Table 13 Stratified Analysis of Cardiac Events According to Treatment of AMI

	Cardiac events during initial 6 months (incidence)	χ^2 test p value	Details of events (cases)				
			Death	MI	PIA	u-PCI	u-CABG
Heparin							
No	20.1% (32/159)	0.0432	24	1	2	4	1
Yes	13.8% (100/724)		53	10	24	11	2
Max daily dosage (units)							
<12,000	15.7% (51/325)	0.1857	29	5	13	3	1
≥12,000	12.3% (49/399)		24	5	11	8	1
Duration of infusion (days)							
<5	11.1% (60/539)	0.0001	28	8	14	9	1
≥5	22.7% (40/176)		25	2	10	2	1
Antiplatelet							
No	62.5% (20/32)	<0.0001	17	0	0	2	1
Yes	13.1% (112/852)		60	11	26	13	2
Aspirin							
No	43.5% (27/62)	<0.0001	21	0	0	5	1
Yes	12.8% (105/822)		56	11	26	10	2
Ticlopidine							
No	21.1% (68/322)	<0.0001	45	4	8	10	1
Yes	11.4% (64/562)		32	7	18	5	2
Coronary angiography							
No	37.5% (6/16)	0.0106	4	0	1	1	0
Yes	14.5% (126/868)		73	11	25	14	3
Vessel (s) with significant stenosis							
0	40.0% (4/10)	<0.0001	1	2	1	0	0
1	7.9% (36/453)		16	4	10	6	0
2	13.3% (32/240)		15	2	7	7	1
3	32.0% (49/153)		38	3	5	1	2
LMT	36.4% (4/11)		3	0	1	0	0
Reperfusion therapy							
No	25.0% (18/72)	0.0124	9	2	6	1	0
Yes	14.0% (114/812)		68	9	20	14	3
Time from admission (h)							
<6	13.5% (91/675)	0.4474	57	9	13	9	3
≥6	16.1% (19/118)		8	0	6	5	0
<12	13.9% (98/705)	0.9461	60	9	15	11	3
≥12	13.6% (12/88)		5	0	4	3	0
PTCA							
No	20.9% (18/86)	0.0517	11	1	2	3	1
Yes	13.2% (96/726)		57	8	18	11	2
Stent							
No	13.7% (39/284)	0.8535	23	3	6	6	1
Yes	14.2% (75/528)		45	6	14	8	2
ICT							
No	14.7% (109/743)	0.0895	66	9	18	14	2
Yes	7.2% (5/69)		2	0	2	0	1
IVCT							
No	13.3% (100/752)	0.0313	61	9	15	12	3
Yes	23.3% (14/60)		7	0	5	2	0
CABG							
No	13.0% (101/778)	<0.0001	56	9	20	14	2
Yes	38.2% (13/34)		12	0	0	0	1
Stenosis (%) of culprit vessel before reperfusion therapy							
0	0.0% (0/0)	0.0012	0	0	0	0	0
25	20.0% (1/5)		0	1	0	0	0
50	100.0% (2/2)		1	1	0	0	0
75	8.3% (1/12)		0	0	0	1	0
90	20.4% (21/103)		9	1	6	5	0
99	9.4% (20/212)		12	3	5	0	0
100	14.2% (67/472)		44	5	8	7	3
Stenosis (%) of culprit vessel after reperfusion therapy							
0	11.5% (40/348)	0.3249	21	4	7	8	0
25	12.9% (38/294)		20	3	10	3	2
50	19.0% (15/79)		9	1	3	2	0
75	7.1% (1/14)		1	0	0	0	0
90	0.0% (0/5)		0	0	0	0	0
99	10.0% (1/10)		1	0	0	0	0
100	26.7% (4/15)		2	1	0	1	0
TIMI flow grade past the culprit lesion before reperfusion therapy							
0	13.6% (62/455)	0.0307	40	5	7	7	3
1	7.2% (6/83)		4	0	2	0	0
2	11.5% (15/130)		9	3	3	0	0
3	20.8% (27/130)		11	3	7	6	0

TIMI flow grade past the culprit lesion after reperfusion therapy							
0	*15.0% (3/20)*		*1*	*1*	*0*	*1*	*0*
1	*15.4% (2/13)*	*0.8950*	*2*	*0*	*0*	*0*	*0*
2	*9.3% (4/43)*		*3*	*0*	*1*	*0*	*0*
3	*12.0% (80/664)*		*46*	*8*	*13*	*11*	*2*

Abbreviations as in Tables 2,8,12.

pectoris, a history of MI, hypertension, renal disease, detection of acute heart failure on admission, and complete left bundle branch block on ECG.

The treatments associated with a significantly lower incidence of cardiac events were heparin, oral antiplatelet drugs (aspirin or ticlopidine), coronary angiography, and reperfusion therapy. Cardiac events even occurred in some AMI patients without significant stenosis on initial coronary angiography.

Discussion

Our questionnaire survey revealed that fewer patients are hospitalized with UA than with AMI in Japan. Among several overseas studies that simultaneously investigated the number of UA and AMI patients, a Spanish study of inpatients reported similar results, with an approximate ratio of 1:2 for UA patients vs AMI patients[8] Of 2 investigations of patients admitted to the ICU or CCU, 1 showed that the number of AMI patients was more than twice that of UA patients[9] and the other revealed that UA patients outnumbered AMI patients by a ratio of 1.5 to 1[10] The ratio of UA vs AMI patients obtained in the present study, which involved inpatients only, might be different if those treated as outpatients had been included. Because the largest percentage of UA patients in the case report investigation had Braunwald class III disease, those with Braunwald class I or II disease may have been treated on an outpatient basis. This finding should be taken into consideration when developing the recommendations for treatment of UA because inpatient care is standard management for suspected UA.

