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Reduction of neointimal hyperplasia after coronary stenting by pioglitazone in nondiabetic patients with metabolic syndrome

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Background This study investigates whether pioglitazone reduces neointimal hyperplasia after coronary stenting in nondiabetic patients with metabolic syndrome (MS) using intravascular ultrasound (IVUS). Pioglitazone, a novel insulin-sensitizing thiazolidinedione, has been shown to reduce neointimal hyperplasia after coronary stenting in patients with type 2 diabetes. However, the effect of pioglitazone on in-stent restenosis in nondiabetic patients with MS remains unknown.

Methods and Results Twenty-eight nondiabetic patients with MS after bare-metal stent implantation were randomized to 6-month treatment with or without 30 mg/d of pioglitazone (pioglitazone group [PIO] of 14 patients with 16 lesions and control group [CONT] of 14 patients with 16 lesions). At baseline and at 6-month follow-up, assessment of insulin resistance and visceral fat accumulation, quantitative coronary angiographic analysis, and IVUS measurements were performed. Pioglitazone treatment improved insulin resistance and decreased visceral fat accumulation without significant changes in plasma glucose levels, glycosylated hemoglobin A_{1c} levels, and lipid profiles. Intimal index (intimal area / stent area) and intimal area were reduced in PIO compared with CONT (13% ± 7% vs 21% ± 13%, $P = .033$; 1.28 ± 0.76 mm^2 vs 1.90 ± 1.16 mm^2, $P = .084$; respectively). Binary restenosis rate was 0% in PIO versus 31% in CONT ($P = .043$).

Conclusions This is the first randomized, prospective IVUS study demonstrating that pioglitazone reduces neointimal hyperplasia after coronary stenting in nondiabetic patients with MS. Our data suggest that pioglitazone treatment may represent a novel therapeutic tool to target in-stent restenosis in nondiabetic patients with MS. (Am Heart J 2007;153:762.e1-762.e7.)

Restenosis after percutaneous coronary intervention (PCI) remains a challenging problem. Restenosis rates after coronary stenting were 20% to 40% at 6 months in the bare-metal stent (BMS) era,[1] and even drug-eluting stent (DES) has not cured this problem, although restenosis rates in the DES era have decreased to less than half compared with that in the BMS era.[2] The primary cause of in-stent restenosis is neointimal hyperplasia due to vascular smooth muscle cell proliferation.[1-4] Recent studies showed that diabetes and insulin resistance are independent predictors of early restenosis after coronary stenting.[5,6] Metabolic syndrome (MS), a multiplex risk factor of coronary artery disease, is also a potent cause for restenosis after PCI because of its close link to insulin resistance.[6,7]

It has been demonstrated that thiazolidinediones (TZDs) including pioglitazone and rosiglitazone reduce restenosis and neointimal hyperplasia after coronary stenting in patients with type 2 diabetes and nondiabetic patients.[8-11] A recent study has shown that rosiglitazone may improve clinical outcomes in nondiabetic patients with MS after DES implantation.[12] However, the effect of pioglitazone on neointimal hyperplasia after coronary stenting in nondiabetic patients with MS remains unknown. Because there is a growing number of patients with MS and of revascularization in these patients,[13] it is critical to provide insights into a novel

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therapeutic approach for in-stent restenosis in patients with MS.

Methods

Patients and design

Nondiabetic patients with MS after BMS implantation were eligible for this randomized, prospective study. Metabolic syndrome was diagnosed based on the Third Report of the National Cholesterol Education Program Adult Treatment Panel III.[7] Between April 2002 and March 2005, patients with stable angina pectoris were included into the study when ≥3 of the following risk determinants were present: (1) abdominal obesity defined as visceral fat area of ≥100 cm^2 measured by abdominal computed tomography (CT) as a surrogate for waist circumference [14]; (2) triglyceride level of ≥150 mg/dL; (3) high-density lipoprotein cholesterol (HDL-C) level of <40 mg/dL in men, <50 mg/dL in women; (4) blood pressure of ≥130/≥85 mm Hg; (5) fasting glucose level of ≥110 mg/dL. Exclusions in this study were as follows: (1) previously treated diabetes or newly diagnosed diabetes by a 75-g oral glucose tolerance test (OGTT); (2) acute coronary syndrome; (3) chronic heart failure; (4) liver or renal dysfunction; (5) ostial or bifurcation lesions; (6) chronic total occlusions; (7) lesions treated with >2 stents; (8) small-vessel lesions (reference diameter <2.5 mm); (9) lesions treated with DES. Before coronary stenting, patients were randomly assigned to 2 treatment groups: the pioglitazone group (PIO) of 16 patients with 18 lesions and the control group (CONT) of 16 patients with 18 lesions. All patients were successfully treated with intravascular ultrasound (IVUS)-guided coronary stenting. After coronary stenting, patients in PIO were treated with 30 mg/d of pioglitazone in combination with standard medications for 6 months, whereas patients in CONT were treated with standard medications only. All patients were examined monthly to check their general conditions and to monitor adverse reactions of pioglitazone. All subjects signed an informed consent document approved by the Jichi Medical University Committee on Clinical Investigation before randomization.

Baseline and follow-up assessment

All patients underwent a 75-g OGTT and blood chemistry analyses before coronary stenting and before follow-up angiography. Blood samples were collected from each patient, and plasma glucose (PG) levels and immunoreactive insulin (IRI) levels were measured by the enzymatic method and radioimmunoassay at baseline, 0.5, 1, and 2 hours after oral glucose load. According to the World Health Organization criteria,[15] normal fasting glucose (fasting PG of <110 mg/dL), impaired fasting glucose (fasting PG of ≥110-126 mg/dL), impaired glucose tolerance (IGT; fasting PG of <126 mg/dL and 2-hour PG of ≥140-200 mg/dL), and diabetes mellitus (fasting PG of ≥126 mg/dL and/or 2-hour PG of ≥200 mg/dL) were diagnosed. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula: HOMA-IR = fasting PG (mg/dL) × IRI (μU/mL) / 405.[16] Levels of glycosylated hemoglobin A_{1c} (HbA_{1c}), total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, and triglyceride were measured by the enzymatic method. To assess visceral fat area, abdominal CT was performed at the same time.

Coronary stenting

In all patients, PCI was performed with the conventional femoral approach by cardiologists who were unaware of the patients' treatment assignments. After administration of intracoronary isosorbide dinitrate, a 40-MHz IVUS catheter (Boston Scientific, Natick, MA) was advanced to the distal side beyond the target lesion, and IVUS images were recorded on S-VHS videotape for offline analysis using automatic pullback (0.5 mm/sec). The lesion was defined as the site with smallest lumen, and the references were defined as the sites with the largest lumen within 10 mm proximal and distal to the lesion. Stents (7 different kinds of BMS) were implanted based on IVUS measurements as previously described.[17] All patients received aspirin (81 mg/d) indefinitely and ticlopidine (200 mg/d) for 4 weeks after coronary stenting.

Quantitative angiographic analysis

Quantitative coronary angiography (QCA) was performed using an automated digital edge-detection system (General Electric Medical Imaging Systems, Waukesha, WI) by 2 observers who were unaware of the patients' treatment assignments as previously described.[17] In-stent restenosis was evaluated with late loss of minimum lumen diameter (MLD) and percent diameter stenosis. Binary restenosis was defined as a diameter stenosis of ≥50% at follow-up angiography.[18]

Evaluation of quantitative IVUS measurements

According to American College of Cardiology Task Force on Clinical Expert Consensus Documents on IVUS,[19] quantitative IVUS measurements were performed by a single observer who was blinded to the patients' treatment assignments using a computer-assisted imaging analyzer (3D Netra IVUS, SciImage, Los Altos, CA) as previously described.[17] Results of IVUS measurements were reproduced by measuring cross-sectional stent areas (stent CSAs) and cross-sectional lumen areas (lumen CSAs) on dual images at baseline and at 6-month follow-up. The same anatomical segments in which stents were implanted were identified on the basis of vessel landmarks, such as calcium deposit and side branches. An intimal area was calculated as stent CSA minus lumen CSA, and an intimal index was defined as intimal area divided by stent CSA. Maximal intimal area and maximal intimal index were defined as intimal area and intimal index of the lesion with minimal lumen area, respectively.

