Original Article

Effect of Acarbose, an Alpha-Glucosidase Inhibitor, on Serum Lipoprotein Lipase Mass Levels and Common Carotid Artery Intima-Media Thickness in Type 2 Diabetes Mellitus Treated by Sulfonylurea

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Aim: Previous reports indicate that serum lipoprotein lipase mass levels (LPL mass) and common carotid artery intima-media thickness (CCA-IMT) are independent predictors of atherosclerotic diseases. The aim of this study was to examine the effects of combination therapy of sulfonylurea and acarbose on LPL mass and CCA-IMT.

Methods: Eighty-four patients with type 2 diabetes mellitus, who were treated with only sulfonylureas and showed CCA-IMT of more than 0.9 mm at baseline, were selected and randomly divided into two groups. One group was administered acarbose 300 mg/day for 12 months (acarbose group, $n=41$), and the other group was not administered acarbose (non-acarbose group, $n=43$).

Results: After 12 months, a significant increase in LPL mass and a significant decrease in CCA-IMT were observed in the acarbose group (1.024 to 0.964 mm), but no significant changes were observed in the non-acarbose group. In a subgroup analysis of patients with HbA1c improved by 0.5% or more, the increase of LPL mass and decrease of CCA-IMT was significantly greater in the acarbose group than in the non-acarbose group although the changes in HbA1c were similar in two groups.

Conclusions: We concluded that reducing postprandial hyperglycemia might increase LPL mass levels and might be useful to prevent macroangiopathy in type 2 diabetic patients treated by sulfonylurea.

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Key words; Type 2 diabetes mellitus, CCA-IMT, LPL mass, Postprandial hyperglycemia, Acarbose

Introduction

The advent of high-resolution B-mode ultrasonography has allowed noninvasive observation of atherosclerosis in the carotid arteries. Common carotid artery (CCA) wall thickness is evaluated by measuring its intima-media thickness (IMT). CCA-IMT has been reported to be an independent predictor of coronary heart disease and stroke^{1,2}. Recent reports have shown that postprandial hyperglycemia is associated with in-

creased CCA-IMT^{3,4} and a higher prevalence of atherosclerotic plaques in the CCA^{4,5}.

Acarbose is an alpha-glucosidase inhibitor that specifically reduces postprandial glucose excursion by delaying the release of glucose from disaccharides and complex carbohydrates in the upper part of the small intestine⁶. Recently, acarbose administration has been shown to delay the progression of IMT in subjects with impaired glucose tolerance³.

Lipoprotein lipase (LPL), which catalyzes the hydrolysis of triglycerides in blood, exists on the endothelial surface and is released into the blood by heparin injection. Analysis of LPL function is important to elucidate lipid metabolism; however, there is almost no LPL activity in the serum under physiological conditions, because LPL remains on the surface of endothelial cells and expresses enzyme activity on the vas-

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cular surface. Therefore, in clinical studies, LPL has been analyzed using serum obtained after intravenous injection of heparin (postheparin LPL), since LPL is detached from the endothelial surface and released in to blood after heparin injection. A considerable level of LPL activity can be observed in serum after intravenous injection of heparin. Postheparin LPL is considered to reflect the amount of LPL production in the whole body.

Recently, a sensitive immunoassay system using specific monoclonal antibody against LPL demonstrated the presence of LPL protein in serum without intravenous injection of heparin (LPL mass)^{7, 8)}. Tornvall *et al.* have indicated that LPL mass might be LPL protein detached from the endothelial surface into blood after degradation⁸⁾. We have previously reported that LPL mass was significantly lower in patients with type 2 diabetes mellitus compared to healthy subjects^{9, 10)} and that low serum levels of LPL mass might be a high-risk factor of coronary atherosclerosis^{11, 12)}. These results suggest that LPL mass may be useful as a predictor of atherosclerosis.

We hypothesized that acarbose administration could increase LPL mass and show an anti-atherosclerotic effect in type 2 diabetes mellitus. We therefore examined the effect of combination therapy of sulfonylurea and acarbose on CCA-IMT and LPL mass in type 2 diabetes mellitus.