The results of both the questionnaire survey and the case report investigation indicate that non-ST-elevation AMI accounts for 15–20% of all AMI, a finding that is also consistent with overseas data[11] The present study showed that both ST-elevation and non-ST-elevation AMI are managed in Japan according to the principle of early invasive therapy as first-line treatment. Although the ACC/AHA and Japanese guidelines for the management of ACS recommend different treatment pathways for ST-elevation and non-ST-elevation AMI, it seems acceptable in practice to make no distinction between the 2 types of AMI in Japan. Compared with the USA and Europe, in Japan a larger proportion of institutions are capable of providing coronary intervention relative to the number of patients with ischemic heart disease.

The present study also showed a trend toward early invasive treatment of UA, especially those cases of Braunwald class III disease (median time from admission to conduct the invasive treatment of Braunwald classes I, II and III was 144, 79 and 34 h, respectively). However, UA patients received invasive treatment later than AMI patients and were usually given drug treatment immediately after arrival at hospital. Because the present study investigated the medical management of UA in 2000, these patients may now receive invasive treatment earlier because of subsequent improvements in the devices for coronary intervention and the skills of the interventionists. However, a GUSTO-IV substudy recently demonstrated that patients with non-ST-elevation ACS showing low levels of biomarkers have a very low 1-year mortality with medical management, and early invasive procedures appear to increase their overall risk of mortality[12] That study may be a warning against early invasive management in UA patients.

According to the present case report data, approximately 70% of patients received continuous infusion of heparin immediately after admission, which indicates that heparin is regarded as essential if medical treatment is used to stabilize the patient in the early hospital phase.

In UA patients, the incidence of cardiac events was 2% at 1 month and 9% at 6 months, which is lower than in AMI patients for both time intervals. Previous overseas studies of patients with non-ST-elevation ACS have revealed a higher incidence of cardiac events[13,14] suggesting that the prognosis of UA may be better in Japanese patients. Although many of the patients were treated with antithrombotic drugs, such as heparin, aspirin, and ticlopidine, only 4% of them experienced major bleeding events during hospitalization and none of them developed intracranial hemorrhage, which suggests that the use of antithrombotic drugs was appropriate, with adequate precautions taken to prevent bleeding complications.

The results of the stratified analyses of the 6-month outcomes in UA patients should be interpreted carefully because of the nonrandomized, retrospective design of this study. For example, our analysis in UA patients failed to detect any significant difference in the incidence of cardiac events between patients undergoing or not undergoing PCI and between those with or without heparin infusion. Patients who undergo PCI or who receive heparin usually have more severe disease than those not receiving these treatments and this difference in severity may have masked the beneficial effect of such treatments on the outcome.

On the other hand, the incidence of cardiac events in patients using any calcium-channel blocker was significantly lower than those in patients who had not received calcium-channel blocker and these 2 subgroups had similar clinical profiles (data not shown). This suggests that calcium-channel blocker therapy may be an independent determinant of the prognosis of UA. Because it has long been known that coronary vasospasm plays a greater role in the etiology of ischemic heart disease in Japanese than in Caucasians, the reduction of cardiac events observed in patients treated with calcium-channel blockers may reflect the ability of drugs in this class to suppress coronary vasospasm[15] However, a randomized clinical trial should be carried out to confirm the efficacy of calcium blockers in the treatment of ACS in Japanese patients.

There was no significant difference in the stratified analysis of cardiac events by infarct location (Table 12). Concerning the relationship between infarct location and long-term prognosis, there is not consensus. Kandzari et al reported that the long-term prognosis of anterior infarction was worse than for other infarction sites[16] whereas Karlson et al

reported that there was no significant difference in long-term prognosis by infarct location![7]

As shown in Table 13, there was no apparent difference in the incidence of cardiac events between patients obtaining Thrombolysis In Myocardial Infarction (TIMI) 3 flow after reperfusion therapy and patients who did not. It is well known that TIMI 0 to TIMI 2 flow is an independent predictor of prognosis[18,19] and the reason why our result was different from previous reports is unclear.

With the cooperation of many cardiovascular specialists, the present nationwide investigation has provided the first insight into the actual management of ACS (including UA) in Japan. It is of great importance to develop appropriate treatments for Japanese patients based on the specific characteristics of this population.

Therefore, more studies in patients with ACS and AMI should be performed in the future. In particular, it would be valuable to collect information about medical treatment with nicorandil, ACE inhibitors, ARBs and statins, and invasive treatments (drug-eluting stents, distal protection devices, thrombectomy, etc).

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Validation of the Association Between the Gene Encoding Proteasome Subunit α Type 6 and Myocardial Infarction in a Japanese Population

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Background Recently, a large case-control study (2,851 cases and 2,592 controls) reported that a functional single nuclear polymorphism (SNP) in the proteasome subunit α type 6 gene (*PSMA6*) conferred a risk of myocardial infarction (MI) in a Japanese population. The SNP (exon 1, –8C/G) is located in the 5' untranslated region of exon 1, and the risk-conferring allele G appears to enhance the transcription of *PSMA6*, which may exaggerate inflammation through activation of nuclear factor-κ β protein. The frequency of the risk conferring genotype (GG) in cases was reported to be greater than that in controls (12.4% vs 8.9%). The purpose of the present study was to validate this observation in our study population.

Methods and Results Subjects with MI (n=433) were recruited from the outpatient clinic of the National Cardiovascular Center. Control subjects (n=2,186) were recruited from the Suita study. The frequencies of the GG genotype did not significantly differ between the control (9.8%) and MI groups (10.6%). Moreover, this genotype was not associated with C reactive protein levels in the Suita study. However, the GG genotype was significantly associated with greater intima-media thickness (n=2,051, p=0.015) after adjusting for blood pressure, sex, body mass index and age in the Suita study.

