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ORIGINAL ARTICLE

ADDITIONAL EFFECT IN REDUCTION OF ALBUMINURIA DUE TO THE COMBINATION THERAPY OF ANGIOTENSIN-CONVERTING-ENZYME INHIBITOR AND ANGIOTENSIN Ⅱ TYPE 1 RECEPTOR BLOCKER IN TYPE 2 DIABETIC PATIENTS WITH THE ANGIOTENSINOGEN 235T ALLELE

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Abstract We evaluated the combination therapy of angiotensin-converting-enzyme inhibitor (ACE-I) and angiotensin II type 1 receptor blocker (ARB) offered an additional effect in reduction of albuminuria in type 2 diabetic patients with angiotensinogen (AGT) M235T polymorphism. The study subjects were type 2 diabetic patients with nephropathy who were attending Hirosaki University Hospital. Fifteen patients with 235T allele (TT genotype 9, MT genotype 6) were evaluated who had diabetic nephropathy in stage 2 or 3 and already treated with ACE-I. Each patient administrated ARB (20-40 mg of termisartan) in addition to ACE-I for 16 weeks as the combination therapy. The addition of termisartan induced a significant reduction in systolic blood pressure (BP) of 14.0 mmHg and diastolic BP of 5.4mmHg. The urinary albumin-creatinine ratio (ACR) was also reduced to 48.9%. There was no significant correlation between the reduction rate of ACR and the antihypertensive response of systolic blood pressure (BP) (rs = 0.1277) and of diastolic BP (rs = 0.1420) by the addition of termisartan. These results indicated that the combination of ACE-I and ARB had an additional effect on urinary albumin excretion in type 2 diabetic patients with AGT 235T allele.
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Key words: renin-angiotensin system; angiotensinogen 235T allele; type 2 diabetic nephropathy; angiotensin-converting-enzyme inhibitor; angiotensin II type 1 receptor blocker.

原 著

アンギオテンシノーゲン遺伝子 235T allele を有する 2 型糖尿病腎症患者における ACE 阻害剤と ARB 併用によるアルブミン尿改善効果

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抄録 高血圧の危険因子であるアンギオテンシノーゲン遺伝子 (ACT) 235T allele を有する 2 型糖尿病 (DM) 腎症患者において ACE 阻害剤と ARB 併用によるレニン－アンギオテンシン系の強力な抑制は，アルブミン尿に対して有効なのか検討した．すでに ACE 阻害剤を内服している 2 － 3 期の腎症を合併した，かつ，235T allele を有する DM 患者 15 名 (TT 9 名，MT 6 名) を対象とした．ARB を併用し，併用前と併用 16 週間後に評価した．併用前後で，収縮期血圧は有意に改善し，拡張期血圧は改善傾向を認めた．また，尿中アルブミン／クレアチニン比 (ACR) も有意に改善した。しかし，ACR の改善率と血圧変化率とのあいだには有意な相関関係を認めなかった．以上から，ACE 阻害薬と ARB 併用によるアルブミン尿改善は降圧以外の効果が関与しており，ACT 235T allele を有する DM 患者においても有効と考えられた．
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キーワード：レニン—アンギオテンシン—アルドステロン系；アンギオテンシノーゲン遺伝子 235T allele；アンギオテンシン変換酵素阻害薬；アンギオテンシンⅡ受容体拮抗薬．

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INTRODUCTION

The renin-angiotensin system (RAS) plays an important role in the onset of hypertension. Many experimental and clinical studies indicated that the activity of RAS was regulated by the genetic variants involved in the renin-angiotensin cascade. A polymorphism in exon 2 of the angiotensinogen (AGT) gene, threonine to methionine substitution at position 235 (M235T), has been associated with higher blood pressure (BP) and higher AGT levels[1]. Pereira et al.[2] indicated a linear relation between the AGT 235T allele dosage and BP and confirmed its role as an independent risk factor for hypertension. Moreover it was shown that TT genotype was associated with a faster progression to end-stage renal disease (ESRD)[3], and the AGT 235T allele was transmitted significantly more frequently to patients with chronic renal failure than expected for no association[4]. These results suggest that the genetic variant of AGT gene might not only play an important role in the onset of hypertension but also the development of renal disease.

