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[Original Article]

SUPPRESSION OF ADVANCED GLYCATION AND LIPOXIDATION END PRODUCTS BY ANGIOTENSIN II TYPE-1 RECEPTOR BLOCKER CANDESARTAN IN TYPE 2 DIABETIC PATIENTS WITH ESSENTIAL HYPERTENSION

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Abstract : Objective : This study investigated whether the angiotensin II type-1 receptor blocker (ARB) candesartan affects markers of oxidative stress in type 2 diabetes mellitus (DM) patients with essential hypertension (EH).

Methods : Urinary excretion of pyrraline (PR), pentosidine (PT), acrolein (AC), and 8-hydroxy-2′-deoxyguanosine (8-OH-dG) and microalbuminuria were assessed in patients with DM complicated by EH who were treated with candesartan 4 mg/day for 3 months.

Results : In a total of 25 patients urinary excretion of PR (nmol/g・cr), PT (pmol/g・cr), and 8-OH-dG (ng/mg・cr) was significantly (all $P < 0.05$) decreased from (mean ± SEM) 11.9 ± 1.9, 30.6 ± 2.4, and 7.9 ± 0.6, respectively, at baseline to 8.4 ± 1.4, 27.1 ± 2.0, and 6.9 ± 0.6, respectively, at 3 months. Meanwhile, excretion of AC was unaltered from 209.6 ± 40.0 to 189 ± 24.8 nmol/mgcr (P = NS). Urinary albumin excretion was significantly ($P < 0.05$) reduced from 27.7 ± 4.6 to 14.1 ± 1.1 mg/g・cr. There were weak but statistically significant positive correlations between the change of urinary 8-OH-dG excretion and that of albumin ($r = 0.414$; $P < 0.05$) and change of hemoglobin (Hb) A_{1c} ($r = 0.45$; $P < 0.05$).

Conclusion : Candesartan exerts protective effect(s) on the cardiovascular system by suppression of oxidative stress — mainly through inhibiting production of advanced glycation end products (AGEs) rather than of advanced lipoxidation end products (ALEs) — in type 2 DM patients with EH.

Key words : type 2 diabetes mellitus (DM), essential hypertension (EH), candesartan, advanced glycation end products (AGEs), advanced lipoxidation endproducts (ALEs), microalbuminuria, oxidative stress

INTRODUCTION

Hyperglycemia plays a major role in the pathogenesis of diabetic complications through nonenzymatic glycation and oxidation of proteins and lipids, resulting in accumulation of irreversibly formed advanced glycation end products (AGEs)[1-4] and advanced lipoxidation end products (ALEs). Especially, the association of AGEs with nephropathy is highly convincing since AGE accumulation was observed in kidney-injured diabetic patients[5]. A morbidly increased intracellular synthesis of AGEs seems to cause progression of diabetic complications. However, the clinical signifi-

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cance of serum and urinary levels of AGEs and ALEs is not clear. We examined the effects of candesartan, an angiotensin II type-1 receptor blocker (ARB), on biomarkers of AGEs and ALEs in patients with type 2 diabetes mellitus (DM) complicated by essential hypertension (EH).

PATIENTS AND METHODS

Objective and Patients

We attempted to clarify the effect of 3 months treatment with candesartan 4 mg/day and no other treatment on oxidative stress markers in patients with physician-diagnosed DM and EH.

This study was approved by Institutional Review Board (IRB) of the Fukushima Medical University and all patients provided written informed consent to participate prior to its inception.

Design and Methods

Using high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) methods[6-10], levels of urinary excretion of AGEs such as pyrraline (PR) and pentosidine (PT) and of the ALE acrolein (AC) were measured in patients with DM and EH before and after treatment with candesartan 4 mg/day for 3 months. Urinary 8-hydroxy-2′-deoxyguanosine (8-OH-dG) level was also determined as a common final biomarker of AGEs and ALEs. Urine specimens were collected in the morning before a meal.

Statistical Analysis

Date before and after candesartan treatment were compared using paired t-tests.

Correlations between changes in various variables HbA1c value were assessed using Spearman's correlation coefficients calculation.