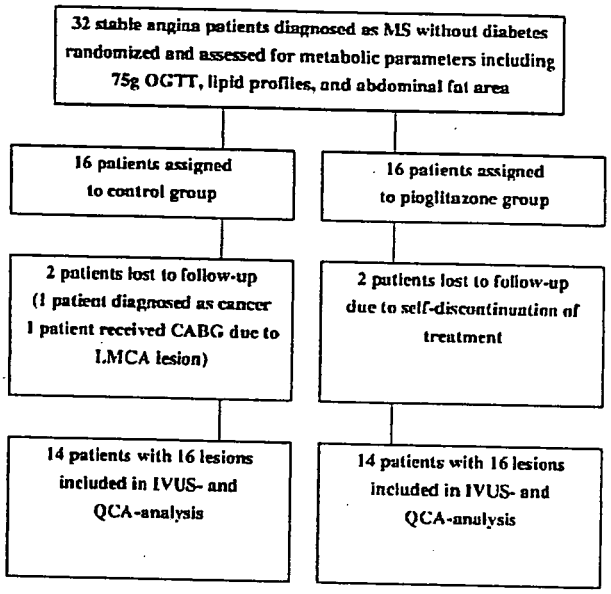
Study end points

The primary end point of this study was reduction of neointimal hyperplasia evaluated by intimal index, a prespecified parameter for evaluating neointimal hyperplasia by IVUS regardless of stent CSA levels.[8,20] Intimal index rather than intimal area is considered as a more reliable parameter for the assessment of neointimal hyperplasia because most of recent IVUS studies have mainly reported and compared percentage in-stent neointimal hyperplasia volume, a 3-dimensional IVUS measure of intimal index.[2] The secondary end points were intimal area, late loss of MLD, percent diameter stenosis, binary restenosis rate, and target vessel revascularization (TVR).

Statistical analysis

Sample size calculation was based on previous observations showing reduction of in-stent neointimal hyperplasia (40%

Figure 1



Flow chart of the study protocol. *CABG*, Coronary artery bypass grafting; *LMCA*, left main coronary artery.

reduction in intimal index and intimal area) by troglitazone and pioglitazone in patients with type 2 diabetes.[8,9] It was estimated that pioglitazone treatment would reduce intimal hyperplasia after BMS implantation in patients with MS by 40% compared with control subjects. Therefore, a minimum of 15 stented lesions in each group would be required to detect such a difference, with a power of 0.80 and a 2-tailed α significance level of .05. These assumptions are consistent with the sample size calculations by Mehran et al,[21] showing that a minimum of 12 stented lesions per treatment arm would be required to show a 50% reduction in intimal hyperplasia volume measured by IVUS as a primary study end point.

Statistical analysis was performed using StatView 5.0 software (SAS Institute, Cary, NC). Quantitative data are shown as mean ± SD. Differences in continuous variables were analyzed by Mann-Whitney *U* test (variables with asymmetric distribution) or Student *t* test (variables with normal distribution), and differences in categorical variables were compared by χ^2 analysis or by Fisher direct test. In all statistical testing, a 2-tailed *P* value of <.05 was considered to be statistically significant. The study was designed on an intention-to-treat basis; however, data were analyzed on a per protocol analysis because of loss to follow-up. We performed lesion-based analysis for the assessment of the study end points because lesion-to-lesion independence of restenosis after BMS implantation within the same patient was validated by a previous study.[22]

Results

Thirty-two nondiabetic patients with MS after coronary stenting (16 patients in PIO and 16 patients in CONT) were enrolled in this study. In PIO, one patient who complained of dizziness and headache and one patient who experienced systemic edema after receiving pioglitazone were excluded from the study because of self-discontinuation of treatment. In CONT, one patient who was diagnosed with cancer during follow-up and one patient who developed severe stenosis of the left main coronary artery were excluded from the study. Finally, 28 patients (14 patients with 16 lesions in PIO and 14 patients with 16 lesions in CONT) completed the study protocol (Figure 1).

Table I. Baseline clinical characteristics

	PIO (16 lesions)	CONT (16 lesions)	P
Age (y)	60.1 ± 15.3	61.3 ± 3.8	.45
Sex (male/female)	15/1	14/2	.99
Risk factor (n [%])			
Hypercholesterolemia	9 (56)	2 (13)	.023
Hypertension	12 (75)	14 (88)	.65
Current smoking	12 (75)	14 (88)	.65
Treatment (n [%])			
Nitrate	5 (31)	11 (69)	.076
β-adrenergic blocker	11 (69)	1 (6)	.001
Calcium antagonist	5 (31)	7 (44)	.71
Statin	14 (88)	10 (63)	.22
IGT (n [%])	14 (88)	11 (69)	.39
Fasting PG (mg/dL)	98.0 ± 12.1	104.8 ± 15.0	.22
2-h PG (mg/dL)	169.2 ± 54.4	175.9 ± 56.8	.76
Fasting IRI (μU/mL)	12.1 ± 4.4	8.6 ± 4.1	.049
2-h IRI (μU/mL)	120.9 ± 84.1	115.6 ± 50.3	.84
HOMA-IR	2.88 ± 1.03	2.28 ± 1.23	.19
HbA_{1c} (%)	5.47 ± 0.40	5.36 ± 0.46	.55
Total cholesterol (mg/dL)	186.2 ± 37.6	193.6 ± 38.4	.62
LDL-C (mg/dL)	119.9 ± 32.8	115.4 ± 33.8	.73
HDL-C (mg/dL)	39.8 ± 10.5	42.0 ± 14.4	.73
Triglyceride (mg/dL)	150.9 ± 72.1	157.3 ± 107.9	.86
Visceral fat area (cm^2)	167.2 ± 61.6	139.6 ± 45.1	.23

Data are mean ± SD.

Clinical characteristics, blood chemical analyses, and visceral fat measurements

Baseline clinical characteristics are shown in Table I. The proportion of patients with hypercholesterolemia and of patients receiving β-adrenergic blockers was significantly higher in PIO compared with CONT. There was no significant difference in the percentage of IGT between the 2 groups. There were no significant differences in fasting PG levels, 2-hour PG levels, and HbA_{1c} levels at baseline and at follow-up between the 2 groups. On the other hand, fasting IRI levels at baseline were significantly higher in PIO compared with CONT, and 2-hour IRI levels at follow-up were lower in PIO compared with CONT (67.1 ± 28.8 μU/mL vs 151.9 ± 185.7 μU/mL, *P* = .027). There was a nonsignificant decrease in HOMA-IR at follow-up in PIO,

Table II. Changes of blood chemical data and visceral fat measurements from baseline values at follow-up

	PIO (16 lesions)	CONT (16 lesions)	P
Change in fasting PG (mg/dL)	−7.2 ± 8.4	−6.9 ± 13.2	.94
Change in 2-h PG (mg/dL)	−14.2 ± 38.2	−23.4 ± 48.6	.59
Change in fasting IRI (μU/mL)	−2.3 ± 5.1	0.70 ± 5.9	.18
Change in 2-h IRI (μU/mL)	−53.8 ± 70.8	36.3 ± 180.8	.080
Change in HOMA-IR	−0.74 ± 1.22	0.020 ± 1.71	.21
Change in HbA_{1c} (%)	−0.10 ± 0.39	−0.040 ± 0.24	.65
Change in total cholesterol (mg/dL)	−1.7 ± 20.4	−1.1 ± 37.1	.79
Change in LDL-C (mg/dL)	−8.2 ± 18.7	−0.40 ± 30.1	.44
Change in HDL-C (mg/dL)	9.3 ± 8.2	5.8 ± 10.4	.34
Change in triglyceride (mg/dL)	−34.0 ± 32.1	−13.9 ± 74.4	.13
Change in visceral fat area (cm^2)	−32.8 ± 28.4	−7.1 ± 18.9	.019

Data are mean ± SD.

whereas no decrease was observed in CONT (Table II). There were no significant differences in plasma lipid profiles including total cholesterol levels, LDL-C levels, HDL-C levels, and triglyceride levels between the 2 groups. Visceral fat areas measured by abdominal CT were significantly decreased at follow-up in PIO compared with CONT (Table II).

Angiographic and procedural characteristics

All 28 patients underwent coronary stenting, and procedural success was achieved in all 32 lesions without major inhospital complications. There was no significant difference between the 2 groups as to the proportion of thinner-strut stents, which elicit less angiographic and clinical restenosis than thicker-strut stents.[23] Findings of lesion characteristics and procedural data are shown in Table III. Late loss of MLD and percent diameter stenosis were significantly decreased in PIO compared with CONT (Figure 2, *A* and *B*). Binary restenosis rate was 0% in PIO versus 31% in CONT (P = .043). Three patients in CONT underwent TVR, whereas no patient in PIO required it (P = .226).

Quantitative IVUS measurements

There were nonsignificant increases in stent CSA and lumen CSA at follow-up in PIO compared with CONT (9.7 ± 2.7 mm^2 vs 9.2 ± 3.2 mm^2, P = .66; 8.4 ± 2.6 mm^2 vs 7.3 ± 3.2 mm^2, P = .30; respectively). Averaged intimal index and maximal intimal index were significantly reduced in PIO compared with CONT (Figure 3, *A*). Averaged intimal area and maximal intimal area were also reduced in PIO compared with CONT but not statistically significant (Figure 3, *B*).