Recent studies suggested that the combination therapy of angiotensin-converting-enzyme inhibitor (ACE-I) and angiotensin II (Ang II) typel (AT1) receptor blocker (ARB) was more effective than the monotherapy of each drug in the patients with renal disease. Nakao et al.[5] reported that the combined therapy of ACE-I and ARB induced a 75.6% reduction in urinary protein excretion in patients with non-diabetic renal disease. In type 1 diabetic patients with nephropathy, the combination therapy of ACE-I and ARB was reported to induce an additional reduction in urinary albumin excretion (UAE) of 43% as compared with any type of monotherapy[6]. These results suggest that the dual blockade of RAS is superior to monotherapy by ACE-I or ARB for lowering albuminuria in type 2 diabetic patients with nephropathy.

Therefore, we evaluated whether the combination therapy of ACE-I and ARB (termisartan) was more effective for reducing albuminuria in type 2 diabetic patients from the point of the AGT M235T polymorphism.

SUBJECTS and METHODS

After informed consent was obtained, AGT polymorphism was identified in 90 patients with type 2 diabetes in Hirosaki University Hospital. AGT M235T polymorphism genotype was determined by polymelase chain reaction of genomic DNA and restriction fragment length polymorphism in genomic DNA, as previously described[7]. The polymorphism was identified as follows: 60 patients with TT, 24 with MT and 6 with MM. Our study included 15 patients (TT genotype 9, MT genotype 6) who have diabetic nephropathy in stage 2 (urinary albumin-creatinine (Cr) ratio (ACR) 30~300 mg/g・Cr) or stage 3 (ACR>300 mg/g・Cr and serum Cr level<1.5 mg/dl) and ACE-I monotherapy had been prescribed. There was no candidate with MM genotype. We defined ACR as urinary albumin concentration divided by urinary Cr level. They received 16 weeks treatment with the combination therapy of ARB (termisartan 20 or 40 mg once a day) in addition to ACE-I. At the start and the end point of treatment, HbA1c (%), serum albumin (g/dl), serum Cr (mg/dl) and plasma potassium (mEq/L) were measured. Arterial BP (mmHg), urinary albumin (mg/dl) and Cr (mg/dl) were determined by the average of two consecutive measurements.

Assessment of retinopathy was performed by fluorescein angiography. Retinal photography was performed and evaluated by ophtalmologists in Hirosaki University Hospital, according to Fukuda's classification[8] and modified Airlie House Classification[9]. Neuropathy was assessed according to the recommendations made by the American Diabetes Association and American Academy of Neurology at the San Antonio

Conference on Diabetic Neuropathy[10].

Values are expressed as the means±SD. Differences in numerical data among the groups were evaluated by Mann-Whitney's U test and Wilcoxon signed-ranks test. Correlations between the reduction rate of ACR and the antihypertensive response of BP were calculated Spearman's rank correlations.

RESULTS

There was no significant difference in gender, age, body mass index, duration, treatment, the prevalence of retinopathy and neuropathy, and lipid profile between the patients with TT and MT genotypes (Table 1). In the patients with both TT and MT genotypes, BMI, HbA1c, serum Cr and plasma potassium did not differ significantly between the start and the end point of treatment period (Table 2).

In 15 patients with AGT 235T allele (TT+MT genotypes), significant reduction of systolic (S) BP (14.0mmHg, p=0.023) and diastolic (D) BP (5.4mmHg, p=0.044) were observed and

Table 1. Clinical characteristics of type 2 diabetic patients with angiotensinogen TT and MT genotypes

	TT	MT
N	9	6
Male / Female	6 / 3	4 / 2
Age (yrs)	64.0±14.2	65.2± 7.9
BMI (kg/m^2)	24.3± 4.0	23.9± 3.2
Duration (yrs)	19.0± 5.5	14.5± 7.5
Treatment (D/O/I)	6 / 5 / 4	0 / 3 / 3
Retinopathy (%)	88.9	66.7
Neuropathy (%)	66.7	100
T.chol (mg/dl)	190.5±34.0	191.0±34.0
TG (mg/dl)	111.6±49.0	114.8±74.5
HDL-chol (mg/dl)	56.9±15.1	56.0±19.8

Results are expressed as mean±S.D..
Abbreviations: BMI=body mass index, D=dietary therapy alone, O=oral hypoglycemic agent, I=insulin therapy, T.chol=total cholesterol, TG=triglyceride, HDL-chol=high density lipoprotein-cholesterol.
There was no difference in clinical characteristics between TT and MT genotypes.