RESULTS

In all, 25 patients with concurrent DM and EH (16 men, 9 women ; ages 33-69 years old, mean age, 55.1 years) whose average duration of DM and baseline hemoglobin (Hb) A_{1c} measured according to the Japan Diabetes Society (JDS) were 4.3 years and 6.9%, respectively, were enrolled.

HbA_{1c} [JDS](%)×1.02+0.25%=National Glycohemoglobin Standardization Program [NGSP](%)

At 3 months, both systolic and diastolic blood pressures were significantly decreased from (mean

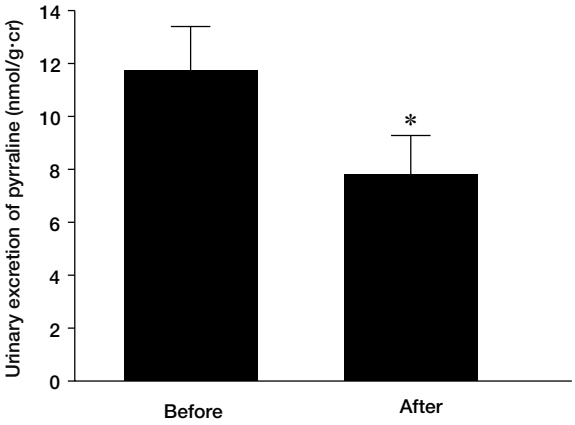

Fig. 1. Urinary excretion of pyrraline (PR) (mean ± SEM) before and after treatment with candesartan 4 mg/day for 3 months in 25 patients with essential hypertension associated with type 2 diabetes. *$P < 0.05$.

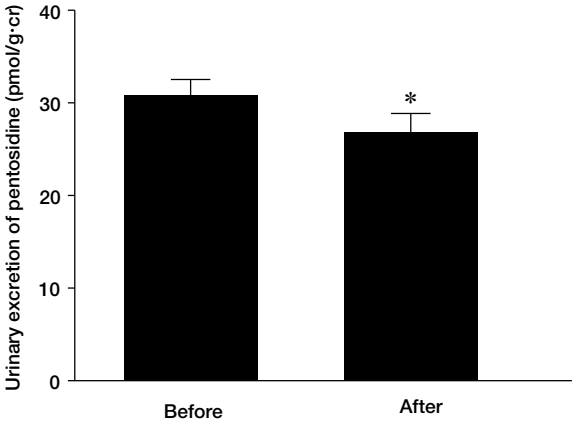

Fig. 2. Urinary excretion of pentosidine (mean ± SEM) before and after treatment with candesartan 4 mg/day for 3 months in 25 patients with essential hypertension associated with type 2 diabetes. *$P < 0.05$.

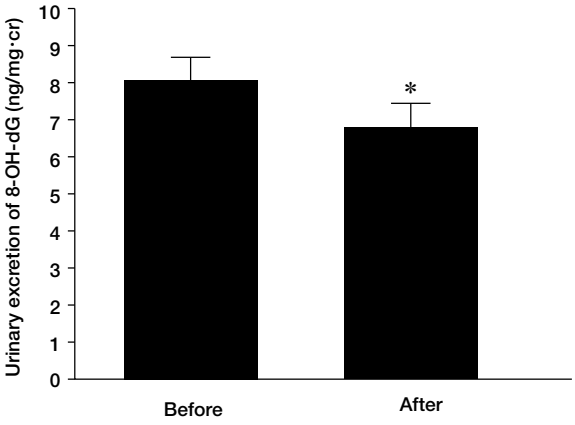

Fig. 3. Urinary excretion of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) (mean ± SEM) before and after treatment with candesartan 4 mg/day for 3 months in 25 patients with essential hypertension associated with type 2 diabetes. *$P < 0.05$.

