Original Article

Effect of Combination Therapy of Benidipine Hydrochloride and Candesartan Cilexetil on Serum Lipid Metabolism and Blood Pressure in Elderly Hypertensive Patients with Type 2 Diabetes Mellitus

Hidehisa Sasaki[1], Saburo Kanai[1], Tomokazu Oyama[2], Yoh Miyashita[2], Shigeo Yamamura[3], and Kohji Shirai[2]

[1]Departments of Pharmacy, Toho University, Sakura Hospital, School of Medicine, Chiba, Japan.
[2]Internal Medicine, Toho University, Sakura Hospital, School of Medicine, Chiba, Japan.
[3]School of Pharmaceutical Sciences, Toho University, Chiba, Japan.

The effects of combination therapy of angiotensin II receptor blockers (ARBs) and a calcium antagonist, benidipine hydrochloride, on glucose and lipid metabolism and pulse pressure were studied in elderly hypertensive patients with type 2 diabetes mellitus. Twenty-five hypertensive diabetic patients aged 65 years or older, who had been receiving candesartan cilexetil, were administered benidipine hydrochloride (4 mg/day) and followed for 4 months. After 4 months, systolic and diastolic blood pressure decreased significantly from 154/91 mmHg to 139/78 mmHg ($p<0.01$ versus before benidipine hydrochloride administration). Body mass index (BMI) and glycosylated hemoglobin (HbA1c) were apparently reduced but the changes were not statistically significant. The serum lipid profile showed no significant changes in serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Serum lipoprotein lipase mass levels (preheparin LPL mass) increased significantly from 51 to 59 ng/dL ($p<0.01$ versus before benidipine hydrochloride administration), and the LDL/HDL motility ratio calculated from PAG disc electrophoresis decreased significantly ($p<0.05$ versus before benidipine hydrochloride administration). When patients were divided into a systolic hypertension group (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure < 90 mmHg) and non-systolic hypertension group (others), preheparin LPL mass was significantly lower in the systolic hypertension group, and the decrease in pulse pressure and increase in preheparin LPL mass were significantly greater in the systolic hypertension group. Stepwise regression analysis showed that low preheparin LPL mass at baseline was associated with a decrease in pulse pressure. Add-on benidipine hydrochloride therapy in elderly hypertensive patients with type 2 diabetes mellitus significantly decreases the LDL/HDL motility ratio and pulse pressure, and significantly increases preheparin LPL mass, in addition to improving blood pressure control. These findings suggest that combination therapy with benidipine hydrochloride and candesartan cilexetil may contribute to the suppression of arteriosclerosis and may be useful for elderly hypertensive patients with diabetes mellitus.

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Key words; Benidipine hydrochloride, Preheparin serum lipoprotein lipase mass, Hypertensive diabetic patient, Elderly

Introduction

Blood pressure increases with age, and approximately 60% of elderly people have hypertension. The incidence of elderly patients with hypertension accounts for approximately half of all patients with hypertension in Japan[1)], and further increase is expected in the future. Hypertension in elderly people is char-

Address for correspondence: Yoh Miyashita, Department of Internal Medicine, Sakura Hospital, School of Medicine, Toho University, 564-1 Shimoshizu, Sakura-shi, Chiba 285-8741, Japan.
E-mail: mumon@sf6.so-net.ne.jp
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Table 1. Characteristics of patients before benidipine hydrochloride administration.

Background factor	All subjects	All subjects	
		systolic type	non-systolic type
n (M/F)	25 (10/15)	12 (6/6)	13 (4/9)
Age (y.o)	72.2 ± 1.2	73.0 ± 1.9	71.4 ± 1.6
BMI (kg/m^2)	23.3 ± 0.6	22.9 ± 0.8	23.8 ± 1.0
BP: systolic (mmHg)	154 ± 2.3	158 ± 2.1	156 ± 3.7
BP: diastolic (mmHg)	91 ± 2.2	81 ± 1.9**	98 ± 2.4
Pulse pressure (mmHg)	63 ± 2.6	77 ± 2.8**	58 ± 3.6
HbA1c (%)	6.7 ± 0.1	6.7 ± 0.2	6.8 ± 0.2
TC (mg/dL)	215 ± 8.3	210 ± 12	219 ± 12
TG (mg/dL)	174 ± 18	191 ± 29	159 ± 23
HDL-C (mg/dL)	49 ± 2.4	43 ± 3.1*	54 ± 3.0
LDL-C (mg/dL)	118 ± 5.3	116 ± 6.6	119 ± 8.4
Preheparin LPL mass (ng/mL)	50.4 ± 2.8	44.6 ± 4.0*	55.8 ± 3.5
Rm ratio	0.35 ± 0.01	0.36 ± 0.01	0.35 ± 0.01

