt-sensitive (Dahl S) rats with salt-induced hypertension. In their experiments, abdominal aortas of Dahl S rats with a high-salt diet showed sixfold greater HO-1 protein concentrations than a low-salt group. It has also been reported that angiotensin II-induced hypertension can increase the expression of HO-1 [10]. Therefore, inducible HO-1 may play a part in hypertension.

In the present study, we screened for sequence variations in the promoter region of the HO-1 gene (*HMOX-1*) and evaluated the significance of polymorphism in essential hypertension.

Methods

Participants

We selected 1998 consecutive patients without any cardiovascular complications, from the Suita study. The selection criteria and design of the Suita study have been described previously [11]. In the present study, participants' information was made anonymous. The study was approved by the Ethics Committee of the National Cardiovascular Centre and by the Committee on Genetic Analysis and Genetic Therapy of the National Cardiovascular Centre. Informed consent to genetic analysis was obtained from about 3700 individuals, and the genotype of *HMOX-1* was determined in 1998 consecutive individuals. Participants were categorized as hypertensive when they had a systolic blood pressure greater than 140 mmHg or a diastolic blood pressure greater than 90 mmHg. Individuals who were taking antihypertensive medication were also categorized as hypertensive.

DNA studies

Genomic DNA from 96 individuals was used as a template for sequence analysis. The promoter region (up to −1.4 kb) was sequenced. (The primer sequences are available on request.) Single-nucleotide polymorphisms were determined using the TaqMan system (PE Applied Biosystems) (Table 1) and $(GT)_n$ repeat length polymorphism was determined on an ABI 3700 DNA sequencing system using GeneScan software after amplification by polymerase chain reaction with a fluorescence-labelled sense primer, P1-S (5′-AGAGCCTGC AGCTTCTCAGA-3′), and an antisense primer, P1-AS (5′-ACAAAGTCTGGCCATAGGAC-3′) (Fig. 1).

Expression study

To explore the regulatory effects of a *T(−413)A* polymorphism in the promoter region, we constructed *HMOX-1* promoter/luciferase fusion genes. The promoter region between −517 and +76 was amplified by PCR with a sense primer, P2-S (5′-GTGAGGAGG CAAGCAGTCAGCAGAGGATTC-3′) and an antisense primer, P2-AS (5′-GTGCTGGGCTCGTTCGT GCTGGCTCC-3′) (Fig. 1) and subcloned into pGL2-Enhancer DNA (Promega), which does not contain any promoter sequence. Transfection was performed in bovine aortic endothelial cells (BAECs) with PRL-CMV vector (Promega) as an internal standard. *Photinus* and *Renilla* luciferase activities were measured with a dual luciferase kit (PG-DUAL-SP, Toyo Ink, Co).

Statistical analysis

Values are expressed as mean ± SE. All statistical analyses were performed using the JMP statistical software package (SAS Institute Inc., Cary, North Carolina, USA). Multiple logistic analyses were performed with other covariates. Differences in numerical data among the groups were analysed by one-way/two-way analysis of variance and the unpaired *t*-test. Differences in frequencies among the groups and the degree of linkage disequilibrium were tested by a contingency table analysis. A value of $P < 0.05$ was considered statistically significant.