Conclusion The reported genotype in *PSMA6* appears not to contribute appreciably to MI, but may contribute slightly to atherosclerosis in the present study population. (*Circ J* 2007; **71**: 495–498)

Key Words: Genetic; Inflammation; Myocardial infarction; *PSMA6*

Myocardial infarction (MI) is a multifactorial disease caused by environmental and genetic factors. There are an increasing number of studies that identify genes that contribute to the incidence of MI; it is possible that these genes can be targeted for personalized prevention of MI.[1–3] Recently, a large case-control study (2,851 cases and 2,592 controls) showed that a functional single nuclear polymorphism (SNP) in the proteasome subunit α type 6 gene (*PSMA6*) conferred a risk for MI in a Japanese population.[4] The SNP (exon 1, –8C/G) is located in the 5' untranslated region of exon 1, and the risk-conferring allele G appears to enhance the transcription of *PSMA6*, which may increase inflammation through activation of nuclear factor-κ β (NF-κB) protein.[5,6] However, because the contribution of a common allele to the pathogenesis of MI appears to be small, validation is necessary in other study populations. The purpose of the present study was to validate the findings of Ozaki et al in a Japanese population and to evaluate the importance of *PSMA6* in the pathogenesis of MI.

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Methods

Study Population

The selection criteria and design of the Suita Study have been described previously.[7–9] Genotypes were determined in 2,500 subjects recruited from the Suita Study between April 2002 and February 2004. The MI group consisted of

Table 1 Characterization of Study Population

	Suita study	*MI subjects*	*p value*
Number	*2,186*	*433*	
Male (number)	*992 (45.38%)*	*370 (86.0%)*	*<0.0001*
Age (years)	*5.35±10.90*	*65.85±9.46*	*0.38*
BMI	*22.84±3.34*	*23.74±2.97*	*<0.0001*
HT (%)	*36.37*	*52.42*	*<0.0001*
DM (%)	*19.81*	*41.51*	*<0.0001*
HLP (%)	*62.44*	*73.31*	*0.0004*
TG (mg/dl)	*106.34±68.40*	*127.62±77.31*	*<0.0001*
TC (mg/dl)	*208.97±32.84*	*199.05±39.73*	*<0.0001*
HDL-C (mg/dl)	*60.05±15.41*	*43.91±13.09*	*<0.0001*
Smoking (%)	*15.74*	*57.60*	*<0.0001*
MI (number)	*34 (1.6%)*	*433 (100%)*	

Values are mean±standard deviation (SD).
MI, myocardial infarction; BMI, body mass index; HT, prevalence of hypertension; DM, prevalence of diabetes mellitus; HLP, prevalence of hyperlipidemia; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; Smoking, current smoking.

Table 2 Association Between MI and rs1048990

Genotype	Suita study*				MI				p value**	p value***
	CC	CG	GG	Total	CC	CG	GG	Total		
Number (%)	1,010 (46.93)	931 (43.3)	211 (9.8)	2,152 (100)	195 (44.3)	192 (45.0)	46 (10.6)	433 (100)	0.73	
Male (%)	44.9	45.5	40.8	44.7	84.6	85.4	89.1	85.5	<0.0001	0.45
Smoking (+) (%)	13.9	17.4	18.0	15.8	53.3	60.4	52.17	57.0	<0.0001	0.97
DM (+) (%)	22.0	17.7	17.1	19.7	38.5	43.2	39.1	40.6	<0.0001	0.089

*Subjects without cardiovascular disease.
**p values are for the comparison between the Suita study and MI subjects.
***p value are for the comparison among genotypes.
Abbreviations see in Table 1.

Table 3 Logistic Analysis of MI

MI	Chi-square	p value	Odds ratio	95%CI
Sex (F)	75.15	<0.0001	0.23	0.16–0.32
Age (years)	7.97	0.0048	3.13	1.42–6.94
Smoking (+)	103.2	<0.0001	3.99	3.06–5.21
Diabetes and/or hyperglycemia (+)	42.77	<0.0001	3.18	2.27–4.56
PSMA6 (GG)	0.02	0.88	0.97	0.63–1.44

Diabetes and/or hyperglycemia (+), subjects diagnosed as having diabetes and/or hyperlipidemia.
CI, confidence interval; PSMA6, proteasome subunit α type 6. Other abbreviation see in Table 1.

Table 4 Association Between *PSMA6* Polymorphism and Intima-Media Thickness

	CC	GC	GG	p value*	p value**
Number	938	884	195		
IMT-mean (mm)	0.79±0.14	0.78±0.13	0.81±0.13	0.025	0.024
Residual IMT-mean	-0.007±0.11	0.005±0.12	0.014±0.11	0.015	0.0073
IMT-max (mm)	1.26±0.53	1.30±0.66	1.24±0.48	0.32	0.38
Residual IMT-max	-0.014±0.469	0.026±0.606	-0.052±0.412	0.099	0.28

Values are mean±SD.
IMT, intima-media thickness. Other abbreviation see in Tables 1,3.
Residuals of IMT were calculated by adjusting for age, systolic blood pressure, sex and BMI.
*p values are for the comparison among CC, CG and GG genotypes.
**p value are for the comparison between CC and GC+GG genotypes.

Table 5 Association Between *PSMA6* Polymorphism and hCRP

	CC	GC	GG	p value*	p value**
Number	1,009	931	210		
hCRP (mg/dl)	0.15±0.47	0.15±0.43	0.11±0.18	0.43	0.69
Log transferred hCRP	-2.79±1.15	-2.76±1.15	-2.80±1.02	0.86	0.71

Values are mean±SD.
hCRP, high sensitivity C related peptide. Other abbreviation see in Table 3.
*p values are for the comparison among CC, CG and GG genotypes.
**p value are for the comparison between CC and GC+GG genotypes.

433 randomly selected inpatients and outpatients with documented MI (370 men, 63 women) who were enrolled in the Division of Cardiology at the National Cardiovascular Center between May 2001 and April 2003[10,11] All subjects enrolled in the present study provided written informed consent. The present study was approved by the Ethics Committee of the National Cardiovascular Center and by the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center.