* $p < 0.05$ vs non-systolic type, ** $p < 0.01$ vs non-systolic type
Data are presented as the mean ± SE unless specified otherwise. BMI: body mass index, BP: blood pressure, pulse pressure: SBP minus DBP, TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, LPL: lipoprotein lipase

acterized by a higher prevalence of systolic hypertension, greater pulse pressure, more frequent orthostatic hypotension, and complications of decreased vascular elasticity and arteriosclerosis, which are all associated with aging. In particular, increased pulse pressure is known to increase cardiovascular risks[2-5]. Therefore, in order to control blood pressure in elderly patients, it is important to control pulse pressure appropriately in addition to lowering blood pressure.

In diabetic patients, hypertension is a frequent complication and accelerates cardiovascular diseases. In particular, the preexistence of atherosclerosis is expected in many elderly patients with diabetes mellitus. Therefore, when using antihypertensives in elderly patients with diabetes mellitus, safety and beneficial effects on glucose and lipid metabolism are desired. Recently, angiotensin II receptor blockers (ARBs) have been frequently used as the first-choice antihypertensive because they exhibit favorable effects on glucose and lipid metabolism, and protective effects on various organs in addition to a hypotensive effect[6, 7]. If the hypotensive effect of an ARB is insufficient, Ca antagonists are added in many cases. This study assessed the effect of combination therapy with ARB and benidipine hydrochloride, a Ca antagonist, on glucose and lipid metabolism, and also assessed the usefulness of combination therapy for the treatment of systolic hypertension in elderly hypertensive patients with diabetes mellitus.

Subjects and Methods

Subjects

Twenty-five elderly patients aged 65 years or older (10 males and 16 females) with diabetes mellitus and hypertension, who had been receiving candesartan cilexetil as an ARB, were enrolled in the study. They had systolic blood pressure (SBP) of 140 mmHg or greater when measurement was performed on two different days within 4 weeks before the start of the study. Among them, patients with diastolic blood pressure (DBP) below 90 mmHg were defined as having systolic hypertension. Benidipine hydrochloride was administered at 4 mg/day for 4 months. **Table 1** shows the baseline clinical data of the subjects. For at least 6 months before and during this study, all patients maintained the same diet and exercise therapies, and did not change medications. The subjects had been receiving candesartan cilexetil for at least 12 weeks before the start of the study. This study was approved by the Ethics Committee of Toho University. The purpose, nature and potential risks of this study were explained to all patients and their voluntary written consent for participation in the study and also for release of the study data was obtained before they were enrolled.

Body Weight and Blood Pressure

Body weight was measured using the same scales after the patients had fasted for at least 10 hours. Before the measurement of blood pressure, the patients

sat undisturbed for at least 5 min and blood pressure was measured while they were in the sitting position. Body weight and blood pressure were both measured at a fixed time.

Blood Sampling

Blood samples were collected in the morning after 12 hours of fasting. Serum was separated within 1 hour, and samples were used for measuring glycosylated hemoglobin (HbA1c), serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). HbA1c, including stable and unstable fractions, was measured by high-pressure liquid chromatography using Hi-Auto A1c (Kyoto Daiichi Kagaku, Kyoto, Japan). Stable-type data were used in this study. TC, TG and LDL-C concentrations were measured enzymatically using a kit from Nippon Shoji (Osaka, Japan) and an autoanalyzer (Hitachi 7150 from Hitachi Tokyo, Japan). HDL-C was measured by the selective inhibition method (Daiichi Pure Chemicals, Tokyo)[8].

Preheparin LPL Mass Assay

Serum lipoprotein lipase mass levels (preheparin LPL mass) were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a specific monoclonal antibody against lipoprotein lipase (Daiichi Pure Chemicals, Tokyo), as described by Kobayashi *et al.*[9]. In this assay, a linear response was observed from 5 ng/mL to 400 ng/mL. Within-run coefficient variation was 2.8%. Between-day coefficient variation was 4.3%.

