Original Article

Long-Term Effects of Pioglitazone on Carotid Atherosclerosis in Japanese Patients with Type 2 Diabetes without a Recent History of Macrovascular Morbidity

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Aim: No previous studies have evaluated the long-term anti-atherosclerotic effects of pioglitazone in Asian patients with type 2 diabetes. Therefore, the present study investigated the protective effects of pioglitazone on the progression of carotid intima-media thickness (IMT), an established surrogate marker of cardiovascular events in Japanese type 2 diabetic patients without a recent history of cardiovascular morbidity.

Methods: This 2.5–4-year, randomized, open-label, blinded endpoint study was conducted in 6 centers across Japan. Patients received pioglitazone with or without other oral glucose-lowering drugs (excluding another thiazolidinedione) ($n=89$) or oral glucose-lowering drugs, excluding thiazolidinediones ($n=97$). Treatment was adjusted to achieve $HbA_{1c} < 6.5\%$. The primary endpoints of the study were the absolute changes from the baseline to final visit in max- and mean-IMT in the average of bilateral common carotid arteries.

Results: Pioglitazone induced carotid IMT regression compared to baseline measurements (from 1.060 ± 0.2368 to 0.992 ± 0.1921 mm; $p=0.0042$ in max-IMT and from 0.839 ± 0.1873 to 0.780 ± 0.1571 mm; $p=0.0019$ in mean-IMT). Although the between-group difference did not reach statistical significance, the regression of carotid IMT values was greater in the pioglitazone-treatment group than in the non-pioglitazone group, (max-IMT: -0.069 ± 0.2199 mm vs -0.031 ± 0.2327 mm, respectively; $p=NS$, mean-IMT: -0.058 ± 0.1718 mm vs -0.043 ± 0.1644 mm, respectively; $p=NS$).

Conclusions: Pioglitazone induced and maintained the long-term regression of carotid IMT in Japanese type 2 diabetic patients. This suggests that pioglitazone may inhibit the progression of atherosclerosis in this patient group. Further studies are required to verify these findings.

J Atheroscler Thromb, 2010; 17:1132-1140.

Key words; Type 2 diabetes mellitus, Pioglitazone, Carotid intima-media thickness

Introduction

Type 2 diabetes is associated with a markedly elevated risk of cardiovascular (CV) events, which results in significantly increased morbidity and mortality, and also leads to impaired quality of life^{1, 2)}. Many studies have shown a direct link between HbA_{1c} levels and CV risk in type 2 diabetes, and management of glu-

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Received: December 29, 2009

Accepted for publication: June 2, 2010

cose levels represents one of the principal treatment goals in these patients³; however, until recently, evidence of an effect on macrovascular outcomes has been limited, as have data on whether any particular therapy has advantages in this respect⁴⁻⁶.

Pioglitazone, a thiazolidinedione, is an oral anti-diabetic agent frequently used to treat type 2 diabetic patients. It acts by increasing insulin sensitivity, mediating favorable effects on blood glucose levels and lipid profile⁷. In addition to these metabolic actions, pioglitazone has beneficial effects on systemic inflammatory and coagulation markers, and endothelial cell function^{8, 9}. Thus, the overall pattern of changes induced by pioglitazone suggests a general improvement of various risk factors for atherosclerosis. Indeed, pioglitazone has been shown to reduce neointimal thickening after endothelial injury in animal models of atherosclerosis¹⁰. Results from recent randomized clinical trials found that pioglitazone might reduce atherosclerotic change in human arteries^{11, 12}. Furthermore, findings from the large PROactive trial and a meta-analysis of 19 smaller trials demonstrated that pioglitazone had a beneficial effect on ischemic CV events in patients with type 2 diabetes^{13, 14}.