Table III. Findings of lesion characteristics and procedural data

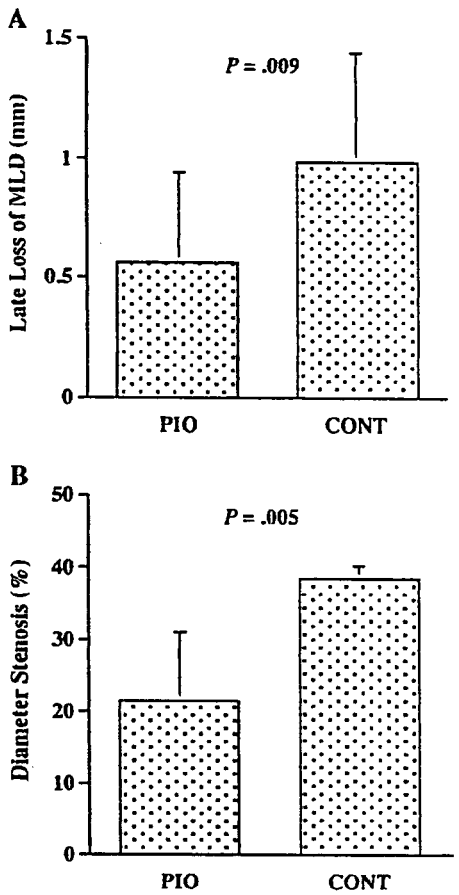
	PIO (16 lesions)	CONT (16 lesions)	P
Target vessel (coronary artery, n) RCA/LAD/LCX	2/6/8	6/4/6	.26
ACC/AHA lesion classification (n) Low risk/moderate risk/high risk	7/9/0	7/9/0	.52
Maximal inflation pressure (atm)	14.9 ± 2.6	13.8 ± 3.1	.27
Stent diameter (mm)	3.41 ± 0.50	3.41 ± 0.46	.91
Stent length (mm)	16.4 ± 4.9	18.2 ± 4.2	.28
Lesion length (mm)	14.1 ± 4.4	16.3 ± 3.8	.11
Reference diameter (mm)			
Before stenting	3.05 ± 0.54	2.99 ± 0.41	.71
After stenting	3.30 ± 0.57	3.23 ± 0.46	.68
Minimum lumen diameter (mm)			
Before stenting	0.98 ± 0.26	0.83 ± 0.40	.23
After stenting	2.79 ± 0.50	2.82 ± 0.53	.89
Diameter stenosis (%)			
Before stenting	67.6 ± 8.7	70.8 ± 14.4	.45
After stenting	14.4 ± 8.6	12.4 ± 8.2	.50
Stent CSA after stenting (mm^2)	9.8 ± 2.5	9.2 ± 3.2	.76
Lumen CSA after stenting (mm^2)	9.8 ± 2.5	9.2 ± 3.2	.76

Data are mean ± SD. *RCA*, Right coronary artery; *LAD*, left anterior descending artery; *LCX*, left circumflex artery; *ACC*, American College of Cardiology; *AHA*, American Heart Association.

Discussion

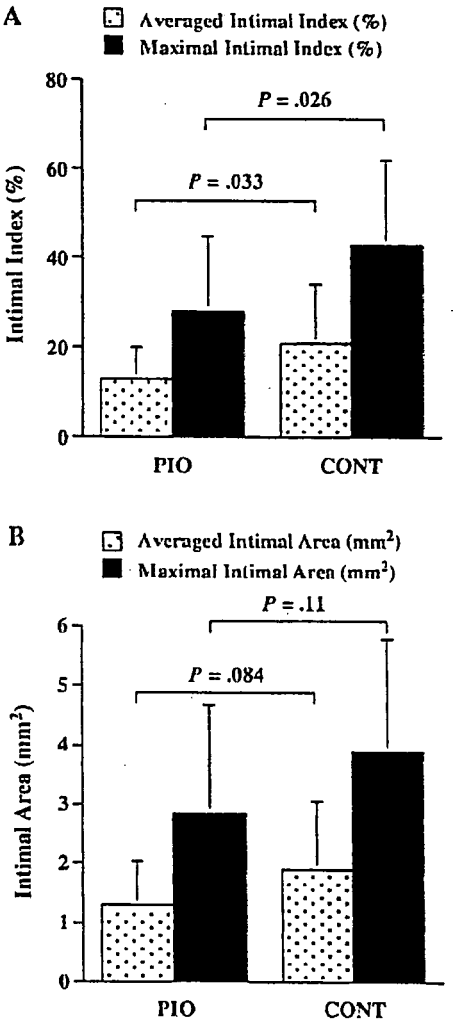
This is the first randomized, prospective IVUS study demonstrating that pioglitazone reduces neointimal hyperplasia after BMS implantation in nondiabetic patients with MS. Neointimal hyperplasia assessed by intimal index was significantly reduced in PIO compared with CONT, and there was a nonsignificant trend toward reduced intimal area in PIO. As to QCA data, late loss of MLD, percent diameter stenosis, and binary restenosis rate were significantly decreased in PIO compared with CONT. These findings indicate that this study has met the primary end point and the secondary end points except for intimal area and TVR. With regard to metabolic changes, pioglitazone treatment improved insulin resistance and decreased visceral fat accumulation, which is closely associated with insulin resistance, without significant changes in PG levels, HbA_{1c} levels, and lipid profiles. Improvement of insulin resistance is supported by the findings that pioglitazone treatment ameliorated a significant increase in fasting IRI levels and significantly decreased 2-hour IRI levels, which is positively correlated with coronary artery disease,[24] and that there was a nonsignificant trend toward decreased HOMA-IR after pioglitazone treatment. Furthermore, Takagi et al[25] have shown that hyperinsulinemia during a 75-g OGTT is associated with increased neointimal hyperplasia after coronary stenting in nondiabetic

Figure 2



Late loss of MLD and percent diameter stenosis assessed by QCA at 6-month follow-up. Late loss of MLD (**A**) and percent diameter stenosis (**B**) were significantly decreased in PIO compared with CONT.

Figure 3



Intimal index and intimal area measured by IVUS at 6-month follow-up. An intimal area was calculated as stent CSA minus lumen CSA, and an intimal index was defined as intimal area divided by stent CSA. **A**, Averaged intimal index and maximal intimal index were significantly reduced in PIO compared with CONT. **B**, There was a nonsignificant trend toward reduced intimal area in PIO compared with CONT.

patients. Taken together, our results indicate that reduction of neointimal hyperplasia by pioglitazone in nondiabetic patients with MS is likely due to the improvement of insulin resistance.

However, several studies demonstrated pleiotropic protective effects of TZDs on the development and progression of atherosclerosis as well as the inflammatory process in addition to improving glucose and lipid metabolism.[26,27] It has been shown that TZDs inhibits migration and proliferation of vascular smooth muscle cells by decreasing matrix metalloproteinase production and by inducing cell cycle arrest or apoptosis.[28,29] Recently, Marx et al[11] have demonstrated that pioglitazone reduces neointima volume after coronary stenting in nondiabetic patients. Although they neither performed a 75-g OGTT nor evaluated insulin resistance, the lack of significant changes in PG levels, IRI levels, HbA_{1c} levels, and lipid profiles suggests the direct inhibitory effect of pioglitazone on neointima formation independent of its metabolic action.

The effect of MS on in-stent restenosis could be influenced by the proportion of patients with diabetes or insulin resistance. Rana et al[13] have shown that MS is not associated with TVR or the combined end point of death, myocardial infarction, and TVR. On the other hand, insulin resistance, a major pathogenesis of MS, has been

demonstrated to be an independent risk factor of restenosis after coronary stenting.[6] In the present study, most nondiabetic patients with MS were diagnosed as IGT by a 75-g OGTT as shown in Table I. Moreover, the best predictor of in-stent restenosis in nondiabetic patients has been reported to be the sum of insulin levels after a 75-g OGTT.[30] Taken together, it is likely that MS is a risk factor of restenosis after coronary stenting in nondiabetic patients when it is associated with insulin resistance such as impaired fasting glucose or IGT.

Our study has demonstrated that pioglitazone reduces neointimal hyperplasia after BMS implantation in nondiabetic patients with MS. Consistent with our results, a recent study from Cao et al[12] has shown that rosiglitazone could reduce the risk of the adverse cardiovascular event and improve clinical outcomes in nondiabetic patients with MS after DES implantation. Based on these findings, it is reasonable to conclude that pioglitazone treatment may represent a novel therapeutic tool to target in-stent restenosis in nondiabetic patients with MS.

Study limitations

Some limitations exist in our study. The proportion of patients with hypercholesterolemia and elevated fasting IRI levels in PIO were higher than that in CONT, although these were not associated with neointimal hyperplasia assessed by linear regression analysis. According to the study from Piatti et al,[6] higher levels of baseline insulin resistance combined with hypercholesterolemia in PIO could be highly predictive of in-stent restenosis at baseline. Nevertheless, neointimal hyperplasia after coronary stenting was significantly reduced in PIO compared with CONT, suggesting a potential effect of pioglitazone on the inhibition of restenosis. As to the differences between the 2 groups in the use of β-adrenergic blockers and nitrate, they were not associated with neointimal hyperplasia as evaluated by linear regression analysis. However, important consideration for these differences should be necessary in a larger trial in which clinical end points are used.