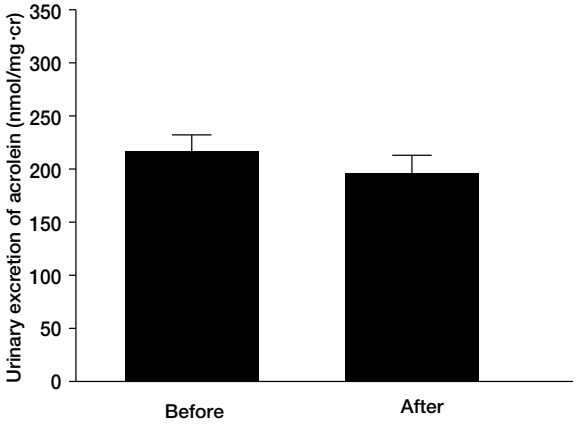

Fig. 4. Urinary excretion of acrolein (AC) (mean ± SEM) before and after treatment with candesartan 4 mg/day for 3 months in 25 patients with essential hypertension associated with type 2 diabetes. *$P < 0.05$.

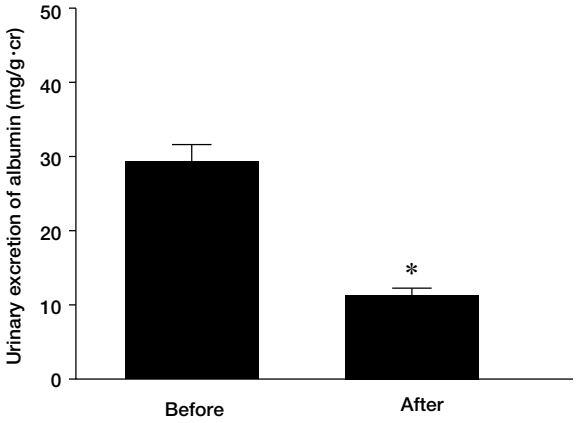

Fig. 5. Urinary excretion of albumin (mean ± SEM) before and after treatment with candesartan 4 mg/day for 3 months in 25 patients with essential hypertension associated with type 2 diabetes. *$P < 0.05$.

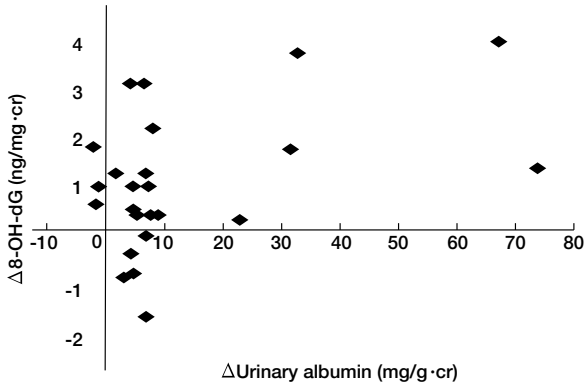

Fig. 6. Correlation between change of urinary 8-hydroxy-2′-deoxyguanosine (8-OH-dG) excretion and that of albumin in 25 patients with essential hypertension associated with type 2 diabetes treated with candesartan 4 mg/day for 3 months ($r = 0.414, P < 0.05$).

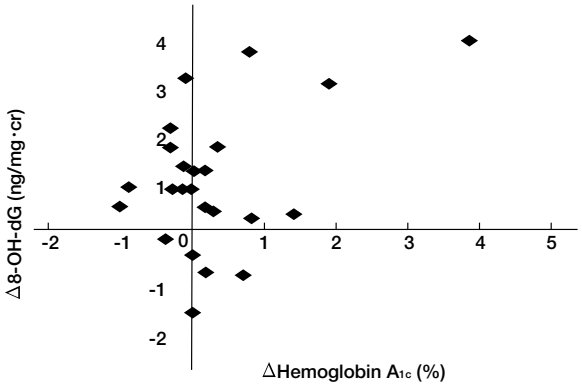

Fig. 7. Correlation between change of urinary 8-hydroxy-2′-deoxyguanosine (8-OH-dG) excretion and levels of hemoglobin (Hb) A_{1c} in 25 patients with essential hypertension associated with type 2 diabetes treated with candesartan 4 mg/day for 3 months ($r = 0.45, P < 0.05$).

± SD) 145.2 ± 7.0 to 128.3 ± 8.6 mmHg and from 92.6 ± 2.3 to 75.8 ±7.1 mmHg ($P<0.01$, respectively).