Methods

Subjects

A randomized, open study was performed. Eighty-four patients with type 2 diabetes mellitus, who attended Sakura Medical Center of Toho University as outpatients, were enrolled. All subjects were treated by only sulfonylureas (glibenclamide) as oral hypoglycemic agents and had IMT thickness above 0.9 mm at baseline, and were divided into two groups by simple randomization using a closed envelope. One group was administered acarbose 300 mg/day for 12 months (acarbose group, $n=41$), and the other group was not administered acarbose (non-acarbose group, $n=43$). The clinical profile of the subjects is shown in Table 1. There were no differences in age, morbidity duration of diabetes mellitus and metabolic parameters between the two groups. There were also no differences in the ratio of subjects using lipid-lowering or blood pressure-lowering drugs between the two groups. All subjects had no previous cardiovascular and cerebrovascular diseases. Subjects were excluded if they received insulin therapy or had diabetic retinopathy and nephropathy. During this study, all subjects maintained

Table 1. Baseline data of two groups

	Non-acarbose group	Acarbose group
n (male/female)	43 (18/25)	41 (12/29)
Age (years)	63 $\pm$ 4	65 $\pm$ 6
Morbidity duration of diabetes (years)	11.3 $\pm$ 5.2	11.7 $\pm$ 6.5
BMI	23.1 $\pm$ 3.2	23.4 $\pm$ 2.5
BP (systolic/diastolic)	132 $\pm$ 13/77 $\pm$ 10	130 $\pm$ 13/76 $\pm$ 9
FBS (mg/dL)	134 $\pm$ 15	141 $\pm$ 19
Basal IRI (μ U/mL)	4.1 $\pm$ 1.2	3.7 $\pm$ 1.5
HbA1c (%)	7.4 $\pm$ 0.7	7.5 $\pm$ 0.8
TC (mg/dL)	198 $\pm$ 36	198 $\pm$ 25
TG (mg/dL)	133 $\pm$ 71	135 $\pm$ 68
HDL-C (mg/dL)	52 $\pm$ 14	53 $\pm$ 16
LDL-C (mg/dL)	114 $\pm$ 26	116 $\pm$ 34
Lipid-lowering drugs (No) (all statins)	10 (20%)	7 (18%)
Blood pressure-lowering drugs (No) (all ARBs)	7 (14%)	4 (10%)

Data are presented as the mean $\pm$ SD. BMI, body mass index; BP, blood pressure; FBS, fasting blood glucose; Basal IRI, basal immunoreactive insulin; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; CCA-IMT, common carotid artery-intima-media thickness; statin, HMG-CoA reductase inhibitor; ARB, angiotensin II receptor antagonist

appropriate diet and exercise therapies, and did not change medications. All subjects received nutrition education from a dietitian every month. The dietitian analyzed a typical meal of the patient and corrected any shortcomings. Exercise therapy was checked by the physician every month. From these check systems, they were confirmed to be following appropriate diet and exercise therapy. This study was approved by the Ethics Committee of Toho University. The purpose 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 study data was obtained before they were enrolled.

Measurement of Body Weight and Blood Pressure

Body weight and blood pressure were measured in the morning after 12 hours of fasting. Blood pressure was measured at least twice in a sitting position, and mean blood pressure was used as data.

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 of fasting blood glucose (FBS), glycosylated hemoglobin

Table 2. Comparison of change of metabolic parameters between the two groups after 12 months

	Non-acarbose group		Acarbose group	
	Change	<i>p</i>	Change	<i>p</i>
BMI	-0.2 ± 0.7	NS	-0.1 ± 1.2	NS
BP (systolic/diastolic)	-2 ± 8/-1 ± 8	NS/NS	-2 ± 10/-1 ± 16	NS/NS
FBS (mg/dL)	-10 ± 8	NS	-7 ± 4	NS
Basal IRI (mU/mL)	-0.1 ± 0.4	NS	-0.9 ± 0.5*	<0.05
HbA1c (%)	-0.3 ± 0.6	<0.05	-0.7 ± 0.8*	<0.05
TC (mg/dL)	2 ± 22	NS	2 ± 20	NS
TG (mg/dL)	-11 ± 43	NS	-28 ± 58*	<0.05
HDL-C (mg/dL)	1.0 ± 4.2	NS	3.5 ± 3.9*	<0.05
LDL-C (mg/dL)	-1 ± 13	NS	1 ± 22	NS