However, there have been no studies evaluating the long-term anti-atherosclerotic effects of pioglitazone in Asian patients with type 2 diabetes; therefore, the present study assessed the protective effects of pioglitazone on the progression of carotid intima-media thickness (IMT), an established surrogate marker of CV events, in Japanese type 2 diabetic patients. The subjects were a subgroup from a clinical trial which evaluated the effects of pioglitazone on macrovascular events in patients with type 2 diabetes treated for 2.5–4 years¹⁵. It was a randomized, controlled clinical study, and the control group consisted of patients treated with oral anti-diabetic therapy, which did not include a thiazolidinedione.

Research Design and Methods

Study Design and Participants

The PROBE (Pioglitazone anti-atherO sclerosis effect on prospective randomized open Blinded Endpoint) trial was a prospective, randomized, open-labeled, blinded endpoint, multicenter study designed to assess the glycemic effects of pioglitazone and their impact on CV outcomes in Japanese patients with type 2 diabetes over a period of 2.5–4 years. The study ran from April 2002 to June 2006 and was performed at 20 centers in Japan (see Appendix 1). The carotid ultrasound substudy was part of the original PROBE clinical trial and was undertaken in six centers in

which ultrasonographic scans of the carotid artery could be completed (Appendix 1). All patients gave written informed consent and the study was performed in accordance with Good Clinical Practice and Good Postmarketing Surveillance Practice guidelines, and the ethical principles set out in the Declaration of Helsinki.

Patients of either sex, aged 35–74 years, with type 2 diabetes ($HbA_{1c} \geq 6.5\%$) and at least 2 of the following risk factors were enrolled in the study: hypertension (either receiving antihypertensive therapy or newly diagnosed with systolic blood pressure (BP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg); dyslipidemia (receiving treatment or newly diagnosed with total cholesterol ≥ 240 mg/dL or LDL-cholesterol ≥ 160 mg/dL or HDL-cholesterol < 40 mg/dL); obesity (BMI ≥ 25 kg/m²); or smoking (≥ 1 cigarette per day). Exclusion criteria were: type 1 diabetes, heart failure, severe arrhythmias, significant renal/hepatic impairment, a BMI < 22 kg/m² with a fasting immunoreactive insulin (IRI) of < 5 μ U/mL. Patients with a recent history (within 6 months of randomization) of CV disorders (specifically, myocardial infarction, coronary artery bypass surgery or percutaneous coronary intervention) or stroke (with the exception of transient ischemic attacks), or who had been hospitalized for acute coronary syndrome within 3 months prior to randomization were also excluded.

Patients, all of whom received diet and exercise instruction, were randomized to either a pioglitazone or a non-pioglitazone (control) group. Randomization used a dynamic allocation method based on the presence/absence of a history of CV events, the number of risk factors present (2 or ≥ 3 , excluding diabetes), age (≤ 59 or ≥ 60 years), sex, and study center. Pioglitazone 15 to 30 mg was orally administered once daily before or after breakfast. The dosage could be titrated to a maximum of 45 mg/day. The recommended starting dose in women and the elderly was 15 mg. Study drug doses were titrated to reach and maintain target glycemic goals, defined as an HbA_{1c} level of $< 6.5\%$. In the control group, either the dosage of current therapy was increased, or a concomitant oral glucose-lowering drug (excluding a thiazolidinedione) was added, or an alternative treatment was administered. In the pioglitazone group, the preferred adjustment was to increase the dose of pioglitazone; however, addition of an alternative glucose-lowering agent (excluding another thiazolidinedione) was permitted. If insulin therapy became necessary, pioglitazone was discontinued; if insulin was later stopped, pioglitazone therapy could be resumed. Antihyperlipidemic, antihypertensive and antithrombotic drugs

were allowed during the study.

Measurement of IMT

Ultrasonographic scans of the carotid artery were performed by certified sonographers who were specifically trained to perform the carotid ultrasound sub-study examination and who were blinded to treatment group allocation. Measurements were taken at baseline, and at 24, 48, 72, 96, 120, 144, 168, and 192 weeks (or at the time of early termination). Each participant was examined by the same sonographer with the same equipment (high resolution B-mode ultrasound scanners equipped with a 7.5-12 MHz linear transducers, where the detection limit was <0.1 mm) throughout the study. Scanning of the extracranial common carotid artery, the carotid bulb, and the internal carotid artery in the neck was performed bilaterally from three different longitudinal projections (i.e., anterior, lateral, and posterior) as well as the transverse projections and the site of the greatest thickness, including a plaque lesion, was sought along the arterial walls. All measurements were performed in the same artery region throughout the study and all scans were stored electronically.