Subjects with a systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure ≥90 mmHg and/or taking antihypertensive medication were categorized as having hypertension[3] Subjects with a fasting blood glucose ≥126 mg/dl, hemoglobin (Hb) A1c ≥6.5% and/or undergoing treatment for diabetes mellitus (DM) were categorized as having DM[3] Subjects with total cholesterol ≥220 mg/dl, triglycerides ≥150 mg/dl and/or taking antihyperlipidemic medication were categorized as having hyperlipidemia[3] The intima-media thickness (IMT), a well-known indicator of coronary atherosclerosis, was measured on a longitudinal scan of the common carotid artery at a point 10 mm proximal from the beginning of the dilation of the bulb[7]

DNA Study

Ozaki et al determined 8 polymorphisms in *PSMA6* genes, and found the most significant association with MI at the polymorphism rs1048990[4] In the present study, we determined the rs1048990 polymorphism using the TaqMan

methods. The following polymerase chain reaction primer and probe set was used: C_11599359_10 (Applied Biosystems, Foster City, USA).

Statistical Analysis

The values are expressed as mean±standard deviation. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc, Cary, NC, USA). Simple correlation analyses and logistic analyses were performed to determine the association between laboratory data and MI cases. Multiple logistic analyses were performed to obtain predictors for MI. Odds ratio and 95% confidence intervals (CI) were also calculated. The continuous phenotypic variables and genotype were compared using one way analysis of variance, adjusting for appropriate confounding factors. Residuals of IMT were calculated by adjusting for age, SBP, sex and body mass index (BMI)[9] C reactive protein (CRP) levels were logarithmically transformed to attain normal distribution.

Results

The characteristics of the present study population are shown in Table 1. In the present study population, the frequencies of the GG genotype in the MI and the control group were 10.6% and 9.8%, respectively (Table 2). No significant difference was observed in the genotype frequency between the 2 groups. The GG genotype was not associated with smoking habits or the prevalence of DM (Table 2). The odds ratio of the GG genotype of *PSMA6* over the CC+CG genotype for MI was 0.97 (95% CI, 0.63–1.44) (Table 3). However, because it was possible that the sample size of the MI group (n=433) was too small to detect the small effects of the risk-conferring alleles, we observed the effects of this genotype on carotid IMT, an excellent non-invasive marker of atherosclerosis. The GG genotype was associated with mean IMT (p=0.025) and greater residuals of mean IMT (p=0.015) after adjusting for age, BMI, sex and SBP (Table 4).

No significant effects from this genotype on CRP levels were observed in the Suita population (Table 5).

Discussion

The purpose of the present study was to validate in our study population the association between *PSMA6* variants and MI that has been reported in a Japanese population. Because the genetic contribution of a single gene to common disease susceptibility appears to be low, as observed in the insertion/deletion polymorphism of the angiotensin converting enzyme gene in cardiovascular disease, validation studies in other study populations are important![12,13]

PSMA6 encodes the proteasome subunit α type 6, a component of the 20S proteasome![14] The 20S proteasome is composed of 7 α and 10 β subunits, and is the core particle for the 26S ubiquitin-proteasome system, which is important in the regulation of the abundance of proteins involved in various cellular functions, including inflammation![15–17] Of note, this system is involved in the degradation of the IκB protein, which inhibits the activation of NF-κB, a central transcriptional factor that regulates the expression of genes related to inflammation[5] Now vascular inflammation is considered a key player for atherogenesis, and CRP levels are a well-known predictor for subsequent MI![18–21]

The reported odds ratio of the GG genotype of *PSMA6* over the CC+CG genotype for MI was just 1.36 (95% CI, 1.12–1.65)[4] Thus, we were unable to detect the association of the GG genotype with MI, probably due to our small sample size. However, we did detect an influence of this genotype on IMT, a well-known index of atherosclerosis of coronary arteries![22–25] This may indicate that the influence of this gene may be directed to the pathogenesis of atherosclerosis.

The influence of the *PSMA6* genotype on the residuals of IMT-mean was significant but slight (r^2=0.0042, p=0.014). The IMT-maximum values may be considered to be more influenced by local micro environmental factors and may be difficult to predict using classical risk factors. Indeed, the r^2 values for IMT-maximum by confounding factors (age, gender, SBP and HbA1c) was 0.181, which is smaller than the r^2 values for IMT-mean (r^2=0.237) by confounding factors (age, gender, BMI, SBP and HbA1c). Therefore, a slight influence of the *PSMA6* genotype may not be detected in the IMT-maximum.

Ozaki et al reported that the frequencies of the genotype GG in the MI and the control groups were 12.4% and 8.9%, respectively[4] However, in the present study population, the frequencies of the GG genotype in the MI and the control group were 10.6% and 9.7%, respectively, with no significant differences between groups. Ozaki et al speculated that the effects of *PMSA6* might be due to potentiation of inflammation[4] CRP levels are known to be a good indicator of future MI![20] However, in the present study, the GG genotype was not associated with the CRP levels. The precise mechanism of how the GG genotype might accelerate atherosclerosis or infarction awaits further investigation.

In conclusion, the reported genotype in *PSMA6* appears not to contribute appreciably to MI, but may contribute slightly to atherosclerosis in the present study population.

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Nifedipine retard prevents hospitalization for angina pectoris better than angiotensin-converting enzyme inhibitors in hypertensive Japanese patients with previous myocardial infarction (JMIC-B substudy)

Yoshiki Yui[a], Eiji Shinoda[b], Kazuhisa Kodama[c], Atsushi Hirayama[d], Hiroshi Nonogi[e], Kazuo Haze[f], Tetsuya Sumiyoshi[g], Saichi Hosoda[g] and Chuichi Kawai[h], and the Japan Multicenter Investigation for Cardiovascular Diseases B (JMIC-B) Study Group

Objectives and background We previously reported that nifedipine retard showed comparable efficacy to angiotensin-converting enzyme (ACE) inhibitors for the prevention of cardiac events in hypertensive patients with coronary artery disease during the Japan Multicenter Investigation for Cardiovascular Diseases B study. In the nifedipine group, patients with a history of myocardial infarction (MI) showed a significant reduction in hospitalization for angina pectoris compared with the ACE inhibitor group. We investigated whether this difference was related to the progression of coronary arteriosclerosis.