Polyacrylamide Gel (PAG) Disc Electrophoresis of LDL

The size of LDL particles was analyzed by PAG disc electrophoresis using the Lipo Phor™ system (Quantimetrix, CA, USA; Jyohkoh, Tokyo, Japan)[10]. The pattern was recorded with a densitometer (Densitron 20HR; Jyohkoh). In this method, the mobility ratio (Rm ratio) of LDL negatively correlated with the particle size (**Fig. 1**)[11-13].

Statistical Analysis

All data are expressed as the mean ± S.E. StatView ver. 4.51 (Abacus Concepts, Inc.) for Macintosh was used for statistical processing. Comparisons were performed by *t*-test, chi-square test, Bonferroni/Dann method and stepwise regression analysis. A p value less than 0.05 was considered significantly different.

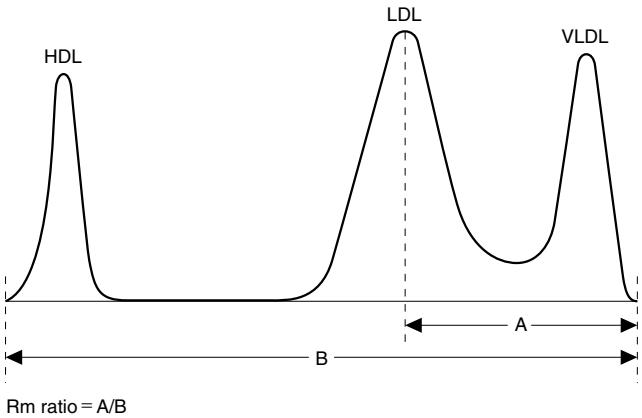

Fig. 1. Definition of relative mobility ratio (Rm ratio) of LDL. LDL relative mobility ratio is calculated from the densitometric patterns of lipoprotein on polyacrylamide gel disc electrophoresis, and is defined as A/B, where A is the distance between VLDL and LDL and B is the distance between VLDL and HDL.)

Results

Changes of BMI During Benidipine Hydrochloride Therapy

After benidipine hydrochloride administration, a gradual decrease in BMI was observed for the first two months; however, the changes were not statistically significant (**Fig. 2A**).

Changes of HbA1c During Benidipine Hydrochloride Therapy

After benidipine hydrochloride administration, a gradual slight decrease in HbA1c was apparent; however, the changes were not statistically significant (**Fig. 2B**).

Changes of Blood Pressure During Benidipine Hydrochloride Therapy

Significant decreases in blood pressure were observed after 1 month of benidipine hydrochloride administration and continued up to 4 months; the mean SBP decreased from 154 mmHg to 139 mmHg and mean DBP from 91 mmHg to 78 mmHg ($p < 0.01$ versus before administration, **Fig. 3**).

Changes of Serum Lipids During Benidipine Hydrochloride Therapy

After 4-month treatment with benidipine hydrochloride, TC decreased by 4 mg/dL, TG decreased by 7 mg/dL, HDL-C increased by 1 mg/dL and LDL-C decreased by 3 mg/dL; however, not all changes were statistically significant (**Fig. 4**). Preheparin LPL mass in-

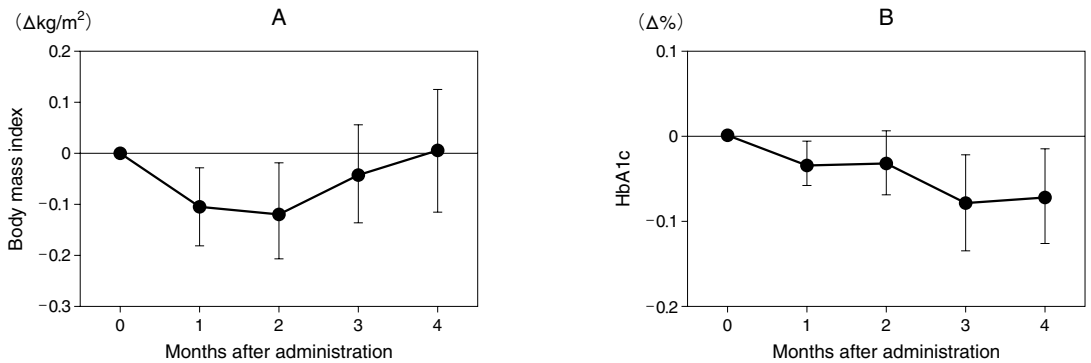

Fig. 2. Changes in BMI (A) and HbA1c (B) during 4 months of benidipine hydrochloride administration.
Data are presented as the mean ± SE.