The images were read by the IMT Evaluation Committee members, who were blinded to treatment allocation, in batch fashion and in random order. To avoid inter-reader variability, batches were read by two expert readers who were blinded according to visit and treatment group, using automated digital edge-detection software (Intimascope; MediaCross, Tokyo, Japan). The IMT was measured as the distance between 2 parallel echogenic lines corresponding to the blood-intima and media-adventitia interface on the posterior wall of the artery. Three determinations of IMT were conducted at the site of the thickest point, maximum IMT (max-IMT) and two adjacent points (located 1 cm upstream and 1 cm downstream from this site). These three determinations were averaged (mean-IMT). The greatest reading among the three max- or mean-IMTs from three selected angles was used as the representative value for each artery, and the average of the right and left values was used as the individual value.

Reproducibility analysis of 60 replicate measures at baseline resulted in absolute mean differences of 0.01 ± 0.03 and 0.01 ± 0.07 mm for max-IMT and mean-IMT, respectively. The intra-class correlations were 0.99 and 0.99 for max-IMT and mean-IMT, respectively.

The primary endpoints of the substudy were the absolute changes from the baseline to final visit in max- and mean-IMT in the average of bilateral com-

mon carotid arteries. Pre-specified subgroups for analysis included the presence/absence of a history of CV events, the number of risk factors present (2 or ≥ 3 , excluding diabetes), gender, age (≤ 59 ≥ 60 years), baseline IMT, presence/absence of hypertension, presence/absence of dyslipidemia, history of smoking, duration of diabetes, body mass index, and HbA1c values.

Laboratory Measurements

All patients underwent pre-treatment screening during a 4-week observation period. Throughout the study HbA1c, blood pressure (BP), and LDL-cholesterol were measured every 12 weeks. Assessments of fasting blood glucose (FBG), fasting IRI, proinsulin, total cholesterol, HDL-cholesterol, and triglycerides were performed every 24 weeks. These measurements were performed by SRL Medisearch, Inc. (Tokyo, Japan). If treatment was discontinued early, all parameters were assessed at the time of discontinuation. HbA1c was measured with HPLC methodology according to Japanese Diabetes Society standards¹⁶.

Statistical Analysis

It has been reported that IMT was reduced by -0.084 ± 0.023 mm (mean $\pm$ SEM) when pioglitazone was administered to patients with type 2 diabetes for 6 months, and increased by 0.022 ± 0.006 mm in a non-pioglitazone (control) group¹⁷. The upper confidence limit of changes in IMT in the pioglitazone-treated group was calculated to be -0.054 mm and the lower confidence limit of changes in IMT in the non-pioglitazone group was calculated to be 0.014 mm. Based on these findings, it was calculated that the inclusion of 106 patients or more in the two groups would be required to obtain a significant difference if it is assumed that the standard deviation common to both groups is 0.106 mm, and α -level and power are 5% and 90%, respectively.

Unless otherwise stated, analyses were based on intention-to-treat and last-observation-carried-forward principles. For the primary endpoints of the substudy, the absolute changes in max- and mean-IMT of the common carotid arteries during the follow-up period were analyzed. The absolute changes in IMTs in each treatment group were analyzed using paired *t*-tests and the difference between treatment groups was analyzed using unpaired *t*-tests. The generalized estimating equations (GEE) model was applied to model the treatment effect on the absolute changes in IMTs, adjusting for covariates, such as the presence/absence of a history of CV events, the number of risk factors present, gender, age, and baseline IMT. Differences in

Table 1. Baseline characteristics and concomitant medications of the 'full analysis set' population