Methods To evaluate coronary arteriosclerosis, we performed coronary angiography (CAG) and a quantitative analysis of coronary angiograms.

Results The cumulative incidence of hospitalization for angina was significantly lower in the nifedipine group (log-rank test $P = 0.013$). The etiology of angina requiring hospitalization was determined on the basis of CAG findings. Its incidence secondary to the development of new lesions or the progression of existing lesions was significantly lower in the nifedipine group than in the ACE inhibitor group (log-rank test $P = 0.042$ and $P = 0.028$, respectively). Using quantitative coronary analysis, changes in the coronary artery luminal diameter were compared between the nifedipine and ACE inhibitor groups. The minimum coronary lumen diameter did not show a significant change in the nifedipine group, whereas it decreased significantly in the ACE inhibitor group (paired t-test $P = 0.002$), and there was a significant difference between the two groups by analysis of covariance ($P = 0.047$).

Conclusion These results indicate that nifedipine more effectively prevented admission for angina pectoris by inhibiting the progression of coronary artery disease in patients with a history of MI. *J Hypertens* 25:2019–2026

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Keywords: angina pectoris, angiotensin-converting enzyme inhibitor, coronary angiography, myocardial infarction, nifedipine retard

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Introduction

According to Western and Japanese epidemiological data, the incidence of cardiac events is clearly higher in patients with a history of myocardial infarction (MI) than in those without [1–3].

Several large-scale clinical studies [4–6] have shown that angiotensin-converting enzyme (ACE) inhibitors are useful for improving the long-term prognosis of patients after MI, whereas calcium antagonists have no such beneficial effect [7–9]. Those studies were, however, conducted using short-acting calcium antagonists, so it remains unclear whether the results are also applicable to long-acting calcium antagonists.

It has been reported that vasospasm is closely related to the occurrence of MI in Japanese patients [10]. Therefore, long-acting calcium antagonists with a strong antispastic effect [11] and a mild antihypertensive effect may possibly be an appropriate treatment for patients after MI.

In the Japan Multicenter Investigation for Cardiovascular Diseases B (JMIC-B) study, we found that nifedipine had

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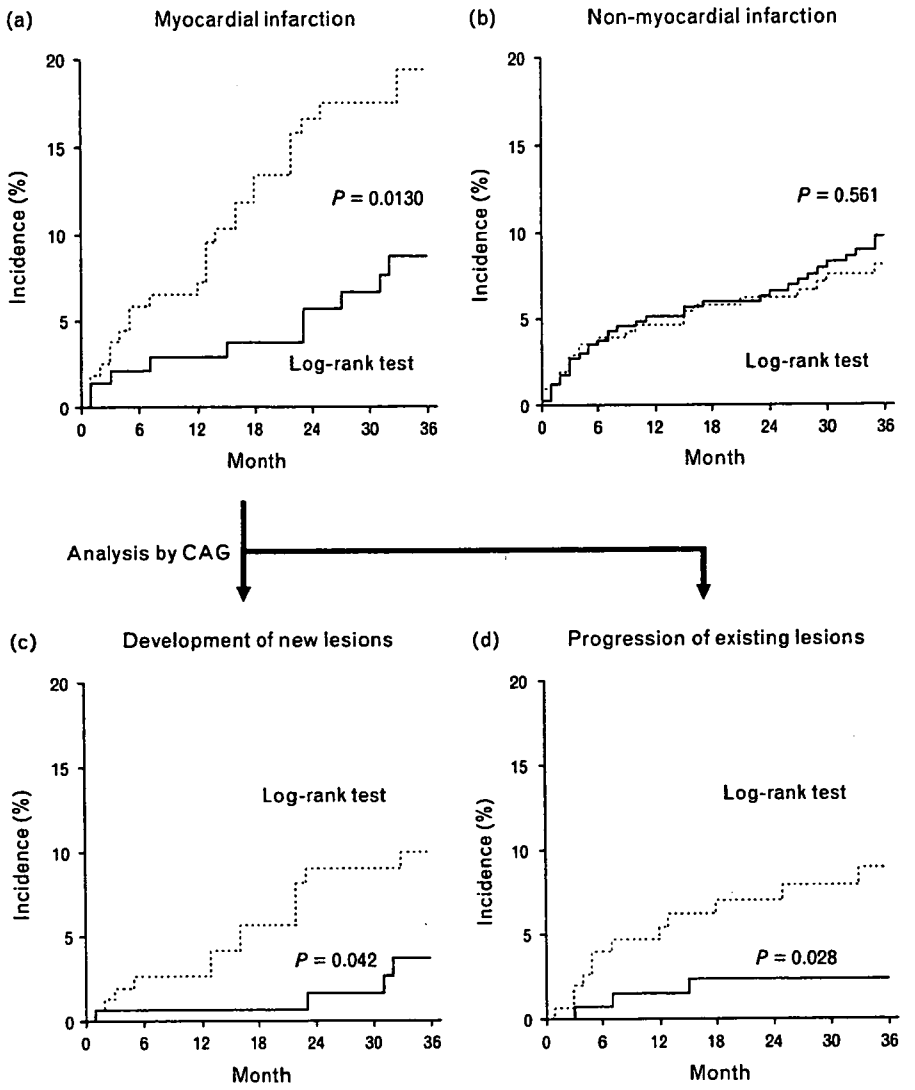
Fig. 1



Kaplan–Meier analysis of the incidence of angina pectoris requiring hospitalization in patients with or without a history of myocardial infarction. (a) and (b) The incidence of angina pectoris requiring hospitalization in patients with a history of myocardial infarction was significantly lower for the nifedipine group than for the angiotensin-converting enzyme (ACE) inhibitor group. (c) The incidences of angina pectoris requiring hospitalization as a result of the development of new lesions and (d) angina pectoris requiring hospitalization as a result of the progression of existing lesions were both significantly lower in the nifedipine group than in the ACE inhibitor group. CAG, Coronary angiography. (a, c and d) —— Nifedipine ($n=148$); – – – – ACE inhibitors ($n=170$); (b) —— Nifedipine ($n=418$); – – – – ACE inhibitors ($n=337$).

coronary intervention, the angiograms obtained immediately before percutaneous coronary intervention were used for evaluation.