This is not a double-blind, placebo-controlled study, and the number of study patients is small. Nonsignificant reduction of intimal area and TVR are presumably due to the small sample size. Although IVUS- and QCA-driven end points suggest the antirestenotic effect of pioglitazone in patients with MS, a double-blind, placebo-controlled study in a large population of DES-treated patients with MS is necessary to show an important clinical benefit of pioglitazone treatment. We tried to assess an independent predictor of reduction in neointimal hyperplasia after coronary stenting in patients with MS because reduction in insulin levels and in visceral fat area seemed to be associated with reduction in neointimal hyperplasia. However, linear regression analysis showed no significant correlation between these parameters.

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Comparison of Once-Daily Glargine Plus Sulfonylurea with Twice-Daily 70/30 Aspart Premix in Insulin-Naive Japanese Patients with Diabetes

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ABSTRACT

Background: Type 2 diabetes patients insufficiently controlled with sulfonylurea (SU) are commonly treated by switching to twice-daily premix insulin replacing SU. The efficacy of glargine (GL) added on to SU compared with the premix therapy has not been analyzed in Japan.

Methods: The open-label two-arm study was conducted in 30 type 2 diabetes patients poorly controlled [hemoglobin A_{1c} (HbA_{1c}) >7.5%] with SU with or without other oral hypoglycemic agents (OHAs). The GL group injected once-daily GL in addition to the OHAs. The aspart 70/30 (70/30) group discontinued SU among the OHAs and injected twice-daily 70/30. Patients were recommended either method in a block random method, and if twice-daily 70/30 was rejected, once-daily GL was selected only at the first time. The insulin dose was titrated to achieve a target fasting plasma glucose of <120 mg/dL and/or HbA_{1c} of <7%.

Results: Nineteen of 20 patients treated with GL and 11 of 14 patients treated with 70/30 completed the 6-month study. Mean HbA_{1c} improved from 8.45% to 7.5% in the GL group and from 9.13% to 7.93% in the 70/30 group. The mean HbA_{1c} decrease during 6 months was −0.95% in the GL group and −1.20% in the 70/30 group ($P = 0.49$). Mean insulin doses at 6 months were 12.0 units/day for the GL group and 26.7 units/day for the 70/30 group. Both therapies were well tolerated without severe hypoglycemia.

Conclusion: Once-daily GL injection added on to OHAs was equally safe and effective compared with twice-daily injection of aspart 70/30 premix replacing SU in type 2 patients insufficiently controlled with OHAs.

INTRODUCTION

THE NUMBER OF PATIENTS WITH DIABETES is increasing dramatically. The number of patients who require insulin injection for blood sugar control is also increasing beyond the capacity of the specialists. Therefore, simple and reliable methods to start insulin applicable by the general physician are required.

For those patients who are inadequately controlled by oral hypoglycemic agents (OHAs), replacing the OHA with premix insulin before breakfast and dinner is the commonly used method in Japan. One problem with this re-

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placement method is the lack of a reliable method to predict exactly what unit dosage of insulin is required. To avoid unexpected hypoglycemia, the starting dose is usually set to a low level and titrated based on self-monitored blood glucose (SMBG). However, without frequent dose adjustment, blood glucose control may be rather deteriorated during the first 1–2 months. This is why hospitalization is still preferred by the common practitioner when insulin therapy is started. This is an obstacle to the timely addition of insulin, which is important to keep good blood glucose control.[1–3] On the other hand, addition of a small dose of basal insulin to OHAs is easier to start without hospitalization. Starting evening premix injection with glimepiride compared with insulin alone was shown to cause fewer dropouts and comparable blood glucose control with less insulin.[4]

In many cases, patients who need insulin therapy are already treated with a combination of several OHAs. What kind of OHA should be used with insulin is another problem. Raskin et al.[5] compared once-daily glargine (GL) and twice-daily aspart 70/30 premix (70/30) in patients poorly controlled with OHAs after the metformin titration period during when sulfonylurea (SU) was discontinued. In their study, 70/30 achieved better control compared with GL. However, the effect of GL in combination with SU was not known. Janka et al.[6] compared GL plus OHA (glimepiride and metformin) versus switching to twice-daily human premixed insulin. In their study, the GL plus OHA group had more pronounced mean hemoglobin A_{1c} (HbA_{1c}) reduction and fewer confirmed nocturnal hypoglycemic episodes. Roach and Malone[7] compared twice-daily lispro mixture 25/75 (25% insulin lispro and 75% NPL) plus OHA (metformin and/or SU) and once-daily GL plus OHA. In their study, end-point HbA_{1c} was lower in the lispro premix group, and mean postprandial plasma glucose was lower during the test-meal period. Mild hypoglycemia was noted in eight of 10 patients in the lispro premix group and three of 10 patients in the GL group.

The introduction of GL alleviated the concern about nocturnal hypoglycemia when used with an OHA.[8,9] Addition of GL to SU plus metformin combination therapy was compared with addition of rosiglitazone.[10] The reduction of HbA_{1c} was greater and body weight gain was less with GL compared with rosiglitazone. Studies analyzing insulin combination therapy with metformin, thiazolidinedione glinides, and α-glucosidase inhibitors have generally reported improvement in glycemic control.[11–17] Metformin, glitazones, and glimepiride are reported to reduce insulin requirements in patients poorly controlled with insulin.[14–17] Combination of SU with premix insulin may increase the risk of severe hypoglycemia. However, metformin, pioglitazone, or α-glucosidase inhibitors used with premix insulin may be of benefit without risk of severe hypoglycemia.

Therefore, in this study we compared once-daily GL added to OHAs (including SU) and replacing SU with twice-daily 70/30 aspart premix combined with OHAs other than SU.

SUBJECTS AND METHODS

Patient recruitment

Male or female patients 40–75 years old with type 2 diabetes' duration over 1 year and treated with OHAs including SU were recruited. The dose of SU was either glibenclamide >5 mg/day or glimepiride >3 mg/day used over 12 weeks. Combination with metformin, pioglitazone, or α-glucosidase inhibitor was allowed. Further inclusion criteria included HbA_{1c} levels between 7.5% and 12.0% and fasting plasma glucose (FPG) >140 mg/dL (>7.8 mmol/L). Exclusion criteria included prior use of insulin within 12 weeks or fasting serum C-peptide levels less than 0.7 ng/mL. The study protocol was approved by the ethical board of Jichi Medical University. The initial intent was to include 40 patients, 20 in each group. This number was calculated to detect the difference of absolute difference of 1.3% for HbA_{1c} reduction with an α error of 0.05 (two sided) and a β error of 0.20. Patients who came to the clinic of this institute from September 2004 to August 2005 were screened for the inclusion criteria. The initial 30 patients were recruited during this period, and then the recruitment period was extended a further 6

months. In total, 20 patients for the GL group and 14 patients for the 70/30 group were enrolled.

Study design

This is an open-label two-arm study that was conducted in a single center. The primary efficacy measure was the change of HbA_{1c} from baseline at 6 months after start of insulin therapy. The secondary efficacy measurements were percentage of patients who achieved the target HbA_{1c} or FPG. The dose of insulin and number of hypoglycemic episodes were also recorded. All the participants provided written informed consent before entry into the study. The GL group started once-daily GL injection in addition to the currently used OHAs. The 70/30 group discontinued SU among the OHAs and started twice-daily 70/30 injection. Therefore, metformin, pioglitazone, or α-glucosidase inhibitor was continued if it was used at the entry time. Patients were recommended to start insulin injection by either method in a block random fashion. If twice-daily 70/30 was rejected, once-daily GL was selected as a second choice. This decision was made only at the start point. No switching to the other group was allowed during the study period. Training for insulin injection and self-monitoring of blood glucose was given by a teaching nurse. The insulin injection device was the OptiClick® (sanofi-aventis, Tokyo, Japan) for the GL group and the Flexpen® (Novo Nordisk, Tokyo) for the 70/30 group. The initial dose of insulin was 6–8 units for the GL group and 10–16 units divided 1:1 or 2:1 before breakfast and dinner for the 70/30 group. The initial dose was chosen dependent on the patient's body weight. Patients were asked to come 1 or 2 weeks after starting insulin injection, when the patient's injection procedure was checked and the dose was re-adjusted. Thereafter, patients were followed up every month, and insulin doses were titrated to achieve a target FPG of <120 mg/dL and/or a HbA_{1c} of <7.0%. Patients' home blood glucose monitoring data were also used for the titration. Patients were asked to monitor their blood glucose once, before breakfast, in the GL group and twice, before breakfast and dinner, in the 70/30 group. The guideline for GL dose titration was as follows: FPG >140 mg/dL, increased by 4 U/day; FPG >120–140 mg/dL, increased by 2 U/day; FPG >100–120 mg/dL, increased by 1 U/day. For the 70/30 group, the morning and evening doses were increased dependent on before-dinner plasma glucose and FPG, respectively: plasma glucose >140 mg/dL, increased by 4 U per injection; plasma glucose >120–140 mg/dL, increased by 2 U per injection; plasma glucose >100–120 mg/dL, increased by 1 U per injection. Therefore, the dose increment for the 70/30 group was doubled as compared with the GL group when plasma glucose was high. Body weight was measured at 3 and 6 months after starting insulin. A questionnaire about hypoglycemia was done 3 months after starting insulin.