Characteristic	Pioglitazone group (n = 89)	Control group (n = 97)	Homogeneity <i>p</i> -value
Sex (male/female) [%]	60.7/39.3	64.9/35.1	0.5466
Age (years)	56.7	57.2	0.7495
Duration of diabetes (< 5/≥ 5 years) [%]	26.9/71.9	22.7/77.3	0.6134
Diabetic complications ^a (%)	98.9	100.0	0.2952
History of CV events ^b (%)	11.2	6.2	0.2198
History of smoking (%)	33.7	38.1	0.5290
Body weight (kg)	70.4	70.8	0.8522
BMI (kg/m ²)	26.91	27.19	0.5350
CIMT for Common Carotid Artery (Mean)	0.839	0.818	
CIMT for Common Carotid Artery (Max)	1.06	1.02	
BP (SBP/DBP; mmHg)	139.1/82.8	135.1/82.0	0.0990/0.6437
HbA _{1c} (%)	7.53	7.55	0.8839
Total cholesterol (mg/dL)	205.7	213.9	0.1338
LDL-cholesterol (mg/dL)	123.7	130.7	0.1163
HDL-cholesterol (mg/dL)	50.4	50.4	0.9857
Triglycerides (mg/dL)	159.3	174.6	0.5797
Fasting blood glucose (mg/dL)	157.9	168.3	0.0554
Fasting IRI (μU/mL)	11.098	11.778	0.5739
Proinsulin (pmol/L)	33.92	35.90	0.5534
Concomitant medications ^c			
Sulphonylureas (%)	73.0	75.3	0.7291
Biguanides (%)	42.7	63.9	0.0037
α-Glucosidase inhibitors (%)	57.3	64.9	0.2849
Rapid-acting insulin-secretagogue drugs (%)	5.6	13.4	0.0729
Statins (%)	41.6	30.9	0.1309
Angiotensin-converting enzyme inhibitors (%)	15.7	11.3	0.3806
Angiotensin II antagonists (%)	33.7	36.1	0.7344
Calcium-channel blockers (%)	46.1	47.4	0.8532
Fibrates (%)	9.0	11.3	0.5968

Mean values are presented unless stated otherwise.

^aMost common: hypertension (74.7% of total population), diabetic microangiopathy (74.2%), hyperlipidemia (72.0%).

^bStroke (excluding TIA), angina pectoris, myocardial infarction and coronary intervention procedure.

^cTaken at least once during the study

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, LDL: low-density lipoprotein, HDL: high-density lipoprotein, IRI: immunoreactive insulin.

parameters at baseline between 2 treatment groups were compared by the unpaired *t* test, the χ^2 test or the Wilcoxon rank sum test. Other parameters were generally analyzed using summary statistics and two-sided confidence intervals. The level of significance was set at 5%.

Results

Baseline Characteristics and Concomitant Medication

A total of 589 qualifying patients were randomized to treatment in the original study, and the carotid

ultrasound substudy was performed in 186 patients (89 in the pioglitazone group and 97 in the control group) at six centers.

Demographic and baseline characteristics of the 'full analysis set' population are summarized in **Table 1**. The frequency of concomitant medication with biguanides was significantly ($p < 0.05$) greater in the control group than in the pioglitazone group. There were no significant differences between groups with respect to the other parameters. Twenty-nine patients, 15 in the pioglitazone group and 14 in the control group, dropped out.

Fig. 1. Change From Baseline to Week 192 in Max and Mean carotid IMT of the Common Carotid Artery.