The chi-squared test was employed to compare baseline clinical characteristics (Table 1) and concomitant drugs (Table 2). The unpaired *t*-test was used for the comparison of blood pressure and heart rate findings over time with the baseline data (Table 3). During the evaluation of QCA data, the paired *t*-test was used for comparison between the baseline and follow-up minimum lumen diameter (MLD; percentage diameter stenosis; %DS) in each treatment group (Table 5). Changes in the MLD and percentage stenosis (%DS) in the individual patients were compared between groups (Table 6) by analysis of covariance (ANCOVA). As covariates, baseline MLD, hyperlipidemia, smoker, concomitant use of α-blockers, achieved SBP, and achieved DBP were employed. Data are expressed as the mean ± standard deviation (SD). All statistical analyses were performed using SAS software (version 6.14; SAS Institute Inc., Cary, North Carolina, USA).

Table 4 Patients with primary or secondary endpoints in the previous myocardial infarction subgroup

	Nifedipine *n* (%)	ACE inhibitors *n* (%)	Relative risk (95% CI)	*P* value
Number	315	381		
All cardiac events	52 (16.5)	66 (17.3)	0.92 (0.63–1.33)	0.64
Cardiac death and sudden death	2 (0.63)	3 (0.79)	1.28 (0.17–9.98)	0.81
Myocardial infarction	9 (2.86)	10 (2.62)	1.15 (0.46–2.87)	0.76
Angina pectoris requiring hospitalization	13 (4.13)	34 (8.92)	0.42 (0.22–0.80)	0.01
Heart failure requiring hospitalization	5 (1.59)	6 (1.57)	0.58 (0.16–2.04)	0.39
Serious arrhythmia	2 (0.63)	2 (0.52)	0.43 (0.05–4.00)	0.46
Coronary intervention	35 (11.11)	49 (12.86)	0.82 (0.53–1.28)	0.39
Cerebrovascular accidents	9 (2.86)	8 (2.10)	1.52 (0.56–4.11)	0.41
Worsening of renal dysfunction	3 (0.95)	1 (0.26)	2.35 (0.22–24.6)	0.48
Total mortality	6 (1.90)	7 (1.84)	1.05 (0.32–3.53)	0.93

ACE, Angiotensin-converting enzyme; CI, confidence interval. The relative risk and *P* values were determined by using the Cox proportional hazard model with adjustment for history of hyperlipidemia, smoker, concomitant use of α-blocker, and achieved blood pressure. Coronary intervention: percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stenting.

In the MI group, the achieved blood pressure was 134 ± 13/76 ± 8 mmHg in the nifedipine group and 136 ± 16/78 ± 8 mmHg in the ACE inhibitor group. SBP tended to be lower in the nifedipine group than in the ACE inhibitor group ($P = 0.086$), whereas DBP was significantly lower in the former group ($P = 0.003$). Among non-MI patients, the achieved blood pressure was 138 ± 14/77 ± 8 mmHg in the nifedipine group and 140 ± 15/79 ± 9 mmHg in the ACE inhibitor group. Both SBP and DBP were significantly lower in the nifedipine group than in the ACE inhibitor group (unpaired *t*-test $P < 0.01$). No significant changes in heart rate were seen throughout treatment with any drug in the patients with or without MI.

Endpoints and previous myocardial infarction

Among the MI patients, comparison between the two treatment groups showed differences in the incidence of hyperlipidemia, smoker, concomitant use of α-blocker, achieved SBP, and achieved DBP. These parameters were therefore entered into the Cox proportional hazard model. Table 4 shows a comparison of the incidence of each event between treatment groups for the MI patients. The incidence of angina pectoris requiring hospitalization was significantly lower in the nifedipine group than in the ACE inhibitor group. Among the non-MI patients, there were no differences in the same endpoints between the two treatment groups.

Cumulative incidence of angina pectoris requiring hospitalization in relation to coronary angiography findings

The cumulative incidence of angina pectoris requiring hospitalization determined by the Kaplan–Meier method (Fig. 1a) was significantly lower in the nifedipine group (log-rank test $P = 0.013$).

Among the non-MI patients, there was no difference in the incidence of angina pectoris requiring hospitalization between the two treatment groups (Fig. 1b).

When the aetiology of angina pectoris requiring hospitalization in the patients with previous MI was determined on the basis of CAG findings, the incidence of angina pectoris requiring hospitalization as a result of new lesions and that caused by the progression of existing lesions were both significantly lower in the nifedipine group than in the ACE inhibitor group (log-rank test $P = 0.042$ and $P = 0.028$, respectively; Fig. 1c,d).