Biochemical values and other determinants

The HbA_{1c} measurement was performed by the high performance liquid chromatography method (Arkray, Kyoto, Japan). Plasma glucose and serum lipids were analyzed by an automated blood chemistry analyzing system (JEOL, Tokyo). SMBG measurement was performed using the Diameter (Arkray).

Statistical analysis

For statistical analysis, two-tailed t test was used for continuous values. For comparison of frequency, the χ^2 test was used. A value of $P < 0.05$ was noted as significant.

RESULTS

Patient characteristics

In total, 34 patients were enrolled in the study. All except for two patients started insulin as an outpatient without hospitalization. Nineteen of 20 patients treated with GL and 11 of 14 patients treated with 70/30 completed the 6-month study (Table 1). In the GL group one patient could not come to this hospital and was treated by a local physician for several months. When she started GL injections, her HbA_{1c} was 13.2%. When she returned to this hospital again, her HbA_{1c} was 8.3%; however, the GL dose was fixed at 16 U/day and had not been increased for 7 months. This patient was excluded from the analysis. In the 70/30 group,

TABLE 1. PROFILES OF PATIENTS WHO COMPLETED THE STUDY

	GL + OHA	*70/30 + OHA other than SU*	P *value*
Age (years)	61.7 ± 8.4	55.9 ± 8.8	0.083
Sex (male/female)	13/6	6/5	0.45
Disease duration (years)	10.4 ± 7.4	9.8 ± 6.2	0.82
OHAs			
Glimepiride	15	11	
Glibenclamide	4	0	
Metformin	14	8	0.95
Pioglitazone	5	1	0.26
α-Glucosidase inhibitor	7	4	0.98

two patients discontinued the study because of their own decision, and one patient switched to once-daily NPH injection because of an inability to inject twice and fear of hypoglycemia. This patient was excluded from the analysis. The imbalance of numbers was caused by the strategy to allow the GL method when the patient initially refused twice-daily 70/30 injection. This problem caused a deviation of sex and age in the two groups. Percentages of females and younger patients were higher in the 70/30 group, although statistically not significant. We considered the difficulty of completing the study according to the initial plan. Therefore we decided to present the results as a pilot study of this kind of treatment in Japan. Before starting the study, all the patients were treated with at least one SU with or without other OHAs. Twenty-six patients were taking glimepiride (mean 5.7 mg), and four were taking glibenclamide (mean 6.9 mg). Twenty-four patients were taking metformin (mean 560 mg), pioglitazone (mean 27.5 mg), or both in combination with SU. Thirteen patients were taking α-glucosidase inhibitor in combination with SU. Unexpectedly, HbA_{1c} at baseline was significantly lower in the GL group ($P = 0.04$). There was no significant difference in the FPG and lipid profiles at baseline (Table 2).

TABLE 2. COMPARISON OF HbA_{1c}, FPG, BODY WEIGHT, BMI, AND LIPID PROFILES

	GL + OHA (n = 19)	*70/30 + OHA other than SU (n = 11)*	P *value*
HbA_{1c} (%)			
At baseline	8.45 ± 0.59	9.13 ± 1.13	0.04
At 6 months	7.50 ± 0.48[a]	7.93 ± 1.05[b]	0.19
Decrease	−0.95 ± 0.84	−1.2 ± 1.06	0.49
FPG (mg/dL)			
At baseline	184.1 ± 42.1	183.3 ± 54.6	0.90
At 6 months	136.0 ± 40.3[c]	141.4 ± 59.8[b]	0.79
Body weight (kg)			
At baseline	68.1 ± 14.0	61.3 ± 13.6	0.22
At 6 months	68.5 ± 13.4	61.8 ± 13.0	0.20
BMI (kg/m^2)			
At baseline	25.5 ± 4.1	23.9 ± 4.4	0.33
At 6 months	25.6 ± 3.9	24.1 ± 4.3	0.33
Total cholesterol			
At baseline	195.7 ± 27.1	201.6 ± 35.6	0.61
At 6 months	184.4 ± 26.7	201.8 ± 39.2	0.16
HDL cholesterol			
At baseline	49.7 ± 11.4	51.5 ± 17.1	0.72
At 6 months	46.2 ± 8.9[c]	53.1 ± 14.8	0.12
Triglyceride			
At baseline	139.4 ± 62.8	140.5 ± 90.0	0.97
At 6 months	107.2 ± 35.6[c]	101.7 ± 43.5	0.71

[a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$ compared with baseline in each group.

Changes in HbA_{1c}, FPG, body weight, and lipid profiles

During the 6 months, mean HbA_{1c} improved from 8.45 ± 0.59% to 7.50 ± 0.48% (average ± standard deviation, $P < 0.001$) in the GL group and from 9.13 ± 1.13% to 7.93 ± 1.05% ($P < 0.01$) in the 70/30 group (Table 2). The curves of HbA_{1c} reduction were almost parallel in the two groups (Fig. 1A). The HbA_{1c} decrease during the 6 months was −0.95% and −1.20% in the GL group and the 70/30 group, respectively (Fig. 1B). There was no statistical difference in the HbA_{1c} decrease. At 6 months after insulin was started, the target HbA_{1c} level of <7.0% was achieved by six patients (31.6%) in the GL group and one patient (9.1%) in the 70/30 group. The high target HbA_{1c} achievement rate of the GL group may be influenced by the lower baseline HbA_{1c}. At 6 months after insulin was started, the target FPG of <120 mg/dL was achieved by seven patients (35.0%) in the GL group and two patients (18.2%) in the 70/30 group. There was no statistical difference in the frequency of those achieving the target HbA_{1c} or the target FPG. In the GL group, high-density lipoprotein (HDL) cholesterol and triglyceride were significantly decreased at 6 months (Table 2). Within this group, one patient was taking bezafibrate at the beginning of the study. In the 70/30 group, total cholesterol and HDL cholesterol did not change significantly. Although triglyceride showed a small decrease in the 70/30 group, this difference did not reach statistical significance. Mean body weight increased during the 6 months by 0.51 kg in the GL group and by 0.42 kg in the 70/30 group. Changes of lipids and body weight from baseline were compared between the two groups. No statistically significant difference was observed.

Age and sex matching

To adjust the sex and age to the 70/30 group, we chose six male patients ranging in age from 40 to 64 years and five female patients ranging in age from 54 to 70 years from the GL group. After this selection, the patients' mean age was 56.8 ± 7.6 years old in the GL group compared with 55.9 ± 8.8 years old in the 70/30 group. However, body weight and body mass index (BMI) were slightly higher and HbA_{1c} was marginally lower (8.35 ± 0.50% vs. 9.13 ± 1.13%, $P = 0.052$) in the GL group. We compared the change of HbA_{1c}, FPG, body weight, and lipid profiles between the age- and sex-matched GL group and 70/30 group. The mean HbA_{1c} decreases from baseline were −0.89% in the GL group and −1.2% in the 70/30 group. There was no statistically significant difference between groups with respect to changes in HbA_{1c}, body weight, and lipid profiles.

Insulin doses

At 6 months the mean insulin dose was 12.0 units for the GL group and 26.7 units for the 70/30 group (Fig. 2). When the patients were divided depending on whether they achieved target HbA_{1c}, the mean insulin dose of achievers in the GL group was 9.7 U/day, while that of non-achievers was 13.1 U/day. In the 70/30 group, the mean insulin dose of achievers was 18 U/day, while that of non-achievers was 27.6 U/day.