Data are presented as the mean ± SD. *p*, baseline vs after 12 months; **p* < 0.05 vs non-acarbose group.

BMI, body mass index; BP, blood pressure; FBS, fasting blood sugar; Basal IRI, basal immunoreactive insulin; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol

(HbA1c), basal immunoreactive insulin (basal IRI), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and LPL mass.

HbA1c and Serum Lipids Analysis

HbA1c, including stable and unstable fractions, was measured by high performance liquid chromatography using the Hi-Auto A1c kit (Kyoto Daiichi Kagaku, Kyoto, Japan). Data of the stable type were used. TC and TG concentrations were measured enzymatically using a kit available from Nippon Shoji (Osaka, Japan) and an automatic analyzer (Hitachi 7150 available from Hitachi Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's method¹³. HDL-C was measured by the selective inhibition method (Daiichi Pure Chemicals, Tokyo)¹⁴.

Assay of LPL Mass

LPL mass was measured by a sandwich enzyme-linked immunosorbent assay using specific monoclonal antibody against lipoprotein lipase (Daiichi Pure Chemicals, Tokyo), as described by Kobayashi *et al.*⁷. The linearity of this assay system was observed from 5 to 400 ng/mL. Within-run coefficient of variation was 2.8%. Between-day coefficient of variation was 4.3%.

Carotid Ultrasonography

Duplex carotid ultrasonography was performed using linear-array 7.5-MHz transducers (EUB-525, Hitachi, Inc., Tokyo, Japan; SSA-260A, Toshiba, Inc., Tokyo, Japan)¹⁵. Briefly, examinations were conducted in a dark room, with the subject lying supine and the head held in the midline position or slightly tilted to

either side. A careful search was performed to image the IMT of the far wall of CCA. When an optimal image was obtained, it was frozen in the end-diastolic phase to minimize variability during the cardiac cycle. IMT was measured twice bilaterally at 5 mm and 10 mm proximal to the dilatation of the CCA. Among the measurements, the highest IMT value was used. The averaged coefficient of variation in all tests was 4.8%.

All measurements of CCA-IMT were performed as a part of the routine clinical work-up by the same technician, and all evaluations were performed by the same clinician. They were blinded to all clinical data of the subjects.

Statistical Analysis

Statistical analysis was performed using Student's *t*-test and paired *t*-test. In all comparisons, values of *p* less than 0.05 were considered significant.

Results

Change of Metabolic Parameters in Acarbose or Non-Acarbose Groups after 12 Months (Table 2)

A significant decrease in HbA1c was observed in the two groups after 12 months; however, the decrease in acarbose group was significantly larger than that in the non-acarbose group. Basal IRI in the acarbose group decreased significantly, but no significant changes were observed in the non-acarbose group. TG decreased in both groups, although the decrease was significant only in the acarbose group. HDL-C increased significantly in the acarbose group, but showed no significant change in the non-acarbose group. No significant changes in BMI, BP, FBS, TC or LDL-C were observed in both groups.