Results

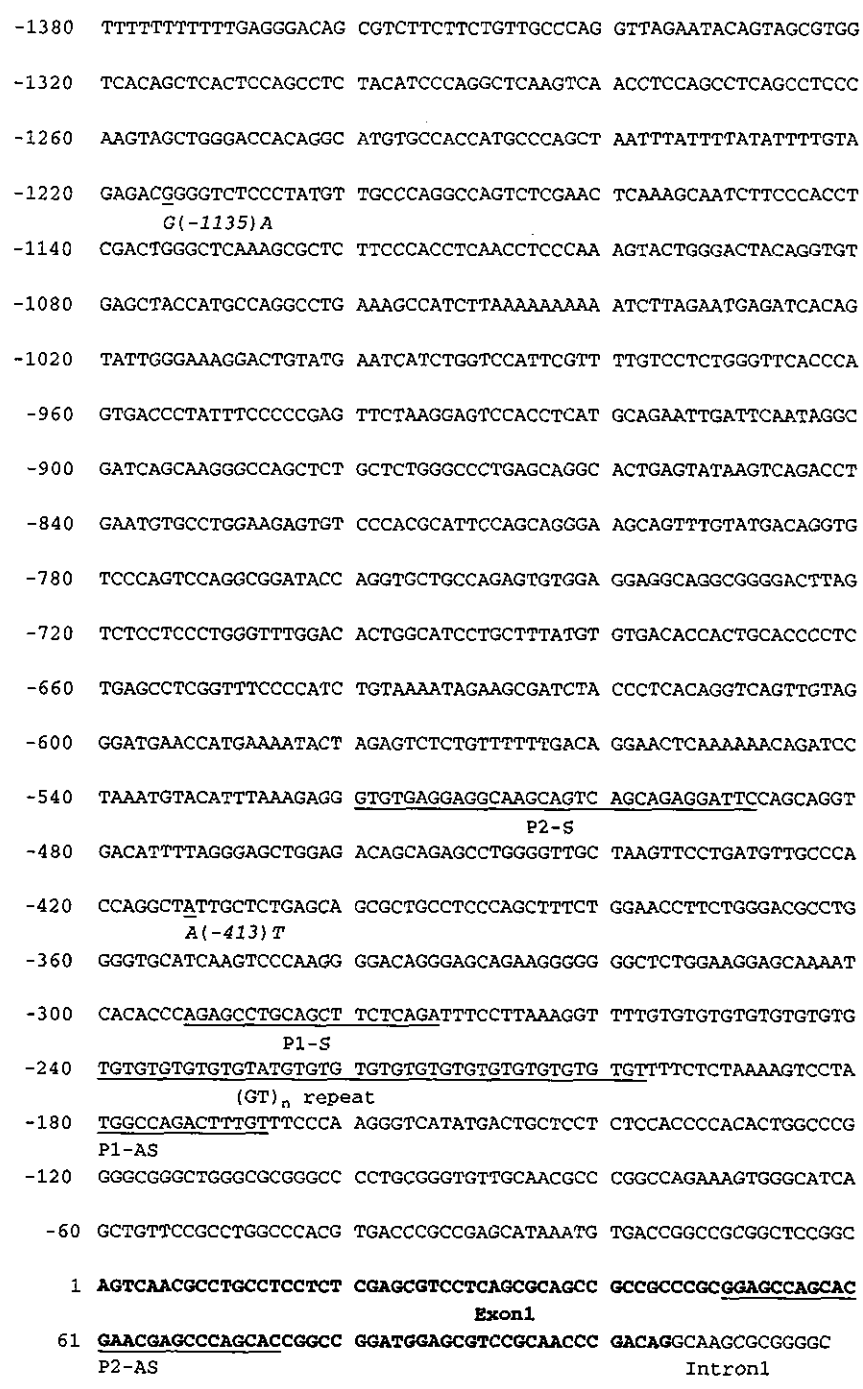
Haeme oxygenase-1 promoter polymorphisms

The nucleotide sequence of the 5′-flanking region and exon 1 of the human *HMOX-1* gene is shown in Figure 1. We found *G(−1135)A* and *T(−413)A* polymorphisms and confirmed the existence of $(GT)_n$ repeat length polymorphism in the promoter region of HO-1. The $(GT)_n$ repeat length in the *HMOX-1* gene ranged from 15 to 43. There were 22 genotypes in $(GT)_n$ repeat polymorphism with frequencies greater than 1% (Table 2).