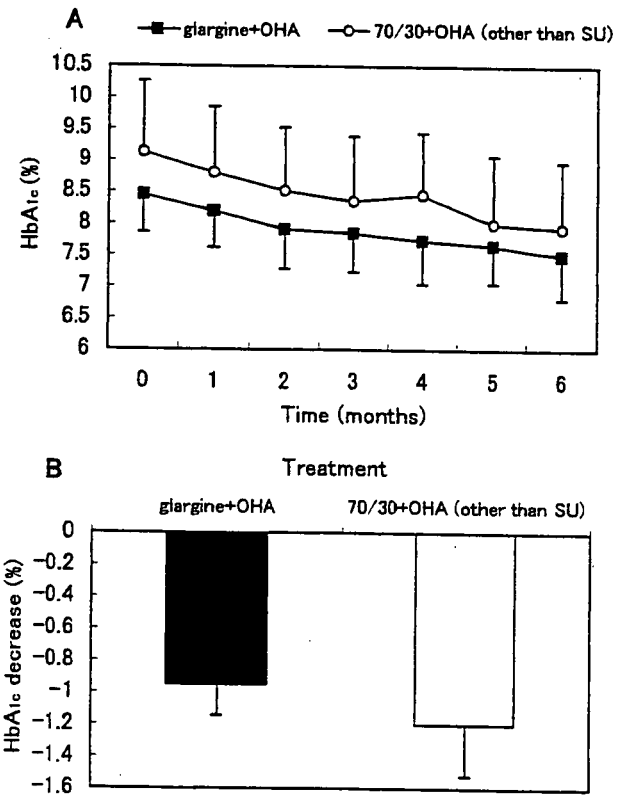

FIG. 1. (A) Change in HbA_{1c} over 6 months (mean ± SD) with insulin GL + OHA and 70/30 premix insulin + OHA other than SU. (B) Improvement in HbA_{1c} (mean decrease from baseline ± SE).

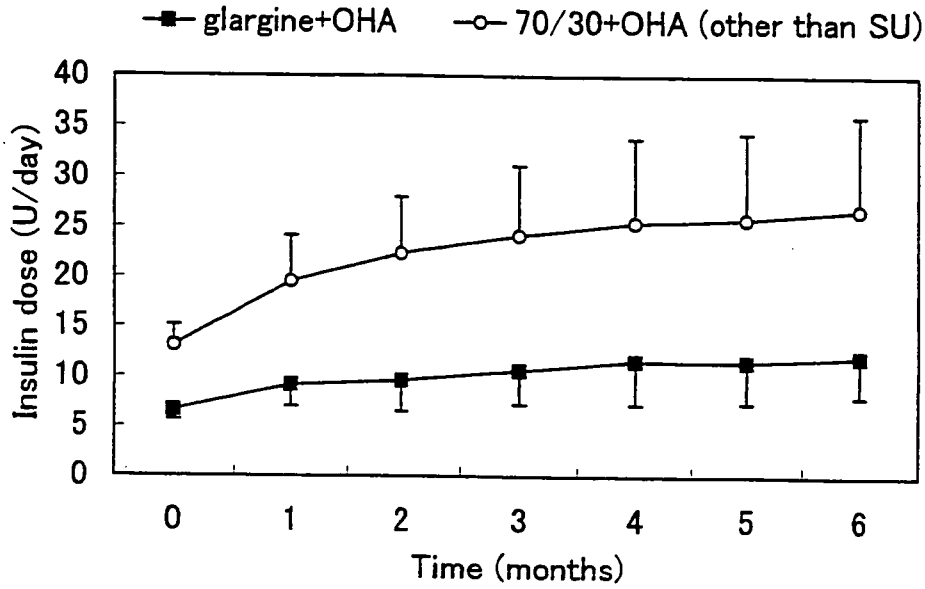

FIG. 2. Insulin dose of GL + OHA (■) and 70/30 + OHA other than SU (O).

Hypoglycemia

The SMBG data were available for seven patients in the GL group and four patients in the 70/30 group. SMBG data lower than 70 mg/dL were observed in 43 episodes in four patients of the GL group and 11 episodes in two patients of the 70/30 group. The questionnaire about hypoglycemia was available from 11 patients in the GL group and five patients in the 70/30 group. Self-reported hypoglycemia was noted in six patients in the GL group and four patients in the 70/30 group. Nocturnal hypoglycemia was reported by two patients in the GL group and three patients in the 70/30 group. The proportion of patients who reported nocturnal hypoglycemia was slightly higher in the 70/30 group; however, it was not statistically significant ($P = 0.094$). Every episode was not severe, and patients could manage their symptom without assistance by another person.

DISCUSSION

The aim of this study was to show that the once-daily injection of GL added to the SU-based OHAs is not inferior to replacing SU with twice-daily premix insulin. During our patient enrollment, a similar study was published by Janka et al.[6] as a well-planned multicenter cooperative study. We considered the difficulty of gathering a sufficient number of patients in a single center and decided to present our results as a pilot study in Japan. The mean decreases of HbA_{1c} levels achieved by 6 months after starting insulin therapy were from 8.45 ± 0.59% to 7.50 ± 0.48% in the GL group and from 9.13 ± 1.13% to 7.93 ± 1.05% in the 70/30 group. We could not find a statistically significant difference between the −0.95% decrease in the GL group and −1.2% in the 70/30 group with this small-scale study. However, larger studies like those presented by Raskin et al.[5] or Janka et al.[6] may prove a significant difference. Addition of GL to SU-based OHAs was well tolerated on an outpatient basis without severe hypoglycemia. The proportions of patients who reported nocturnal hypoglycemia were smaller in the GL group compared with the 70/30 group (two of 11 vs. three of five); however, they were not significantly different between the groups. The proportions of patients who achieved the target HbA_{1c} of <7.0% or the target FPG of <120 mg/dL were higher in the GL group; however, this result may have been influenced by the lower baseline HbA_{1c} level in the GL group. Also, combination of GL with SU resulted in a sparing effect of insulin doses to below half of the premix. This economical benefit is important considering the growing number of patients who need insulin therapy; however, a precise cost/benefit analysis should

depend on effectiveness in controlling complications over a longer interval. We do not take the GL add-on therapy as lifetime treatment. Rather, once-a-day injection is an important way to introduce patients who require insulin to break the psychological barrier to injection. The ease of recruiting the GL group in this study clearly shows this point. We think that the HbA_{1c} decrease by −0.95% in 6 months is acceptable for an introductory method.

There were inevitable biases at the start point. This study was an open-label study, and patients who disliked twice-daily injection were included in the GL group. Female patients and younger patients agreed more readily to the twice-daily injection. Therefore, the average age and BMI were a little lower and the percentage of female patients was larger in the 70/30 group. Although the age and sex differences were not statistically significant, these biases might have resulted in more motivated patients in the 70/30 group. We selected age- and sex-matched patients from the GL group and compared them with the 70/30 group. The HbA_{1c} decrease was somewhat smaller in the GL group; however, the difference was not statistically significant. The doses of insulin used by Japanese patients were smaller compared with Caucasians. This is because the mean BMI is smaller in Japanese patients. The FPG responded well to the GL add-on therapy, and the dose increment was slowed when the FPG was reduced under 120 mg/dL.

There are several limitations to this study. First, we cannot tell whether the once-daily GL injection combined with SU is effective in the long term. Second, postprandial glucose control was not assessed in this study. Third, this study was done on an outpatient basis. Therefore, if the insulin titration was done more vigorously in the hospitalized setting, the twice-daily premix control might have achieved better control. The average dose of GL was smaller compared with the 70/30 premix, and the dose difference between the achievers and non-achievers was also smaller in the GL group. The reason for the small dose difference between achievers and non-achievers in the GL group is that FPG is reduced fairly early in the GL group, and the increase of the dose was limited to avoid early morning hypoglycemia. Also, postprandial glycemic excursion is difficult to control with an SU-stimulated patient's own insulin secretion. To overcome this problem, introduction of premeal rapid-acting insulin should be considered. Recent studies have shown that three times-a-day premeal LysPro or LysPro premix combined was better in controlling postprandial glucose compared with GL when used with an OHA.[18,19] Better control of postprandial glucose is important for preventing complications, and the cost to reduce postprandial glucose may pay in the long run. Valentine et al.[20] analyzed the lifetime cost of twice-daily aspart premix injection and once-daily GL depending on data from Raskin et al.[5] According to their calculation, aspart premix was associated with lower retinopathy and nephropathy, and total lifetime direct costs of aspart premix were £1,319 higher than GL.

Taking these points into consideration, we propose once-daily GL injection added to OHAs as a convenient starting method for insulin treatment on an outpatient basis. Once insulin injection is started and blood sugar control gets better, patients' psychological threshold to multiple insulin injection is lowered. Then we can shift to twice-a-day or more injection therapy depending on the individual patient's need.

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Original Article

Combination Therapy of Angiotensin Converting Enzyme Inhibitor and Angiotensin AT1 Receptor Antagonist in Diabetic Nephropathy

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Background: The present study was undertaken to determine whether combination therapy of angiotensin converting enzyme inhibitor (ACEI) and angiotensin AT1 receptor antagonist (ARA) is a useful tool for reducing albuminuria in diabetic nephropathy.

Methods: Thirty-four subjects with diabetic nephropathy were enrolled in the present study. All the subjects had hypertension and urinary albumin index (UAI) < 1,000 mg/g creatinine. They were divided into three groups. Group 1 of 16 subjects was initially treated with imidapril (5–10 mg). Group 2 of eight subjects had losartan (50–100 mg) added to consecutive therapy with imidapril. Group 3 of 10 subjects had imidapril (5–10 mg) added to consecutive therapy with losartan. Blood pressure and UAI were determined before and 3 and 6 months after the start of the present study.