Table 3. Comparison of baseline and change of CCA-IMT and LPL mass between the two groups after 12 months

	Non-acarbose group			Acarbose group		
	Baseline	Change	<i>p</i>	Baseline	Change	<i>p</i>
LPL mass (ng/mL)	49.0 ± 17.8	3.1 ± 8.2	NS	49.6 ± 16.8	11.5 ± 9.4*	<0.05
CCA-IMT (mm)	0.995 ± 0.21	0.02 ± 0.04	NS	1.024 ± 0.24	-0.06 ± 0.06*	<0.05

Data are presented as the mean ± SD (LPL mass) or SE (CCA-IMT). *p*, baseline vs after 12 months; **p* < 0.05 vs. non-acarbose group. CCA-IMT, common carotid artery-intima media thickness

Table 4. Comparison of baseline and change of data in subjects showing HbA1c decrease >0.5% after 12 months

	Non-acarbose group			Acarbose group		
	Baseline	Change	<i>p</i>	Baseline	Change	<i>p</i>
n (male/female)	21 (7/14)			31 (16/15)		
Age (years)	61 ± 5			59 ± 4		
Morbidity duration of diabetes (years)	11.9 ± 7.1			12.1 ± 6.5		
BMI	22.9 ± 3.2	-0.2 ± 0.5	NS	23.2 ± 3.2	-0.1 ± 0.8	NS
BP (systolic/diastolic)	131 ± 10/74 ± 6	-3 ± 12/-1 ± 10	NS/NS	130 ± 9/75 ± 4	-2 ± 9/-1 ± 12	NS/NS
FBS (mg/dL)	135 ± 15	-12 ± 6	NS	132 ± 13	-9 ± 6	NS
Basal IRI (μU/mL)	4.5 ± 1.2	-0.2 ± 0.4	NS	4.2 ± 1.5	-1.1 ± 0.5*	<0.05
HbA1c (%)	7.5 ± 0.8	-0.8 ± 0.3	<0.05	7.8 ± 0.9	-0.9 ± 0.2	<0.05
TC (mg/dL)	201 ± 34	2 ± 18	NS	210 ± 33	3 ± 22	NS
TG (mg/dL)	137 ± 75	-16 ± 57	NS	140 ± 83	-31 ± 66*	<0.05
HDL-C (mg/dL)	51 ± 12	1 ± 6	NS	54 ± 11	4 ± 5*	<0.05
LDL-C (mg/dL)	117 ± 31	3 ± 15	NS	124 ± 34	2 ± 25	NS
LPL mass (ng/mL)	48.6 ± 10.2	4.6 ± 8.4	<0.05	47.2 ± 11.2	12.3 ± 9.1*	<0.01
CCA-IMT (mm)	0.965 ± 0.11	-0.02 ± 0.03	NS	0.962 ± 0.08	-0.08 ± 0.04*	<0.05
Lipid-lowering drugs (No)	5 (16%)			5 (24%)		
Blood pressure-lowering drugs (No)	4 (13%)			3 (14%)		

Data are presented as the mean ± SD or SE (CCA-IMT). *p*, baseline vs after 12 months; NS, not significant; **p* < 0.05 vs non-acarbose group; BMI, body mass index; BP, blood pressure; FBS, fasting blood sugar; Basal IRI, basal immunoreactive insulin; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; CCA-IMT, common carotid artery-intima media thickness

Baseline Data and Changes of CCA-IMT and LPL Mass in Acarbose or Non-Acarbose Group (Table 3)

The CCA-IMT in the acarbose group decreased significantly after 12 months of acarbose administration, whereas the CCA-IMT increased slightly in the non-acarbose group. This change in CCA-IMT was significantly different between the two groups. A significant increase in LPL mass was observed after acarbose administration for 12 months; however, no significant change in LPL mass was observed in the non-acarbose group.

Effect of Acarbose on Changes of LPL Mass or CCA-IMT Among Patients Showing Improved HbA1c (Table 4)

Next, to clarify whether the effect of acarbose on CCA-IMT is independent of the improvement in

HbA1c, we selected patients with HbA1c who decreased by more than 0.5% after 12 months from among the two groups (31 patients in acarbose group, 21 patients in non-acarbose group), and compared the changes in LPL mass and CCA-IMT between these two groups. The two groups did not differ significantly in any of the baseline parameters. In the acarbose group, a significant increase in LPL mass and a significant decrease in CCA-IMT were observed after 12 months; however, no significant change of CCA-IMT was observed in the non-acarbose group, although the changes in FBS and HbA1c were almost the same in the two groups. The change in TG and increase in HDL-C were also significantly (*p* < 0.05) greater in the acarbose group than in the non-acarbose group, but no significant changes in BMI, BP, TC and LDL-C were observed between two groups.