Table 1 Primers and probes for genotype determination

Haeme oxygenase-1	Sequence
G(−1135)A	
Sense	5′-AGTCGAGGTGGGAAGATTGCT-3′
Antisense	5′-CCACCATGCCCAGCTAATTTA-3′
Probe for G(−1135)	5′-Fam-GAGACCCTGTCTCTACA-MGB-3′
Probe for A(−1135)	5′-Vic-AGACCCCGTCTCTACA-MGB-3′
A(−413)T	
Sense	5′-TGACATTTTAGGGAGCTGGAGACA-3′
Antisense	5′-AGGCGTCCCAGAAGGTTCCA-3′
Probe for A(−413)	5′-Fam-CCCACCAGGCTATTGCTCTGAGCA-Tamra-3′
Probe for T(−413)	5′-Tet-CCCACCAGGCTTTTGCTCTGAGC- Tamra-3′

Fig. 1



Nucleotide sequence of the 5′-flanking region and exon 1 of the human haeme oxygenase-1 gene (GenBank S58267 [12]). The fragment between primers P1-S and P1-AS was amplified by polymerase chain reaction and the $(GT)_n$ repeat length was determined. P2-S and P2-AS were used to construct haeme oxygenase-1 promoter/luciferase fusion genes.

Haplotype frequencies were estimated from data in Table 2 and are shown in Table 3. $A(-413)$-$(GT)_{30}$ and $T(-413)$-$(GT)_{23}$ were the two major alleles.

Association study

Table 4 shows characteristics of the study population. The frequency of hypertensive individuals and the use of antihypertensive drugs were significantly greater in the *AA* genotype than in other genotypes among women: 45.5, 34.2, and 35.0% ($P = 0.0099$) and 23.4, 17.5, and 15.0% ($P = 0.038$), respectively, for the *AA*, *AT*, and *TT* genotypes, respectively. Multiple logistic

Table 2 Number of *A(−413)T* genotypes in each genotype of $(GT)_n$ repeat length polymorphism

$(GT)_n$ repeat length polymorphism	*AA*	*AT*	*TT*
(21,30)	0	25	2
(22,23)	0	1	18
(22,30)	0	16	0
(23,23)	0	7	86
(23,24)	0	5	64
(23,25)	2	5	49
(23,30)	6	217	4
(23,31)	2	29	1
(23,33)	0	21	14
(23,34)	2	3	29
(24,25)	0	2	26
(24,30)	2	71	2
(25,30)	3	66	0
(25,33)	0	13	7
(26,30)	2	24	0
(30,30)	197	13	4
(30,31)	56	4	1
(30,32)	23	3	0
(30,33)	46	16	3
(30,34)	2	52	1
(30,36)	0	24	0

Table 3 Estimated allele frequency

Number of $(GT)_n$repeats	−413	Estimated allele frequency (%)
30	*A*	42.0
23	*T*	28.0
24	*T*	7.2
25	*T*	6.4
31	*A*	4.6
33	*A*	3.6
34	*T*	2.4
33	*T*	1.9
32	*A*	1.8
22	*T*	1.4
30	*T*	1.1

Alleles with a frequency of less than 1% are not shown.

analyses indicated that the *T(−413)A* (*AA/TA+TT*) polymorphism ($P = 0.0058$), age ($P < 0.0001$), and body mass index ($P < 0.0001$) affected the occurrence of hypertension in women. The odds ratio of the *AA* genotype for hypertension in women was 1.59 ($P = 0.0058$; 95% confidence interval 1.14 to 2.20). The lack of a difference in blood pressure among the three genotypes in women may be attributable to the use of antihypertensive drugs. This association was not observed in men. No significant difference was observed in the frequency of the *G(−1135)A* genotype between normotensive and hypertensive individuals.

Functional significance of T(−413)A polymorphism

We next examined the functional significance of *T(−413)A* polymorphism *in vitro* using BAECs. As shown in Figure 2, the promoter activity of the *A(−413)*-$(GT)_{30}$ allele was significantly greater than that of the *T(−413)*-$(GT)_{23}$ allele *in vitro* ($P < 0.01$). Because the basal activity in this promoter region was low [12], we used a vector that contains an SV40 enhancer sequence. The same results were obtained without an enhancer sequence, although the activity was low.