Results: Blood pressure was significantly decreased in Group 1 subjects. Blood pressure was also reduced in Groups 2 and 3, but its reduction was not significant. Imidapril significantly reduced UAI in Group 1, from 213.1 ± 46.6 to 111.6 ± 35.6 ($p < 0.001$) and 114.5 ± 35.4 mg/g creatinine ($p < 0.01$) 3 and 6 months after imidapril treatment, respectively. The addition of losartan further reduced UAI in Group 2, who had been treated with imidapril. UAI decreased from 328.8 ± 87.3 to 185.3 ± 51.4 ($p < 0.05$) and 244.8 ± 88.3 mg/g creatinine 3 and 6 months after the addition of losartan, respectively. In contrast, the addition of imidapril to losartan therapy did not alter UAI in Group 3.

Conclusion: These results indicate that the addition of ARA to consecutive therapy with ACEI augments a protective effect against the progression of diabetic nephropathy. [*Hong Kong J Nephrol* 2007;9(1):31–5]

Key words: albuminuria, angiotensin AT1 receptor antagonist, angiotensin converting enzyme inhibitor, combination therapy, diabetic nephropathy

背景：本研究旨在調查 ACEI (血管緊張素轉化酵素抑制劑) 與 ARA (血管緊張素 AT1 受體拮抗劑) 的合併療法，是否有助於減少糖尿性腎病患者的白蛋白尿症。

方法：研究對象為三組共 34 位糖尿性腎病兼高血壓患者，尿白蛋白指數 (UAI) < 1,000 mg/g 肌酸酐。第一組 ($n = 16$) 接受 imidapril 5—10 mg 治療，第二組 ($n = 8$) 接受 losartan 50—100 mg 添加於原先的 imidapril 療程，第三組 ($n = 10$) 則接受 imidapril 5—10 mg 添加於 losartan 之中。在上述療程開始前及其後 3 及 6 個月，病人得接受血壓與 UAI 的追蹤。

結果：隨著療程的進行，第一組病人的血壓出現明顯下降；其餘組別的病人亦呈現血壓下降的跡象，但未達統計學差異。在第一組的病人間，在 imidapril 治療開始後 3 及 6 個月，UAI 分別從 213.1 ± 46.6 顯著下降至 111.6 ± 35.6 ($p < 0.001$) 及 114.5 ± 35.4 mg/g 肌酸酐 ($p < 0.01$)。在第二組病人間，隨著 losartan 添加於 imidapril 後 3 及 6 個月，UAI 分別從 328.8 ± 87.3 下降至 185.3 ± 51.4 ($p < 0.05$) 及 244.8 ± 88.3 mg/g 肌酸酐。相反的，在第三組接受 imidapril 添加於 losartan 的病人之中，UAI 並未出現明顯改變。

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結論：從本研究的結果可見，對於糖尿性腎病患者，在 ACEI 療程添加 ARA 可達到更進一步的腎臟保護作用。

Introduction

Diabetic nephropathy is the major cause of end-stage renal disease, which is increasing year by year. Intervention for prevention and delaying the progression of end-stage renal disease is an important issue. The evidence that the control of blood glucose and blood pressure is effective for diabetic nephropathy is already established [1–5]. However, there is limited effective intervention, except for plasma glucose and blood pressure control at present. The renal hemodynamic effect of angiotensin II plays a role in the development of nephropathy in diabetic subjects [6]. Angiotensin II is a strong vasoconstrictor hormone. There are afferent and efferent arterioles in glomerulus, and angiotensin II specifically constricts efferent arterioles, and thus provides intraglomerular hypertension. Besides, angiotensin II has many additional actions, including vascular smooth muscle cell proliferation, collagen synthesis, superoxide production, platelet aggregation, plasminogen activator inhibitor-1 (PAI-1) activation, and others [6–8]. It is evident that inhibition of the renin–angiotensin system prevents the development of diabetic nephropathy. There are two classes of drugs, namely, angiotensin converting enzyme inhibitor (ACEI) and angiotensin AT1 receptor antagonist (ARA), and their therapeutic efficacy has been proven in diabetic nephropathy [9–17]. Because there are some differences in the pharmacologic action between ACEI and ARA, renoprotective effect is not always uniform [18]. Several studies have depicted the beneficial effect of a combination therapy of these two agents [17,19, 20]. In the present study, we determined whether combination therapy with ACEI and ARA is effective in reducing albuminuria in diabetic subjects.

Subjects and Methods

Subjects

Thirty-four subjects with type 2 diabetes mellitus were enrolled in the present study between January 2003 and May 2005. There were 19 males and 15 females, with a mean age of 65.4 ± 8.7 years (range, 36–77 years). All subjects had been attending the outpatient clinic of Jichi Medical University Omiya Medical Center. They had hypertension and albuminuria < 1,000 mg/g creatinine. They were subgrouped into three groups according to the administration of imidapril, an ACEI, and/or losartan, an ARA. Group 1 included 16 subjects (7 males; 9 females) who were initially started on imidapril 5 mg/day. The dose was increased to a maximum of 10 mg/day during the observation period. Group 2 included eight subjects (5 males; 3 females). The subjects had taken imidapril 10 mg/day for at least 6 months, and began to take losartan 50 mg/day as a second drug. The dose of losartan was increased to 100 mg/day 3 months after the start of the present study in all eight subjects. Group 3 included 10 subjects (7 males; 3 females). They had taken losartan 100 mg/day for at least 6 months, and then began to take a second agent of imidapril 5 mg/day. The dose of imidapril was increased to 10 mg/day 3 months after the start of the present study in all 10 subjects. The subjects had been treated at our outpatient clinic for an additional 6 months. During this observation period, urinary excretion of albumin was determined at 3 and 6 months. We excluded subjects who had advanced renal diseases with serum creatinine level > 2 mg/dL, chronic liver dysfunction, chronic heart disease, inflammatory disease or malignant tumors. Urinary excretion of albumin was defined as urinary albumin index (UAI), as noted below. Blood samples were collected after an overnight fast to determine fasting plasma glucose, hemoglobin A1c, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, serum sodium, potassium and chloride, blood urea nitrogen (BUN), creatinine and uric acid. The present study was approved by the ethics committee of Jichi Medical University for human studies. We obtained informed consent from all the subjects who joined the present protocol.

Measurements

Spot urine samples were collected in the morning when the subjects were attending the outpatient clinic. Urinary concentration of albumin was determined by latex agglutination immunoassay (Eiken, Tokyo, Japan). Urinary creatinine was measured by enzymatic assay. UAI (mg/g creatinine) was defined as urinary albumin concentration (μg/mL) divided by urinary creatinine level (mg/mL).

Statistical analysis

All values were expressed as mean ± standard error of the mean. The values were analyzed by analysis of variance (ANOVA) and Student's paired *t* test, when appropriate. Categorical variables were analyzed by χ^2 test. Logarithmic transformation of UAI was used because of widely distributed UAI. The statistical package StatView version 5.0 (SAS Institute Inc., Cary, NC, USA) was used for the present analysis. A p value < 0.05 was considered significant.

Results

Table 1 shows the clinical characteristics of all the subjects with diabetic nephropathy. There were no differences in age, systolic and diastolic blood pressure, fasting plasma glucose, hemoglobin A1c, total cholesterol, HDL cholesterol and triglyceride among the three groups of subjects. The numbers of subjects with retinopathy were significantly increased in Groups 2 and 3 compared to in Group 1 ($p < 0.001$), indicating that the subjects of Groups 2 and 3 might have considerably advanced diabetic complications.

Table 2 shows blood pressure, serum electrolytes and renal function during the observation period in the three groups of subjects with diabetic nephropathy. There was no difference in all the basal values among the three groups of subjects. In Group 1, systolic blood pressure was significantly decreased 3 and 6 months after the administration of imidapril. Diastolic blood pressure was also reduced significantly 3 and 6 months after the administration of imidapril. Systolic and diastolic blood pressures were also reduced in Groups 2 and 3, but the reduction was not statistically significant. BUN and serum creatinine were in the normal ranges, and they remained unchanged during the 6-month observation period. Estimated glomerular filtration rate (GFR) was also in the normal range, and they remained unchanged during the 6-month period.