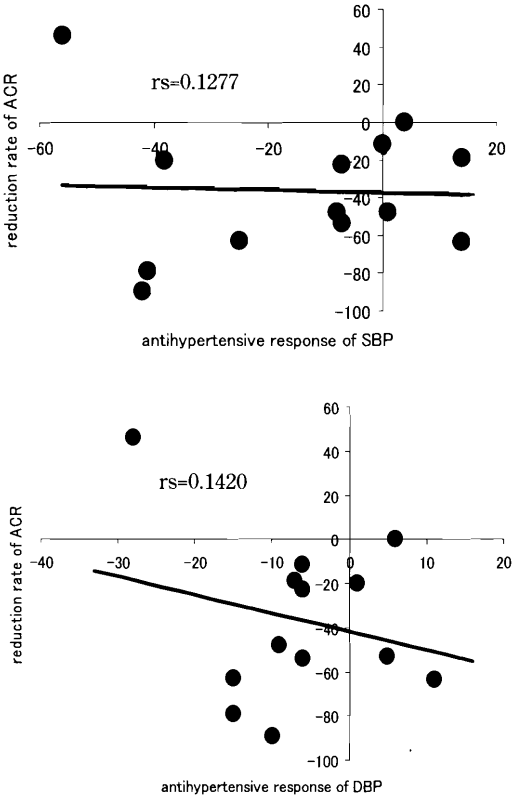

Figure 1. Correlation between the reduction rate of ACR and the antihypertensive response of SBP and of DBP in type 2 diabetic patients with nephropathy.
Abbreviations: ACR=urinary albumin-creatinine ratio (mg/g・Cr), SBP=systolic blood pressure (mmHg), DBP=diastolic blood pressure.

Table 2. Clinical parameters before and after the combination therapy with ACE-I and termisartan in type 2 diabetic patients with angiotensinogen TT and MT genotypes

	TT (n=9)		MT (n=6)		TOTAL (n=15)	
	before	after	before	after	before	after
BMI (kg/m^2)	24.3±4.0	24.2±3.4	23.9±3.2	24.0±3.1	24.1±3.6	24.1±3.3
HbA1c (%)	8.0±1.5	8.0±1.7	8.2±2.0	7.9±2.1	8.1±1.7	8.0±1.8
Serum Cr (mg/dl)	0.91±0.25	0.85±0.19	0.92±0.28	1.03±0.28	0.91±0.25	0.93±0.24
Plasma K (mEq/L)	4.4±0.2	4.5±0.5	4.5±0.3	4.6±0.5	4.4±0.3	4.5±0.4

Results are expressed as mean±S.D..
Abbreviations: BMI=body mass index, Cr=creatinine, K=potassium.
There was no difference in clinical parameters during the treatment between TT and MT genotypes.

Table 3. Arterial blood pressure before and after the combination therapy with ACE-I and termisartan in type 2 diabetic patients with angiotensinogen TT and MT genotypes

	TT (n=9)		MT (n=6)		TOTAL (n=15)	
	before	after	before	after	before	after
SBP (mmHg)	146.9±23.2	135.8±20.6	155.7±21.3	137.3±10.9	150.4±22.1	136.4±16.8[a]
DBP (mmHg)	79.0±8.8	76.3±14.3	89.7±15.6	80.2± 9.3	83.3±12.7	77.9±12.3[b]

Results are expressed as mean±S.D.. Significant differences in arterial blood pressure before and after the combination therapy ([a]p=0.023, [b]p=0.044).
Abbreviations: SBP=systolic blood pressure, DBP=diastolic blood pressure.
Systolic and diastolic blood pressure decreased significantly in TT and MT genotypes.