Quantitative coronary analysis study

Eighty-seven patients with MI (nifedipine group 38, ACE inhibitor group 49) were subjected to QCA analysis. Table 5 shows the changes in coronary artery diameter (ΔMLD and Δ%DS) after treatment in these patients. There were no significant changes in MLD in any segment in the nifedipine group ($P = 0.810$), whereas a

Table 5 Changes in mean minimum lumen diameter and percentage diameter stenosis in myocardial infarction

Segments	Group	Baseline	Follow-up	Change	*P* value
All segments					
MLD mm, mean (SD)	Nifedipine ($n = 38$)	2.13 (0.49)	2.14 (0.44)	0.01 (0.28)	0.810
	ACE inhibitors ($n = 49$)	2.24 (0.47)	2.12 (0.43)	−0.11 (0.24)	0.002
DS (%) mean (SD)	Nifedipine ($n = 38$)	19.34 (5.59)	16.17 (4.46)	−3.17 (4.75)	0.001
	ACE inhibitors ($n = 49$)	16.76 (5.07)	17.62 (7.22)	0.86 (5.40)	0.270
Coronary lesion segments (%DS ≥ 21)					
MLD mm, mean (SD)	Nifedipine ($n = 38$)	1.63 (0.44)	1.76 (0.54)	0.12 (0.28)	0.011
	ACE inhibitors ($n = 44$)	1.68 (0.42)	1.69 (0.60)	0.00 (0.39)	0.953
DS (%) mean (SD)	Nifedipine ($n = 38$)	31.79 (5.38)	23.78 (10.34)	−8.01 (11.06)	0.001
	ACE inhibitors ($n = 44$)	31.70 (6.71)	27.83 (16.05)	−3.87 (15.79)	0.115

ACE, Angiotensin-converting enzyme; DS, diameter stenosis; MLD, minimum lumen diameter. *P* value (paired *t*-test). Changes: (ΔMLD and Δ%DS).

to the occurrence of coronary artery disease in Japanese patients. A joint Japanese/Italian study of patients immediately after acute MI [10] showed that the provocation of coronary vasospasm by acetylcholine was three times more frequent in Japanese patients than in Caucasian patients. Ozaki *et al.* [17] demonstrated by QCA that the progression of coronary stenosis occurs at sites of vasospasm, whereas stenosis improves when vasospasm is treated with calcium antagonists or nitrates.

Accordingly, it is possible that the antispastic effect of nifedipine [11] inhibited the progression of coronary artery lesions and thus reduced the onset of angina pectoris requiring hospitalization in our patients with a history of MI.

It was shown by ENCORE that nifedipine inhibits atherosclerosis by improving coronary endothelial function [18], whereas it inhibited the progression of coronary calcification and the increase in intima–media thickness [19,20] in the INSIGHT side arm study. Such anti-atherosclerotic effects of nifedipine would presumably be beneficial in patients with a history of MI.

This study adds to the growing body of evidence regarding the superior effectiveness of dihydropyridine calcium antagonists for preventing cardiovascular events in (hypertensive) patients with coronary artery disease compared with ACE inhibitors. In the PREVENT study, amlodipine had no demonstrable effect on the angiographic progression of coronary atherosclerosis or the risk of major cardiovascular events, but reduced the incidence of hospitalization for unstable angina and revascularization [21]. In the ALLHAT subanalysis, heart failure was the only secondary outcome for which ACE inhibitors showed superior efficacy compared with the calcium antagonist. In contrast, for stroke and several other 'minor' outcomes (peripheral arterial disease, hospitalized angina, gastrointestinal bleeding, and angioedema), the calcium antagonist was superior to ACE inhibitor therapy [22].

A limitation of this study is that JMIC-B had a prospective, randomized, open-blinded endpoint design. In addition, the present subanalysis is underpowered and randomization seems not to be secured, although the adjustment of covariates was done by Cox proportional hazard model and ANCOVA. Patients for CAG analysis by AHA criteria and QCA analysis were a portion of the total patients. Statistical correction of covariates does not give absolute safety. Less severe baseline characteristics (diabetes mellitus and smoking in Table 1) and greater blood pressure reduction (Table 3) in the nifedipine group might have shown a more marked anti-atherosclerotic effect.

Furthermore, the possibility cannot be ruled out that the effects of unknown factors other than those adjusted this time were confounded. The achieved blood pressure of MI patients was lower in the nifedipine group than in the ACE inhibitor group, but there was no significant difference of events other than angina pectoris requiring hospitalization (Table 4). This may have been partly ascribable to a fewer number of each events.

The subjects in the present study were in the stable phase at least 2 months after acute MI, and patients with cardiac dysfunction were excluded. Accordingly, if cardiac dysfunction is not present after MI, nifedipine seems to be useful for the management of blood pressure and improvement of ischaemic heart disease.

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There is no conflict of interests.

JMIC-B Substudy

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Endpoint committee: Kodama K., Hirayama A., Nonogi H., and Sumiyoshi T.

Quantitative coronary analysis committee: Shinoda E. and Yui Y.

Biostatistician: Origasa H. (University of Toyama School of Medicine).

Safety committee: Kato K. (the Cardiovascular Institute), Nakashima M. (Hamamatsu Institute of Clinical Pharmacology and Therapeutics).

Study secretaries: Hattori R. (Kinki University School of Medicine Nara Hospital) and Morishita H. (Morishita Cardiovascular Clinic).

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Association between insulin resistance and endothelial dysfunction in type 2 diabetes and the effects of pioglitazone

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Abstract

Endothelial dysfunction is regarded as an early stage of atherosclerosis, and plays a role in the development of atherosclerotic diseases. Insulin resistance is related to the atherosclerotic process. In this study, we examined the association between endothelial function and insulin resistance in 48 subjects with type 2 diabetes. In addition, the effects of pioglitazone treatment on endothelial function and insulin resistance were investigated in a subgroup of subjects. Endothelial function of the brachial artery was non-invasively assessed using ultrasound technique. We measured flow-mediated endothelium-dependent vasodilation (FMD) and glyceryl trinitrate-induced endothelium-independent vasodilation (GTN). Insulin sensitivity was measured by the steady-state plasma glucose (SSPG) method. High SSPG levels indicate insulin resistance. There was a significant inverse correlation ($r = -0.462$, $p < 0.001$) between SSPG and FMD. Systolic blood pressure was inversely correlated with FMD ($r = -0.360$, $p < 0.013$). By multiple regression analysis, insulin resistance was the sole predictor of FMD. The effects of chronic treatment with pioglitazone were assessed in 10 subjects with type 2 diabetes. The increase in FMD significantly correlated with the decrease in SSPG. There is a significant association between vascular endothelial dysfunction and insulin resistance in type 2 diabetes. This result was supported by the effects of the insulin sensitizer, pioglitazone.