(A) Sequential change in Max-IMT of subjects with and without pioglitazone. Data are shown as the mean $\pm$ SEM. * indicates significantly different to baseline. (B) Absolute change from baseline to final visit in Max-IMT in the average of bilateral common carotid arteries. During the treatment period, max-IMT significantly decreased in patients in the pioglitazone group ($p=0.0042$) but not in the control group (NS). The regression of max-IMT in the pioglitazone group was greater than in the non-pioglitazone group but the between-group difference did not reach statistical significance (NS). (C) Sequential change in Mean-IMT of subjects with and without pioglitazone. Data are shown as the mean $\pm$ SEM. * indicates significantly different as compared to baseline. (D) Absolute change from baseline to final visit in Mean-IMT in the average of bilateral common carotid arteries. During the treatment period, mean-IMT significantly decreased in the pioglitazone group ($p=0.0019$) and the control group ($p=0.0110$). There were no significant differences in changes in mean-IMT between treatment groups (NS).

Effect of Pioglitazone on Carotid IMT

In patients in the pioglitazone group, both max- and mean-IMT were significantly improved during treatment compared with baseline measurements (from 1.060 ± 0.2368 to 0.992 ± 0.1921 mm; $p=0.0042$ in max-IMT and from 0.839 ± 0.1873 to 0.780 ± 0.1571 mm; $p=0.0019$ in mean-IMT). In patients in the non-pioglitazone group, however, there was no significant difference in max-IMT before and after the treat-

ment period (from 1.021 ± 0.2136 to 0.990 ± 0.2158 mm; $p=NS$), while mean-IMT was significantly improved during treatment compared with baseline (from 0.818 ± 0.1731 to 0.774 ± 0.1570 mm; $p=0.0110$).

The regression in max-IMT in the pioglitazone group was more than twice that achieved in the non-pioglitazone group, but the between-group difference did not reach statistical significance (-0.069 ± 0.2199

Fig. 2. Changes in HbA_{1c} and HDL-cholesterol during long-term treatment.

Error bars indicate SEM.

HbA_{1c} was significantly lower in the pioglitazone group from week 12 to week 180 ($p < 0.05$).

HDL-cholesterol level was significantly higher in the pioglitazone group from week 24 to week 144 ($p < 0.05$).

mm vs -0.031 ± 0.2327 mm, respectively; $p = \text{NS}$). Similarly, pioglitazone tended to induce greater regression of mean-IMT but the between-group difference again did not reach statistical significance (-0.058 ± 0.1718 mm vs -0.043 ± 0.1644 mm, respectively; $p = \text{NS}$) (**Fig. 1**). Similar results were obtained after stratification by baseline characteristics (presence or absence of previous CV events; 2 or ≥ 3 risk factors for macrovascular events; age ≤ 59 or ≥ 60 years; male or female). GEE analysis of the endpoint also produced similar results.

The number of patients given the average dose of pioglitazone less than 15 mg, 15–30 mg, and over 30 mg were 41, 47, and 1, respectively. The changes in max-IMT and mean-IMT showed no significant correlation with the averaged dose of pioglitazone (data not shown).

Metabolic Parameters

HbA_{1c} was significantly ($p < 0.05$) lower in the pioglitazone group than in the control group from week 12 to week 180. The changes from the baseline HbA_{1c} levels were -0.73% at week 48, -0.61% at week 96, and -0.50% at week 144 in the pioglitazone group, while -0.18% , -0.03% , and 0.08% in the control group (**Fig. 2A**).

HDL-cholesterol levels were significantly higher in the pioglitazone group from week 24 to week 144 ($p < 0.05$). The increase from baseline HDL-cholesterol level was 3.7 mg/dL at week 48, 2.7 mg/dL at week 96, and 2.1 mg/dL at week 144 in the pioglitazone group, and -0.4 mg/dL, 0.1 mg/dL, and -0.5

mg/dL in the control group (**Fig. 2B**).

The increase in body weight (from baseline) was significantly greater in the pioglitazone group from week 8 to the end of the study ($p < 0.05$) (mean value ranged from 0.7 to 3.5 kg) compared with the control group (mean value ranged from -1.2 to 0.0 kg). Regarding other metabolic parameters, there were no significant differences between the two treatment groups.