Discussion

In the present study, we found previously unidentified sequence variations in the promoter region of HO-1. We then examined the relationship between these polymorphisms and the occurrence of hypertension. The frequency of hypertensive individuals and the use of antihypertensive drugs were significantly greater in the *AA* genotype of *T(−413)A* polymorphism of the *HMOX-1* gene than in other genotypes among women. However, this association was not observed in men.

Recently, *HMOX-1* gene promoter microsatellite polymorphism has been reported to be associated with emphysema and restenosis after percutaneous transluminal angioplasty [13,14]. In these reports, the number of $(GT)_n$ repeats was divided into three classes. However, no rational explanation was given for this classification. As *A(−413)*-$(GT)_{30}$ and *T(−413)*-$(GT)_{23}$ are the two major alleles, and our promoter assay showed that the promoter activity of the *A(−413)*-$(GT)_{30}$ allele was significantly greater than that of the *T(−413)*-$(GT)_{23}$ allele, determination of the *T(−413)A* genotype should be sufficient to decide whether there is any functional alteration in the *HMOX-1* gene.

Ever since the haeme oxygenase enzyme was isolated in 1968, research in the field has largely focused on the role of this enzyme in haeme metabolism. However, in recent years, as a result of increased awareness of the role of haeme oxygenase in a variety of biological processes, there has been growing interest in its role in maintaining cellular homeostasis. Carbon monoxide, which is one of the products of haeme oxygenase, participates in the control of cardiovascular functions, including the regulation of blood pressure. Carbon monoxide has been shown to induce the relaxation of vascular smooth muscle cells (VSMCs) by stimulating soluble guanylyl cyclase [15]. The activation of soluble guanylyl cyclase converts GTP to cGMP and intracellular cGMP regulates biological functions by activating cGMP-dependent protein kinases [16]. Thus the synchronized activities of the haeme oxygenase–carbon monoxide–soluble guanylyl cyclase–cGMP system may constitute an important metabolic pathway in the modulation of blood pressure.

A previous in-vitro investigation showed that nitric oxide and carbon monoxide activate guanylate cyclase by distinct mechanisms, carbon monoxide being far less potent than nitric oxide [7]. Because of the difference in cyclase-activating properties between these gases, carbon monoxide endogenously generated from VSMCs may modulate cyclase activity by competing with nitric oxide released from endothelial cells [6]. It has also

Table 4 Characteristics of men and women classified by haeme oxygenase-1 genotype