There were also no differences in the ratio of subjects using lipid-lowering or blood pressure-lowering drugs between the two groups.

Discussion

Recently, a prospective study proved that LPL mass could be an independent predictor of coronary heart disease¹⁶. CCA-IMT is also known to be an important predictor of coronary heart disease and stroke^{1, 2}. Therefore, we administered acarbose to type 2 diabetes patients treated by sulfonylurea, and investigated the effect of acarbose on LPL mass and CCA-IMT. The decrease of CCA-IMT and increase of LPL mass were significantly higher in the acarbose group than in the non-acarbose group. These findings suggested that acarbose had anti-atherosclerotic effects in type 2 diabetic patients treated by sulfonylurea.

As for the mechanisms of LPL mass increase in the acarbose group, we speculate that acarbose administration improves insulin resistance, since LPL mass has been reported to possibly reflect insulin sensitivity^{10, 17, 18}. This speculation is supported by recent reports that postprandial hyperglycemia is associated with insulin resistance^{19, 20} and that acarbose improves insulin resistance^{21, 22}. In our data, a significant decrease of basal IRI in the acarbose group also might support the improvement of insulin resistance.

Serum levels of LPL mass are reported to be related to atherosclerosis^{10, 11, 16}. We therefore examined whether the change of LPL mass influences CCA-IMT. An increase of LPL mass was accompanied with a decrease of CCA-IMT. These results suggest that an increase of LPL mass could be consistent with anti-atherosclerotic change and might be a clinical marker for atherosclerotic diseases. The effect of acarbose on IMT has already been reported by Hanefeld *et al.*³ who showed that acarbose slows the progression of CCA-IMT in subjects with impaired glucose tolerance (IGT). Our data indicate that acarbose not only slows the progression but even decreases CCA-IMT. The reason for the difference between our data and the findings of Hanefeld *et al.* might be due to the difference in the subjects studied. The subjects in Hanefeld's study had IGT and the effect on CCA-IMT is largely due to the reduction of postprandial hyperglycemia by acarbose; however, our subjects were patients with type 2 diabetes mellitus, and the effect on CCA-IMT is a compound effect of the reduction of postprandial hyperglycemia by acarbose administration and also a decrease of HbA1c. In other glucose-lowering drugs, biguanide have been reported to attenuate progression of CCA-IMT^{23, 24}, and pioglitazone is known to im-

prove CCA-IMT^{25, 26}. These effects are considered to be due to improved insulin resistance. Acarbose also might improve insulin resistance by decreasing postprandial hyperglycemia.

With respect to serum lipids, HDL-C increased significantly in the acarbose group, while the increase was not significant in the non-acarbose group. TG decreased in both groups, although the decrease was significant only in the acarbose group. Previous reports demonstrated that high levels of TG, and low levels of HDL-C and remnant lipoprotein are generally recognized in patients with low levels of preheparin LPL mass^{27, 28}, and that an increase of preheparin LPL mass is accompanied with a decrease of TG and an increase of HDL-C²⁹; therefore, the improvement of serum lipid metabolism following acarbose administration observed in this study may be explained by an increase of LPL mass.

In this study, decreases of HbA1c were observed in not only the acarbose group but also the non-acarbose group. In addition to diet and exercise therapies, these decreases might be due to the effect of sulfonylurea because all subjects were treated by sulfonylurea, which might influence CCA-IMT and other parameters. The effect of only acarbose treatment should be studied in future.

In summary, our study indicated that in type 2 diabetes mellitus treated by sulfonylurea, an alpha-glucosidase inhibitor, acarbose, increased LPL mass and decreased CCA-IMT. From these results, we conclude that in type 2 diabetes mellitus treated by sulfonylurea, reduction of postprandial hyperglycemia may increase LPL mass and may be useful for the prevention of macroangiopathy.

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