Urinary excretion of PR (nmol/g·cr), PT (pmol/g·cr), and 8-OH-dG (ng/mg·cr) were significantly (all $P < 0.05$) decreased from (mean ± SEM) 11.9 ± 1.9, 30.6 ± 2.4, and 7.9 ± 0.6, respectively, at baseline to 8.4 ± 1.4, 27.1 ± 2.0, and 6.9 ± 0.6, respectively, at study end (Fig. 1-3). Meanwhile, AC was unaltered from 209.6 ± 40.0 to 189 ± 24.8 nmol/mg·cr (P = NS) (Fig. 4). Urinary albumin excretion was significantly ($P < 0.05$) reduced from 27.7 ± 4.6 to 14.1 ± 1.1 mg/g·cr (Fig. 5).

There were weak but statistically significant positive correlations between the change of urinary 8-OH-dG excretion and that of albumin ($r = 0.414$; $P < 0.05$) (Fig. 6) and of HbA_{1c} ($r = 0.45$; $P < 0.05$) (Fig. 7).

We usually checked the marker of diabetic control of HbA_{1c} once a month, therefore, we assessed in the 3- month for sufficient for evaluate this study.

DISCUSSION

DM is a major risk factor for cardiovascular morbidity and mortality[11,12] and the risk is considerably enhanced by the coexistence of hypertension. A common complication of DM is nephropathy, which is manifested initially by microalbuminuria then by clinical proteinuria, and leads progressively to chronic renal failure and end-stage renal disease (ESRD). Microalbuminuria is therefore considered an early indicator of renal endothelial dysfunction and is an independent predictor of cardiovascular risk.

During recent years, a number of studies have shown that tight blood pressure control is required in diabetic patients to provide them maximal protection against cardiovascular events and deterioration of renal function[13-16].

Of note, recent evidence indicates that blockade of the renin-angiotensin-aldosterone system (RAAS) by ARB exerts marked renoprotective effects in patients with DM and EH, both at early and late stages of renal disease[17]. Microalbuminuria in patients with DM is additionally an important marker for pronounced peripheral vasculopathy as well as for nephropathy. Although the best possible glycemic control is important in preventing and ameliorating the course of microalbuminuria, another major treatment strategy is antihypertensive treatment including inhibition of RAAS[18-20]. Numerous studies have shown that not only microalbuminuria but also renal and cardiovascular complications can be adequately controlled by their early detection and treatment. Therefore screening for microalbuminuria should be a strategy in all DM management programs followed by effective intervention[21].

Maillard or browning reactions between reducing sugars and protein lead to formation of AGEs, and these are thought to contribute to the pathogenesis of diabetic complications. ALEs derived from polyunsaturated fatty acids are increased in DM and hyperlipidemia, and this may contribute to development of long-term renal and vascular pathology[22, 23]. 8-OH-dG is a biomarker of oxidative DNA damage. Both 8-OH-dG and PT levels increase similarly in hypercholesterolemia and hypertension[24] and immunohistological detection of AGEs in diabetic tissues using monoclonal antibody to pyrraline has been performed[25].

Increased urinary levels of PT adduct do not seem a consequence of the glycation levels in DM patients, since glycation does not play a major role in production of PT adduct. Instead, increased levels of oxidative conditions, which can be expected in DM, are thought responsible for the observed increase. Regardless of the underlying mechanism, freeform urinary PT may be a useful marker for the assessment of DM and diabetic complications[26]. Moreover, protein-bound AC adduct has been proposed as a marker for oxidative stress[22]. The mechanism of glucotoxicity involves several transcription factors and is, at least in part, mediated by generation of chronic oxidative stress. Lipotoxicity is likely mediated by accumulation of cytosolic signals derived from the fatty acid esterification pathway. Based on recent reports, chronic hyperglycemia, independent of hyperlipidemia, appears toxic for pancreatic β-cell function, whereas chronic hyperlipidemia is deleterious only in the context of concomitant hyperglycemia[27]. Increased AGE levels in DM appear causative of diabetic nephropathy[28-30], while impaired renal function in turn seems to cause increases of AGE levels[31, 32].