Characteristic	Haeme oxygenase-1 genotype			P
	AA	*AT*	*TT*	
Men ($n = 962$)	($n = 194$)	($n = 463$)	($n = 305$)	
Age (years)	61.4 ± 0.88	61.2 ± 0.57	60.7 ± 0.70	0.78
Body mass index (kg/m2)	22.7 ± 0.20	23.3 ± 0.13	23.2 ± 0.16	0.12
Waist-to-hip circumference ratio	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.50
Alcohol consumption (ml/day)	25.2 ± 3.8	25.9 ± 1.2	25.8 ± 1.5	0.94
HbA_{1c} (mg/dl)	5.4 ± 0.06	5.4 ± 0.04	5.4 ± 0.04	0.94
HDL cholesterol (mg/dl)	64.2 ± 1.0	63.8 ± 0.7	62.5 ± 0.85	0.37
Total cholesterol (mg/dl)	201 ± 2.3	205 ± 1.5	205 ± 1.8	0.22
Triglycerides (mg/dl)	142 ± 8.6	148 ± 5.6	144 ± 6.8	0.82
Uric acid (mg/dl)	5.8 ± 0.09	5.9 ± 0.06	5.9 ± 0.07	0.66
Proteinuria (%)	14.5	15.2	17.2	0.66
Current smoking (%)	38.7	38.0	37.2	0.94
Ischaemic heart disease (%)	1.6	1.9	1.9	0.88
Cerebrovascular accident (%)	3.1	3.7	2.3	0.55
Use of antihypertensive drugs (%)	16.0	18.1	19.3	0.63
SBP (mmHg)	128.2 ± 1.4	129.2 ± 0.89	129.0 ± 1.1	0.82
DBP (mmHg)	81.0 ± 0.78	81.3 ± 0.50	80.7 ± 0.62	0.72
Pulse pressure (mmHg)	47.2 ± 1.0	47.8 ± 0.67	48.4 ± 0.83	0.70
Heart rate (beats/min)	67.3 ± 0.59	67.3 ± 0.38	67.2 ± 0.47	0.98
Age- and BMI-adjusted SBP (mmHg)	127.7 ± 1.2	129.2 ± 0.80	129.3 ± 1.0	0.89
Age- and BMI-adjusted DBP (mmHg)	81.3 ± 0.74	81.4 ± 0.48	80.3 ± 0.59	0.71
Hypertension (%)†	37.6	41.7	40.7	0.62
Women ($n = 1036$)	($n = 231$)	($n = 439$)	($n = 326$)	
Age (years)	60.0 ± 0.77	59.1 ± 0.54	58.4 ± 0.65	0.27
Body mass index (kg/m2)	22.3 ± 0.21	22.3 ± 0.14	22.5 ± 0.17	0.72
Waist-to-hip circumference ratio	0.89 ± 0.01	0.89 ± 0.01	0.90 ± 0.01	0.82
Alcohol consumption (ml/day)	5.9 ± 0.76	5.0 ± 0.5	5.4 ± 0.6	0.57
HbA_{1c} (mg/dl)	5.2 ± 0.04	5.3 ± 0.03	5.2 ± 0.03	0.29
HDL cholesterol (mg/dl)	64.2 ± 1.0	63.8 ± 0.7	62.5 ± 0.85	0.37
Total cholesterol (mg/dl)	215 ± 2.1	216 ± 1.5	216 ± 1.8	0.90
Triglycerides (mg/dl)	104 ± 5.2	111 ± 3.6	112 ± 4.4	0.47
Uric acid (mg/dl)	4.5 ± 0.07	4.4 ± 0.05	4.5 ± 0.06	0.058
Proteinuria (%)	12.1	8.1	8.6	0.22
Current smoking (%)	4.8	10.0	9.5	0.053
Ischaemic heart disease (%)	0.9	0.6	0.9	0.88
Cerebrovascular accident (%)	1.7	0.63	1.5	0.31
Use of antihypertensive drugs (%)	23.4	17.5	15.0	0.038
SBP (mmHg)	131.5 ± 1.4	128.7 ± 0.96	128.8 ± 1.2	0.22
DBP (mmHg)	79.6 ± 0.72	78.6 ± 0.50	79.0 ± 0.61	0.51
Pulse pressure (mmHg)	51.9 ± 1.0	50.1 ± 0.70	49.7 ± 0.85	0.23
Heart rate (beats/min)	68.5 ± 0.54	68.3 ± 0.37	68.3 ± 0.45	0.93
Age- and BMI-adjusted SBP (mmHg)	132.9 ± 1.4	128.0 ± 0.84	128.5 ± 1.0	0.35
Age- and BMI-adjusted DBP (mmHg)	80.1 ± 0.68	78.2 ± 0.47	79.0 ± 0.57	0.55
Hypertension (%)†	45.5	34.2	35.0	0.0099