Discussion

Carotid ultrasound measurements of IMT are well-accepted predictors of CV events in subjects with and without type 2 diabetes^{18, 19}. Changes in carotid IMT over time correlate with future CV event rates²⁰. Diabetes, which confers an increased risk of CV disease, accelerates carotid IMT progression²¹. In a recent review of 11 carotid IMT intervention trials in diabetes, the mean carotid IMT progression rate in type 2 diabetic subjects without intervention was 0.034 mm/year (95% CI, 0.029 to 0.039 ; median SD, 0.054)²². Several studies have assessed the effect of various therapeutic agents on carotid IMT as a measure of atherosclerosis. Although pioglitazone was associated with reduced CV events in the PROactive study¹³, few investigations have evaluated its effects on IMT. To date, trials have been confined to 6-month studies comparing pioglitazone with sulfonylurea oral anti-diabetic agents or diet alone^{22, 23}, and the more extensive CHICAGO study. This randomized double-blind trial was performed over a 72-week period in patients with type 2 diabetes in the

Chicago metropolitan area, and reported that pioglitazone significantly reduced carotid IMT compared with glimepiride¹²⁾. Current studies have only been performed in European and American populations^{11, 12)} and no investigations have evaluated the efficacy of pioglitazone against CV events and IMT over a prolonged period of time in Asian patients. The present study was the first to assess the effects of long-term pioglitazone treatment on macrovascular outcomes¹⁵⁾ and the progression of carotid IMT in Japanese type 2 diabetic subjects without a recent history of macrovascular events.

We found that suppression of carotid IMT progression was more marked in the pioglitazone group than in the non-pioglitazone group, compared with baseline measurements, suggesting that pioglitazone may suppress the progression of atherosclerosis in this patient group; however, between-group differences did not achieve statistical significance.

When comparing the results of the current study to previous trials, it appears that the lack of significant differences between groups may be attributable to several factors. For example, we measured IMT at six centers whereas only one facility with a single ultrasonographer was utilized in the CHICAGO study. Whilst the stratified (dynamic) randomization of patients employed in our study should have minimized this source of variation, it is possible that the smaller groups per institution might have resulted in such an imbalance. A second factor is the smaller total sample size in our study: we assessed 186 cases compared with 361 in the CHICAGO study. The number of patients treated was based on the assumption of a change of -0.022 mm in the non-pioglitazone group, whereas the actual differences were -0.031 and -0.043 mm. This significant decrease even in the non-pioglitazone group possibly reflects the more frequent use of biguanides and α -glucosidase inhibitors in the non-pioglitazone group^{24, 25)}. Interestingly, the findings of this substudy mirror the results of the "parent" PROBE trial, which also found a non-significant trend towards improved macrovascular outcomes in the pioglitazone group versus the control group¹⁵⁾. However, the improved outcomes were not as great as those reported in the PROactive study¹³⁾ and this was likely due to the type of patients included in the study (i.e. milder disease with no recent history of CV events) and the high use of concomitant anti-diabetic agents. Also, the minimized number of differences in the drop-out rate (15 in the pioglitazone group and 14 in the control group) may not account for the lack of treatment differences between the groups. These suggestions may help to explain the results obtained in

this substudy investigating changes in IMT. To determine whether there is a clear clinical benefit of including pioglitazone in the treatment regimen for patients with type 2 diabetes and coexistent risk factors for cardiovascular disease, both in terms of macrovascular outcomes and carotid IMT, further well-controlled single-facility studies with larger subject numbers are required.