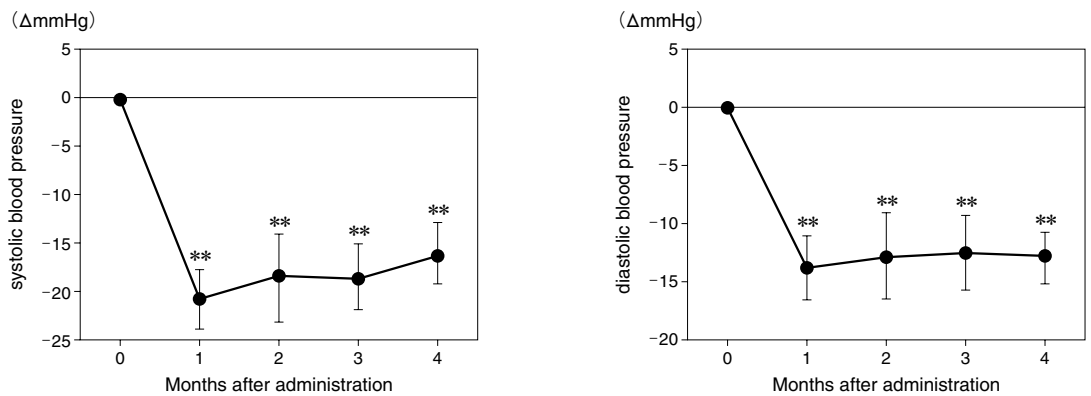

Fig. 3. Changes in systolic blood pressure and diastolic blood pressure during 4 months of benidipine hydrochloride administration.
Data are presented as the mean ± SE. **: $p<0.01$ versus baseline (0 month).

creased significantly from 51 ng/mL to 59 ng/mL ($p<0.01$), and Rm ratio decreased significantly ($p<0.05$) compared to before administration (**Fig. 5**).

Baseline Clinical Characteristics of Patients with Systolic Hypertension and Non-Systolic Hypertension

Table 1 shows the clinical characteristics of patients with systolic hypertension (systolic type) and patients with other types of hypertension (non-systolic type) before the start of treatment with benidipine hydrochloride. SBP did not differ greatly between the two groups, while DBP was significantly lower in the systolic type ($p<0.01$). There was a significant difference in pulse pressure between the two groups at baseline ($p<0.01$). Among the serum lipid parameters, HDL-C and preheparin LPL mass were significantly lower in the systolic type ($p<0.01$ and $p<0.05$, respectively). There were no significant differences between the two groups with respect to the other parameters, but TG was slightly higher in the systolic type.

Changes in Blood Pressure and Preheparin LPL Mass in the Systolic Type and Non-Systolic Type after Benidipine Hydrochloride Administration

The two groups showed a similar time course of change in SBP (**Fig. 6A**). DBP was reduced to a greater extent in the non-systolic type than in the systolic type after benidipine hydrochloride administration (**Fig. 6B**). Pulse pressure decreased significantly relative to the baseline level from 1 month to 4 months of administration in the systolic type, while pulse pressure was only significantly lowered at 1 month in the non-systolic type (**Fig. 6C**). Preheparin LPL mass increased in both groups, but the increase was greater in the systolic type compared to the non-systolic type. At 4 months after benidipine hydrochloride administration, a significant increase compared to the baseline