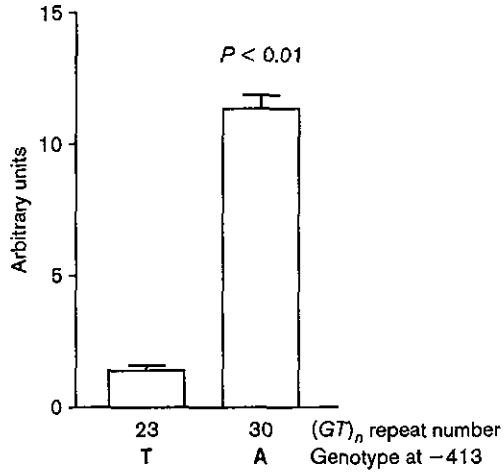
Values are mean ± SE, or %. HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; BMI, body mass index; SBP, DBP, systolic and diastolic blood pressures.
†Percentage of group with hypertension.

been reported that high concentrations of carbon monoxide inhibit NOS activity and nitric oxide generation [8]. These authors also examined the in-vivo effects of an HO-1 inducer, cobalt chloride ($CoCl_2$), and found that carbacol-induced release of nitric oxide from $CoCl_2$-treated rats was significantly reduced compared with that from arterial segments from control rats. In this case, high concentrations of carbon monoxide produced by haeme oxygenase may directly inhibit the generation of nitric oxide and deplete cellular stores of nitric oxide.

Recently, Johnson *et al.* [9] found that expression of vascular HO-1 and production of endogenous carbon monoxide were increased in Dahl S rats with salt-induced hypertension, but not in Dahl salt-resistant rats receiving a high-salt diet. Acute pretreatment with an inhibitor of haeme oxygenase, chromium mesoporphyrin, enhanced vascular responses to N^{ω}-nitro-L-arginine methyl ester (L-NAME) and acetylcholine in both groups, but abolished the differences between high-salt and low-salt arterioles. Therefore, their results suggest that increased concentrations of endogenous carbon monoxide contribute to arteriolar nitric oxide dysfunction *in vivo*.

As men have higher blood pressure than women, it is possible that female hormones may play a part in protecting women from developing higher blood pressures. Oestrogen has been shown to stimulate the production of nitric oxide [17,18]. Huang *et al.* [19] examined the possible effect of oestrogen on flow-induced dilatation of arterioles in four groups of rats: males, ovariectomized females, normal females, and

Fig. 2



Assessment of promoter activity. Transient transfection of haeme oxygenase-1 promoter/luciferase fusion genes was performed in bovine aortic endothelial cells. *Photinus* luciferase activity, which indicated promoter activity of the haeme oxygenase-1 gene, was divided by *Renilla* luciferase activity and expressed as relative luciferase units. The *A(−413)-(GT)*$_{30}$ allele had significantly greater promoter activity than the *T(−413)-(GT)*$_{23}$ allele ($P < 0.01$). $n = 4$ for each experiment.

ovariectomized females with oestrogen replacement. They found a greater flow-induced dilatation of arterioles in rats with high concentrations of oestrogen that was completely eliminated by 10^{-3} mol/l L-NAME. The effect of nitric oxide produced by oestrogen on blood pressure control was also observed in mice deficient in oestrogen β-receptor [20]. Thus the inhibitory effects of carbon monoxide from *HMOX-1* on guanylyl cyclase or eNOS may be more evident in females, as the vascular tone in females may be more dependent on oestrogen-mediated nitric oxide compared with that in males.

We did not observe a difference in the effect of the HO-1 genotype on blood pressure between younger and older groups (data not shown). It has been demonstrated that adaptive structural changes occur in vessels in response to the increased wall stress in hypertension [21]. As the vessel wall thickens and encroaches on the lumen, this adaptive change results in an increased vascular resistance. Therefore, it is possible that an irreversible change may already have occurred during the premenopausal period as a result of a hypertensive status induced by HO-1.

In this study, we found that the *AA* genotype of the *T(−413)A* polymorphism is associated with high blood pressure in women, possibly as a result of the high expression of HO-1. This polymorphism of *HMOX-1* may also be useful for identifying individuals with nitrate tolerance. Future studies may be required in a different group.

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