Although awareness of the importance of successfully normalizing blood pressure level and tight glycemic control has led to improved survival of diabetic patients, mortality remains unacceptably high in those with diabetic nephropathy. New treatment strategies are under investigation, including inhibitors of AGE formation, protein kinase C inhibitors, antioxidants, glycosaminoglycans, PPAR-γ antagonist, and COX-2 inhibitors[33].

Enhanced oxidative stress is involved in the progression of renal disease. Since angiotensin-converting enzyme inhibitors (ACEIs) have been shown to improve antioxidative defense, the short-term effects of the ACEI ramipril on parameters of oxidative stress such as AGEs, advanced oxidation protein products (AOPPs), homocysteine (Hcy), and lipid peroxidation products were examined in a previous report[34-37]. Administration of ramipril resulted in reductions of blood pressure and proteinuria, while creatinine clearance remained the same. A decline in AOPPs and malondialhydrate was noted, suggesting that ramipril administration reduces AGEs probably by decreasing oxidative stress[38].

Although there is some evidence in favor of ACEI exerting beneficial effects against markers of oxidative stress, some experimental reports concerning ARB have been published[39-41]. In most of the reports, indicate ARB improves of endothelial function during hypercholesterolemia. However, these reports show decrease cholesterol, LDL and HDL level, on the contrary, in these results show increase triglyceride level after ARB treatment. Therefore, these reports are not sufficient for oxidative stress on lipoxidation end products.

Statins have been widely used to treat dyslipidemia, and evidence supports in lowering serum lipids levels and regression of arteriosclerosis by decreasing oxidative stress via their ancillary effects[42, 43]. Althogh, there are some kind types of dyslipidemia with diabetes, therefore, the current protocol is designed to provide an ethically justifiable test of combined statin plus fibrate treatment consistent with the highest level of safety and lipid treatment standards of care on Evolution of the

Lipid Trial Protocol of the action to Control Cardiovascular Risk in Diabetes (ACCORD) trial[44] and effects of combination therapy in Type 2 Diabetes Mellitus are published that the combination therapy did not reduce the rate of fatal cardiovascular events, nonfatal myocardial infarction, or nonfatal stroke, as compared with statin alone. These results do not support the routine use of combination therapy with fibrate and statin to reduce cardiovascular risk in the majority of high-risk patient with type 2 diabetes[45].

Recently, effects of fibrate on lipid and glucose metabolism in dyslipidemic patints with diabetes mellitus clinical trial study were reported[46, 47].

Especially, the Japan BEzafibrate cliNical EFectIveness and Tolerability (J-BENEFIT) study[47] shows the control of both triglyceride and blood glucose level is important consideration the treatment of dyslipidemia complicated by diabetes and may be improved modality for amelioration of disease course and improvement of outcome in these patients.

Both AGEs and ALEs interaction experimental report concerning ARB have been published no report to date. In this study, the effects of candesartan on levels of PT, PR, 8-OH-dG, and AC were assessed, and a significant correlation between urinary albumin excretion and urinary levels of PT, PR, and 8-OH-dG was identified. Furthermore, there was a weak but statistically significant positive correlation between change of urinary 8-OH-dG excretion and that of albumin and change of HbA_{1c}. On the other hand, urinary AC excretion was not correlated with that of albumin. These results strongly suggest that ARB exerts protective effects on the cardiovascular system by suppression of oxidative stress mainly through inhibition of AGE production rather than that of ALEs in DM patients with EH.

In light of these reports and in our results, we strongly suggest that combination therapy with both ARB and statin and/or fibrate might be particularly effective at protecting the cardiovascular system by exerting potent suppression of oxidative stress.

CONCLUSION

Our results strongly suggest that the ARB candesartan exerts protective effects on the cardiovascular system by suppression of oxidative stress mainly through inhibition of AGEs production rather than that of ALEs in type 2 DM patients complicated by EH.

The results of this study were mainly presented at the 20th Scientific Meeting of the International Society of Hypertension, Sao Paulo, Brazil, 2004.

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