The mechanisms by which pioglitazone treatment significantly reduces carotid IMT are unclear, but several possibilities have been hypothesized. The reduction of carotid IMT may be related to the improvement of traditional risk factors. As shown in several previous investigations^{7, 8, 12, 13)}, pioglitazone therapy decreases HbA1c levels and increases HDL-cholesterol levels, and similar changes were observed in the present study (**Fig. 2**). Although the difference in the number of metabolic risks or fasting insulin level did not affect the change in IMT in both groups (data not shown), the beneficial effect of pioglitazone on carotid IMT may be at least partially attributed to the modulation of hyperglycemia and/or dyslipidemia. Another possibility is that pioglitazone may have decreased carotid IMT by improving insulin sensitivity, since insulin resistance is reported to be a risk factor for carotid atherosclerosis²⁶⁾. In addition to these metabolic actions, pioglitazone has beneficial effects on endothelial cell function²⁷⁾ and may have mediated the regression of carotid IMT by improving arterial vasodilator capacity, platelet aggregation, and intimal thickening. Indeed, pioglitazone has been shown to reduce neointimal thickening after endothelial injury in animal models of atherosclerosis¹⁰⁾. Furthermore, pioglitazone can modify systemic inflammatory and coagulation markers^{8, 9)}. Recently, pioglitazone was found to modify the progression of carotid atherosclerosis induced by various genetic risk factors²⁸⁾. Taken together, the overall pattern of changes induced by pioglitazone suggests a general improvement in a number of different risk factors associated with atherosclerosis. In this study, endothelial function was not measured and metabolic level was not evaluated. Therefore, other studies are needed to clarify the possibility that pioglitazone induces the regression of carotid atherosclerosis by improving endothelial function or the possibility that the metabolic level may affect the improvement of the carotid atherosclerosis by pioglitazone.

In conclusion, our study demonstrated that pioglitazone induced and maintained the regression of carotid IMT in Japanese patients with type 2 diabetes and coexisting risk factors for cardiovascular disease. Compared with a control group receiving oral anti-

diabetic therapy without a thiazolidinedione, pioglitazone produced greater improvements but did not achieve statistical significance. Larger, well-controlled studies are required to verify the current findings and to fully elucidate the clinical benefits of pioglitazone in this type of patient.

Acknowledgements

This clinical study was supported by a financial grant from Takeda Pharmaceutical Co., Ltd, Osaka, Japan. Editorial assistance was provided by Caroline McGown PhD, ContentEdNet. The authors would like to thank the members of the following committees for their assistance with the clinical trial:

The Independent Safety Data Monitoring Committee members; Toshiaki Hanafusa, First Department of Internal Medicine, Osaka Medical College; Minoru Kubota, Health care center, Kwansei Gakuin University; Kiyoshi Yoshida, Department of Cardiology, Kawasaki Medical School.

The Macrovascular Events Evaluation Committee members; Akira Yamashina, Second Department of Internal Medicine, Tokyo Medical University; Tsutomu Yamazaki, Department of Clinical Epidemiology & Systems, Graduate School of Medicine, University of Tokyo; Shin-ichi Momomura, Saitama Medical Center, Jichi Medical University; Takashi Iwase, Iwase Internal Medicine Cardiology Clinic.

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Appendix 1

Listing of all investigational sites and investigators

Site	Investigator
Kurihara Diabetic Care Clinic	Yoshio Kurihara
Keijinkai Sasaki Diabetic Hospital	Takashi Sasaki
Toei Hospital	Ryo Sugiura
Kenichi Yamada Clinic	Kenichi Yamada
Okuguchi Clinic*	Fuminobu Okuguchi
Nakakinen Clinic*	Takeshi Osonoi
Minamiakatsuka Clinic	Hideo Takahashi
Kawai Clinic	Koichi Kawai
Arisaka Clinic	Tomoyuki Arisaka
Kobayashi Clinic of Internal Medicine and Gastroenterology	Izuru Kobayashi
Aiso Clinic*	Yoshitaka Aiso
Sugawara Medicine Clinic	Masahiro Sugawara
Kenkoukan Suzuki Clinic	Kazuo Suzuki
Matsuba Clinic	Ikuro Matsuba
Okada Clinic	Akira Okada
Abe Diabetes Clinic	Nobuyuki Abe
Jinnouchi Clinic Diabetes Center*	Hideaki Jinnouchi
Heiwadai Hospital*	Syuji Nakamura
Yano Clinic	Nobuki Yano
Tempozan Naika Clinic*	Jun Hashiguchi

*; Six centers where the carotid ultrasound protocol was performed.