Table 1. Clinical characteristics of all subjects with diabetic nephropathy*

	Group 1 ($n = 16$)	Group 2 ($n = 8$)	Group 3 ($n = 10$)
Age, yr	65.9 ± 1.9	63.6 ± 4.6	66.0 ± 2.2
Sex (M/F), *n*	7/9	5/3	7/3
DM retinopathy, *n* (%)	5 (31)	6 (75)†	7 (70)†
Hyperlipidemia, *n* (%)	6 (38)	1 (13)	2 (20)
Ischemic heart disease, *n* (%)	0 (0)	1 (13)	0 (0)
Systolic BP, mmHg	148.3 ± 3.2	156.4 ± 4.6	149.8 ± 4.1
Diastolic BP, mmHg	84.1 ± 1.6	80.1 ± 3.8	79.9 ± 3.7
Fasting plasma glucose, mg/dL	148.8 ± 14.6	185.1 ± 19.1	143.6 ± 11.7
HbA1c, %	7.7 ± 0.5	7.7 ± 0.4	7.7 ± 0.4
Total cholesterol, mg/dL	203.3 ± 6.7	206.1 ± 8.8	202.1 ± 9.7
HDL cholesterol, mg/dL	53.6 ± 2.3	49.4 ± 3.2	61.0 ± 6.0
Triglycerides, mg/dL	139.3 ± 15.5	169.3 ± 22.4	113.4 ± 17.8
Therapy, *n* (%)			
Diet	0 (0)	1 (13)	0 (0)
OHA	13 (81)	5 (63)	9 (90)
Insulin	3 (19)	2 (25)	1 (10)

*Data are presented as mean ± standard error of the mean; †$p < 0.001$ *vs.* Group 1. DM = diabetes mellitus; BP = blood pressure; HDL = high-density lipoprotein; OHA = oral hypoglycemic agents.

Table 2. Blood pressure, serum electrolytes and renal function during the observation period in the three groups of subjects with diabetic nephropathy*

	Group 1			Group 2			Group 3		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
Systolic BP, mmHg	148 ± 3.2	139.8 ± 3.1†	141.3 ± 4.3	156.4 ± 4.6	150.3 ± 4.4	142.0 ± 4.9	149.8 ± 4.1	144.1 ± 4.8	147.6 ± 3.7
Diastolic BP, mmHg	85.1 ± 1.6	77.1 ± 2.2†	79.5 ± 2.4†	80.1 ± 3.8	81.9 ± 2.8	76.8 ± 1.1	79.9 ± 3.7	76.3 ± 2.6	85.6 ± 3.4
Serum Na, mmol/L	140.6 ± 0.8	140.2 ± 0.7	140.9 ± 0.5	140.9 ± 0.7	141.0 ± 0.8	139.9 ± 0.7	139.5 ± 1.1	140.0 ± 0.8	138.5 ± 1.1
Serum K, mmol/L	4.2 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.2	4.4 ± 0.2	4.3 ± 0.1	4.5 ± 0.2	4.4 ± 0.2
Serum Cl, mmol/L	101.3 ± 0.5	102.3 ± 0.9	103.2 ± 0.6	101.5 ± 0.4	103.1 ± 0.9	102.1 ± 0.7	101.3 ± 1.3	102.7 ± 0.8	101.5 ± 0.8
BUN, mg/dL	13.4 ± 0.9	14.8 ± 1.7	15.3 ± 1.1	12.9 ± 1.1	13.7 ± 1.4	15.4 ± 1.8	15.9 ± 1.3	15.0 ± 1.4	18.6 ± 2.1
Serum Cr, mg/dL	0.60 ± 0.05	0.72 ± 0.08	0.69 ± 0.07	0.66 ± 0.06	0.71 ± 0.05	0.68 ± 0.05	0.76 ± 0.05	0.73 ± 0.05	0.83 ± 0.07
Estimated GFR, mL/min	109.0 ± 9.1	100.5 ± 6.9	101.6 ± 6.8	109.3 ± 9.0	104.6 ± 7.6	103.3 ± 6.5	92.7 ± 7.3	92.8 ± 7.7	93.6 ± 8.6
Serum uric acid, mg/dL	5.0 ± 0.5	5.2 ± 0.6	5.3 ± 0.5	5.7 ± 0.5	5.9 ± 0.4	5.7 ± 0.5	4.5 ± 0.2	4.3 ± 0.3	4.9 ± 0.4

*Data are presented as mean ± standard error of the mean; †$p < 0.05$ *vs.* baseline. BP = blood pressure; BUN = blood urea nitrogen; Cr = creatinine; GFR = glomerular filtration rate.

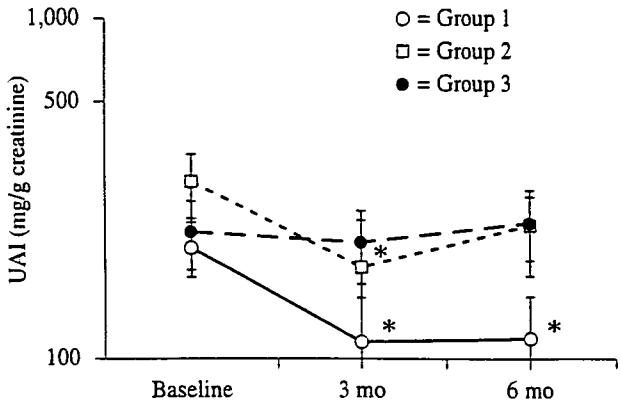

Figure. Urinary albumin index (UAI) during the 6-month observation period in the three groups of subjects with diabetic nephropathy. Group 1 subjects were treated with imidapril. Group 2 subjects had losartan added to consecutive therapy with imidapril. Group 3 subjects had imidapril added to consecutive therapy with losartan. Values are mean ± standard error of the mean. *$p < 0.05$ *vs.* baseline.

The Figure shows UAI during the 6-month observation period in the three groups of subjects. Mean UAI ranged from 213.1 to 328.8 mg/g creatinine before the administration of drugs. Imidapril was initially administered to Group 1 subjects, and UAI gradually decreased during the 6-month observation period (before: 213.1 ± 46.6; 3 months: 111.6 ± 35.6, $p < 0.001$; 6 months: 114.5 ± 35.4 mg/g creatinine, $p < 0.01$). In Group 2 subjects who had taken imidapril, the addition of losartan further decreased UAI from 328.8 ± 87.3 to 185.3 ± 51.4 mg/g creatinine at 3 months ($p < 0.05$), and to 244.8 ± 88.3 mg/g creatinine at 6 months. However, the addition of imidapril did not alter UAI in Group 3 subjects who had taken losartan.

Discussion

The present study demonstrated the therapeutic efficacy of the combination of ACEI and ARA to treat albuminuria in subjects with diabetic nephropathy. The administration of imidapril alone significantly reduced UAI, without any change in BUN, serum creatinine levels and estimated GFR. The drug also decreased systolic and diastolic blood pressure. Group 1 subjects had fewer diabetic complications than Groups 2 and 3 subjects, and the initial use of ACEI had the therapeutic efficacy of reducing UAI in Group 1 subjects. Angiotensin II directly constricts efferent arterioles of glomerulus, and the inhibition of the renin–angiotensin system could release from intraglomerular hypertension and glomerular hyperfiltration [21,22]. Direct renoprotective effect as well as decrease in blood pressure could ameliorate renal impairment in subjects with diabetic nephropathy.

Subjects in Groups 2 and 3 had already been treated with either ACEI or ARA, and thus urinary excretion of albumin might already have been considerably reduced. It did not seem easy that an additional drug would produce further reduction in albuminuria. In Group 2, ARA, added after consecutive therapy with ACEI, significantly decreased UAI without any decrease in blood pressure. The magnitude of its decrement was somewhat equivalent to that in Group 1 subjects, who had initially been treated with ACEI. In contrast, there was no alteration in albuminuria in Group 3 subjects, who were given ACEI after treatment with ARA. The reason for this difference was not determined, but we may consider several possibilities regarding the pharmacologic properties of ACEI and ARA. Chronic administration of ACEI might not totally block the conversion of angiotensin I to angiotensin II, and could relatively upregulate the function of angiotensin AT1 and AT2 receptors. Also, angiotensin II is partially produced by chymase independently of angiotensin converting enzyme [20]. Under these circumstances, the addition of ARA would abolish the alternative changes and further reduce UAI in Group 2. After the inhibition of AT1 receptor binding by ARA, the conversion of angiotensin I to angiotensin II could be augmented. The addition of ACEI diminished its augmented conversion, but there was no benefit in reducing albuminuria when adding ACEI to consecutive ARA therapy in Group 3. Previous studies have demonstrated the efficacy of combination therapy in diabetic nephropathy [24,25]. In those studies, the administration of ACEI and ARA was started simultaneously, and the effectiveness was evaluated by comparing with the single use of either ACEI or ARA. There is only one previous report of either ACEI or ARA added after initial therapy with the other agent [23]. The present finding may thus indicate that ARA has a dominant effect on diminishing albuminuria in diabetic nephropathy. Further studies will be necessary to obtain conclusive evidence of the efficacy of combination therapy with ACEI and ARA.

In conclusion, we determined whether combination therapy with ACEI and ARA is a useful tool for reducing albuminuria in diabetic nephropathy. Initial use of ACEI profoundly decreased albuminuria. The addition of ARA to consecutive ACEI therapy further reduced albuminuria in subjects with advanced renal impairment, but the addition of ACEI to consecutive ARA therapy did not. These results indicate that the addition of ARA to consecutive ACEI therapy augments a protective effect against the progression of diabetic nephropathy.