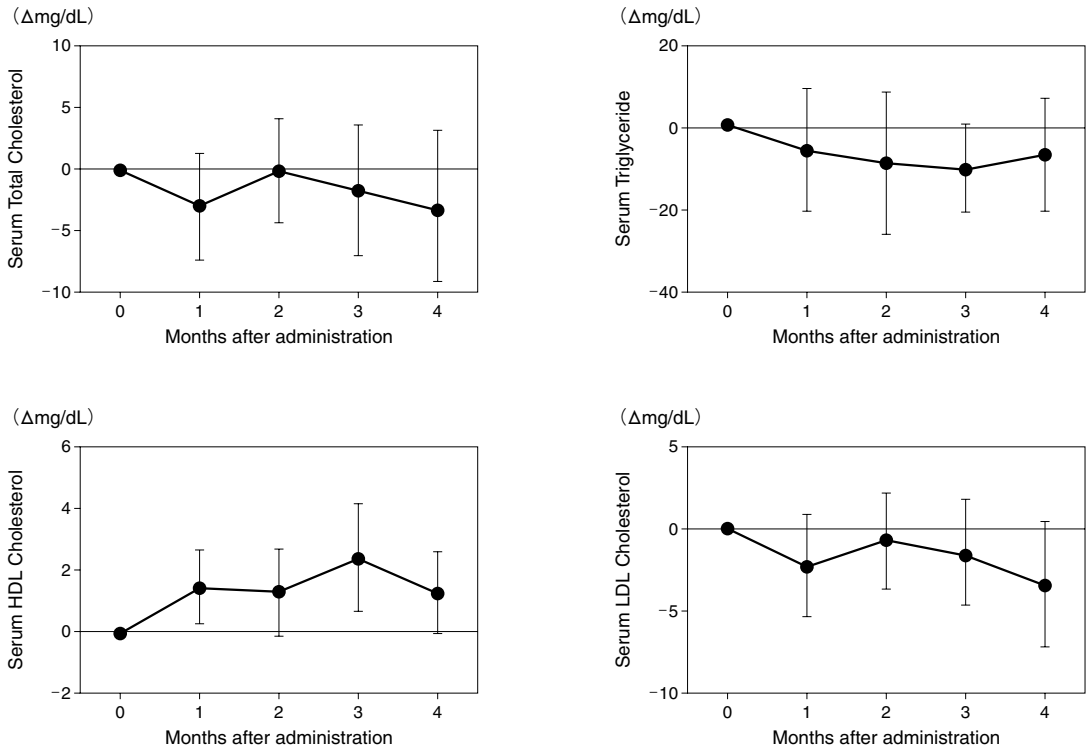

Fig. 4. Changes in total cholesterol, triglycerides, HDL-cholesterol and LDL- cholesterol after 4 months of benidipine hydrochloride administration.

Data are presented as the mean ± SE.

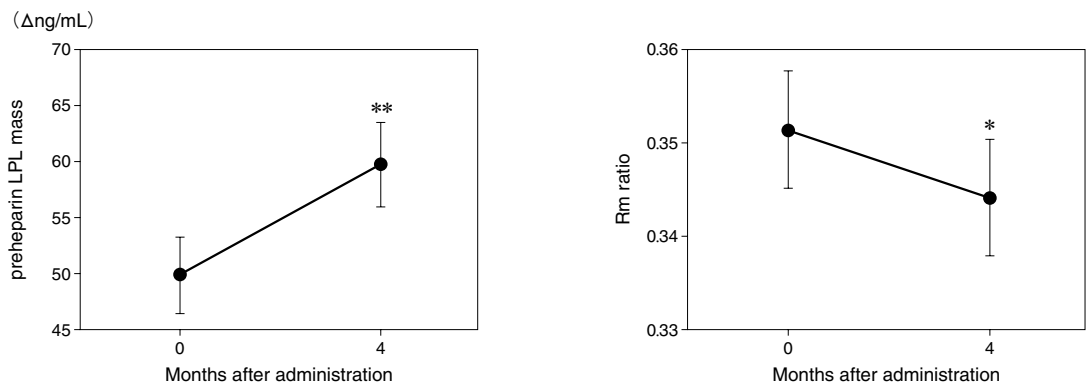

Fig. 5. Changes in preheparin lipoprotein lipase (LPL) mass and LDL relative mobility ratio (Rm ratio) after 4 months of benidipine hydrochloride administration.

Data are presented as the mean ± SE. *: $p<0.05$; **: $p<0.01$.

was observed in the systolic type but not in the non-systolic type (**Fig. 6D**).

Stepwise Regression Analysis of the Association Between Variables and Pulse Pressure

The stepwise regression method was used to select factors significantly associated with changes in pulse pressure. The results of the final model with variables related to changes of pulse pressure are summarized in **Table 2**. BMI, diastolic BP, TG and preheparin LPL mass at the baseline were associated with a decrease in pulse pressure, whereas HbA1c at the baseline was associated with an increase in pulse pressure.