Table 4. Urinary albumin-creatinine ratio before and after the combination therapy with ACE-I and termisartan in type 2 diabetic patients with angiotensinogen TT and MT genotypes

	TT (n=9)		MT (n=6)		TOTAL (n=15)	
	before	after	before	after	before	after
ACR (mg/g・Cr)	393.2±396.4	192.4±163.6	216.6±219.9	123.4±111.6	322.6±339.4	164.8±144.8[a]

Results are expressed as mean±S.D.. Significant differences in ACR before and after the combination therapy ([a]p=0.015).
Abbreviations: ACR=urinary albumin-creatinine ratio.
ACR decreased significantly in TT and MT genotypes.

ACR was also significantly reduced to 48.9% (p=0.015) by addition of termisartan to ACE-I for 16 weeks. However, there was no significant correlation between the reduction rate of ACR and antihypertensive response of SBP (rs=0.1277) or DBP (rs=0.1420) (Figure 1).

When those evaluations were performed in the patients with TT (n=9) and MT (n=6) genotype respectively, the effects of termisartan did not show any significant reduction in SBP, DBP (Table 3) and ACR in each group (Table 4).

DISCUSSION

ACE-I decreases both Ang II formation and the degradation of bradykinin which causes powerful vasodilation, which is not observed with an ARB[11]. However, it was demonstrated that an insufficient response of ACE-I caused incomplete blockade of Ang II generation by ACE-independent pathways such as chymase[12]. In addition, chronic administration of an ACE-I in human was demonstrated to upregulate vascular expression of AT1 receptor[13], and the activation of the receptor was effectively suppressed by an ARB. Therefore, the combination therapy of ACE-I and ARB is considered to have more beneficial effect for renoprotection.

Several reports showed a better outcome for the renoprotective effect of the combination therapy than the monotherapy on diabetic nephropathy[6,14-16]. In type 1 diabetic patients with nephropathy, the combination therapy of ACE-I and ARB was reported to induce an additional reduction in UAE of 43% as compared with any type of monotherapy[6]. As for the renoprotective effects of the combination therapy in type 2 diabetic patients with nephropathy, Rossing et al.[15] evaluated the renoprotective effects in albuminuria of dual blockade of the RAS by adding an ARB to treatment with maximal recommended doses of an ACE-I. In comparison with maximal recommended doses of ACE-I in type 2 diabetic patients with nephropathy, dual blockade of the RAS demonstrated superior renoprotection independently with systemic BP changes. In addition, the combination of half doses of ACE-I and ARB was demonstrated to be effective in reducing albuminuria compared with either full dose alone, despite no significant alteration in BP[16]. In this study, there was no significant

correlation between the reduction rate of ACR and the antihypertensive response of SBP and of DBP by the addition of termisartan. This finding suggested that the local effect of combination therapy of ACE-I and ARB on glomeruli was more profound than its systemic effect on vascular tonus.

Approximately 20% to 60% of the population variability in BP is genetically determined[17]. Jeunemaitre et al.[18] initialy reported genetic linkage to hypertension and suggested that the M235T polymorphism in AGT gene in the homozygous TT state was associated with approximate 20 % increase in plasma AGT. Odds ratio for hypertension in TT is 1.95 compared with the MM wild type. A meta-analysis[17] revealed that AGT M235T polymorphism genotype was associated with the risk of hypertension in both white and Asian subjects. Moreover it was shown that the AGT-TT genotype was associated with a faster progression to ESRD[3], and the AGT 235T allele was transmitted significantly more frequently in the patients with chronic renal failure than expected for no association[4]. In the present study, we evaluated the effects of combination therapy of ACE-I and ARB on reduction of albuminuria in the patients with AGT 235T allele. The observed effects were not different in the patients with TT and with MT genotypes. We could not evaluate the reduction of albuminuria in the patients with MM genotype. However, it was demonstrated that the combination therapy was effective in reducing albuminuria in patients with type 2 diabetes and was independent with AGT 235T allele dosage.

In conclusion, the combination therapy of ACE-I and ARB demonstrated an additional beneficial effect on albuminuria in type 2 diabetic patients with AGT 235 T allele. As there was no significant correlation between the reduction rate of ACR and the antihypertensive response, other factors might induced a reduction of ACR by the addition of termisartan.

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