Table 2. Factors influencing the change in pulse pressure. (stepwise multiple regression)

Factors	Estimate	S.E.	*t* value	*p* value (Prob > |t|)
BMI	2.84327	0.433871	6.55	< 0.0001
Diastolic BP	0.58442	0.111004	5.26	< 0.001
HbA1c	−15.9703	2.112747	−7.56	< 0.001
TG	0.063563	0.016062	3.96	0.0008
Preheparin LPL mass	0.213794	0.092637	2.31	0.0324

Dependent variable: Δ pulse pressure
Explanation factor: Age, BMI, systolic BP, diastolic BP, HbA1c, TC, TG, HDL-C, LDL-C, Rm ratio, preheparin LPL mass, Δ preheparin LPL mass

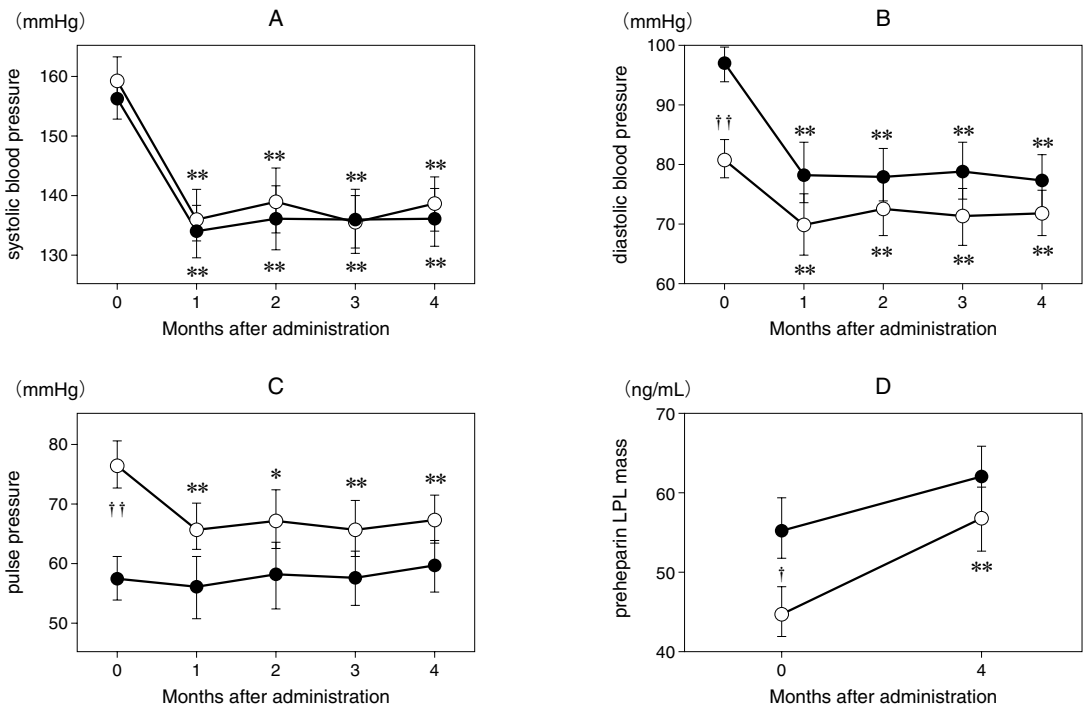

Fig. 6. Comparison of patients with systolic hypertension (open circle) and patients with non-systolic hypertension (closed circle) with respect to changes in systolic blood pressure (A), diastolic blood pressure (B), pulse pressure (C) and preheparin lipoprotein lipase (LPL) mass (D) levels during 4 months of benidipine hydrochloride treatment.

Systolic hypertension is defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure < 90 mmHg (n = 12). Non-systolic hypertension is defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg (n = 13). Data are presented as the mean ± SE. †: $p < 0.05$, ††: $p < 0.01$ versus non-systolic hypertension. *: $p < 0.05$, **: $p < 0.01$ versus baseline (0 month).

Discussion

In this study, 25 elderly patients with hypertension and diabetes mellitus were given benidipine hydrochloride as add-on therapy to candesartan cilexetil. Benidipine hydrochloride add-on therapy improved serum lipid levels, increased preheparin LPL mass, and decreased the LDL Rm ratio obtained from lipoprotein PAG disc electrophoresis. Candesartan cilexetil is known to have beneficial effects on glucose and lipid metabolism[6, 7]. Our study showed that benidipine hydrochloride had an additive effect on improving glucose and lipid metabolism. No change in glucose and lipid metabolism was due to a change in body weight because no significant change in BMI was observed during this study.

In clinical studies, LPL is analyzed using serum obtained after an intravenous injection of heparin (postheparin LPL). Postheparin LPL is considered to reflect the amount of LPL production in the whole body; however, there is a question over whether postheparin LPL accurately reflects the amount of working LPL. Preheparin LPL mass is a completely physiological parameter and is measured without artificial interventions such as heparin injection. Tornvall *et al.*[14] have shown that preheparin LPL mass is LPL protein detached from the endothelial surface into plasma after degradation. Therefore, preheparin LPL mass may reflect the amount of working LPL more accurately than postheparin LPL[15]. In this study, preheparin LPL mass was increased significantly by benidipine hydrochloride administration. This finding suggests that benidipine hydrochloride may increase the amount of working LPL. The mechanisms of the increase of preheparin LPL mass by benidipine hydrochloride might be due to the peripheral vessel dilatation effect of this agent. By the dilatation of peripheral vessels, the catabolism of TG-rich lipoproteins by LPL could be promoted, and as a result, working LPL increased. In this study, a significant decrease in the LDL Rm ratio was found following benidipine hydrochloride treatment, and an apparent decrease in TG and increase in HDL-C were observed, although the differences were not significant. A decrease in the LDL Rm ratio detected by PAG disc electrophoresis indicates an increase in LDL particle size[16, 17]. These improving effects of serum lipid metabolism by benidipine hydrochloride might be explained by an increase of working LPL mass.

We previously demonstrated that high levels of TG, low levels of HDL-C and remnant lipoprotein, which are characteristics of dyslipidemia caused by insulin resistance[8, 16, 18-20], are generally recognized in patients with low levels of preheparin LPL mass[21]. Preheparin LPL mass has also been shown to be low in diabetic patients and to be increased by an insulin-sensitizing agent[22, 23]. Furthermore, preheparin LPL mass has been reported to correlate negatively with the amount of visceral fat and the severity of coronary atherosclerosis[24, 25]. Therefore, preheparin LPL mass may be considered to be a quantitative indicator of insulin resistance throughout the whole body[26, 27], and low levels of preheparin LPL mass could be a high risk for atherosclerosis. Our study showed that preheparin LPL mass levels were significantly lower in patients with systolic hypertension than in patients with non-systolic hypertension before benidipine hydrochloride administration. From our previous reports[21-27], this finding suggests that insulin resistance is more severe in patients with systolic hypertension than in those with non-systolic hypertension before benidipine hydrochloride administration.

Systolic hypertension, which is generally caused by atherosclerosis, is a characteristic of elderly hypertension. Systolic hypertension is characterized by an increase of pulse pressure amplitude. The Framingham study reported that increased pulse pressure amplitude was the most important risk factor for acute coronary syndrome in elderly patients[28-30]. Therefore, successful treatment of systolic hypertension in elderly patients is expected not only to decrease blood pressure but also to decrease pulse pressure amplitude. In this study, benidipine hydrochloride significantly decreased pulse pressure amplitude in patients with systolic hypertension. This finding reflects the beneficial effect of benidipine hydrochloride for elderly systolic hypertension. Stepwise regression analysis showed that a low preheparin LPL mass at baseline was associated with a decrease in pulse pressure. From our previous reports[21-27], low preheparin LPL mass could be consistent with increased insulin resistance. It is known that the sympathetic nerve is activated under the condition of insulin resistance[31-34] and that blood vessels contract by activation of the sympathetic nerve. So, it is expected that the tonus of the sympathetic nerve in patients with systolic hypertension might be higher than that in patients with non-systolic hypertension before benidipine hydrochloride administration, because preheparin LPL mass levels were significantly lower in patients with systolic hypertension than in patients with non-systolic hypertension before benidipine hydrochloride administration. Benidipine hydrochloride treatment might preferentially promote the dilatation of blood vessels in patients with systolic hypertension by improving the tonus of the sympathetic nerve. Furthermore, we speculate that insulin sensitivity may be improved to a greater extent in patients with systolic hypertension than in patients with non-systolic hypertension, because preheparin LPL mass levels increased more markedly in patients with systolic hypertension compared to those with non-systolic hypertension after benidipine hydrochloride administration.

In summary, combination therapy of benidipine hydrochloride with candesartan cilexetil improved glucose and lipid metabolism, and this combination therapy was effective in improving systolic hypertension in elderly hypertensive patients with diabetes mellitus. These results indicate that this combination therapy could be useful for elderly hypertensive patients with diabetes mellitus; however, in this study, the number of subjects was not enough to establish our conclusion as a common theory, and there was a bias such as the

difference in the mean age between the systolic hypertension group and non-systolic hypertension group. A further study of more subjects is thesefore needed.

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