Keywords: Endothelial dysfunction; Insulin resistance; Pioglitazone

1. Introduction

Endothelial dysfunction is thought to be an important early feature in the development of atherosclerosis and occurs in subjects with type 2 diabetes mellitus [1–4]. Insulin resistance is also associated with atherosclerosis and is observed in subjects with type 2 diabetes [5,6]. We previously reported the association between endothelial dysfunction and insulin resistance in patients with essential hypertension [7]. However, the mechanisms responsible for endothelial dysfunction and insulin resistance in hypertension might be different from those of type 2 diabetes. Therefore, we evaluated the relationship between endothelial dysfunction and insulin resistance in patients with type 2 diabetes. Thiazolidinediones, an agonist for the peroxisome proliferator-activated receptor γ (PPARγ), improve insulin resistance. If there is a significant relationship

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between endothelial dysfunction and insulin resistance, thiazolidinediones might influence endothelial function. Therefore, we examined the effects of pioglitazone on endothelial dysfunction and insulin resistance in a subgroup of subjects with type 2 diabetes to verify the relationship between endothelial dysfunction and insulin resistance.

The main purpose of this study was to investigate the relation between vascular endothelial dysfunction and insulin resistance in type 2 diabetes. In addition, the influence of pioglitazone treatment was examined.

2. Subjects and methods

2.1. Subjects

Forty-eight (30 males and 18 females) patients with type 2 diabetes were recruited in the Department of Diabetes and Atherosclerosis of the National Cardiovascular Center. The subjects did not have diabetic retinopathy or nephropathy. Subjects were included on the basis of the following criteria: age between 40 and 79 years, body mass index (BMI) between 17 and 35 kg/m^2, type 2 diabetes confirmed by American Diabetes Association criteria [8]. Subjects were excluded from participation if they had coronary heart, peripheral vascular, renal, hepatic or other endocrine diseases. Subjects were excluded if they had a resting seated blood pressure greater than 150 mmHg systolic or greater than 90 mmHg diastolic, or were taking anti-hypertensive drugs. Diabetes duration was 5.3 ± 1.9 years (3–7 years). Diabetes treatment regimens included diet alone (27 subjects), sulfonylures (18 subjects) and metformin (3 subjects).

The 48 subjects had an average age of 64 ± 1 years, with a mean BMI of 24.6 ± 0.3 kg/m^2, HbA_{1c} of 8.6 ± 0.2%, total cholesterol of 199 ± 5 mg/dl, HDL-cholesterol of 43 ± 2 mg/dl and triglycerides of 137 ± 14 mg/dl. Mean systolic and diastolic blood pressures were 131 ± 3 and 74 ± 2 mmHg, respectively.

Of the 48 diabetic subjects, 10 subjects were started on a single 15 or 30 mg-tablet of pioglitazone (Actos, Takeda Pharmaceuticals, Tokyo, Japan) by mouth each day. Inclusion criteria of the pioglitazone treatment were male, non-smoker, diet alone treatment and mild to severe insulin resistance (SSPG > 160 mg/dl). They received a mean dose of 25.5 ± 2.3 mg/day (30 mg/day: seven subjects and 15 mg/day: three subjects) of pioglitazone for 16.3 ± 1.6 weeks (10–20 weeks). The secondary assessments of endothelial function and insulin sensitivity were performed after the pioglitazone treatments.

The study protocol was approved by the ethics committee of the National Cardiovascular Center. The experiments were conducted with the understanding and the consent of each participant.

2.2. Methods

2.2.1. Assessment of endothelial function

Using the ultrasound method, arterial endothelium and smooth muscle function were measured by examining brachial artery responses to endothelium-dependent and endothelium-independent stimuli. Ultrasound measurements were carried out based on the method described by Celermajer et al. [9] and our method was reported previously [7]. The assessments were performed after an overnight fast in a quiet air-conditioned room (22–23 °C). The diameter of the brachial artery was measured on B-mode ultrasound images, with the use of a 10-MHz linear array transducer (ProSound SSD-5500, ALOKA, Tokyo, Japan). The right brachial artery was scanned in longitudinal sections 1–10 cm above the elbow, after at least 15 min of rest in the supine position. After the detection of the right transducer position, the skin surface was marked and the arm was kept in the same position during the study. All scans were recorded using a super-VHS videocassette recorder (SONY, SVO-9500MD), and analyzed later.

At first, baseline measurements of the diameter were carried out. Endothelium-dependent vasodilation (flow-mediated dilation) was determined by the scans during reactive hyperemia. Because flow-mediated vasodilation was mainly blocked by *N*-monomethyl-L-arginine (an inhibitor of endothelial nitric oxide synthase) this dilation was regarded as endothelium dependent [10]. A pneumatic cuff placed around the forearm was inflated to 220 mmHg and was deflated after 4.5 min. The diameter of the brachial artery was scanned and recorded after deflation. After 10–15 min rest, the second control scan of the diameter and the flow velocity was recorded. Then, sublingual glyceryl trinitrate spray (300 μg) was administered and 3.5–4 min later a final scan of the diameter was recorded.

Measurements of the vessel diameter were taken from the anterior to the posterior 'm' line (interface between the media and adventitia) at endo-diastole, coincident with the R wave on a continuously recorded electrocardiogram. The diameters at four cardiac cycles were measured for each scan, and these results were averaged. Determinations of the flow-mediated dilation were carried out 45–60 s after the cuff release to measure a maximum diameter. Vasodilation by reactive hyperemia (flow-mediated dilation, FMD) or glyceryl trinitrate (GTN) was expressed as the percent change in diameter compared to the baseline values.

2.2.2. Insulin sensitivity test

Glucose utilization in response to insulin was evaluated by a modified steady state plasma glucose (SSPG) method [6,7,11] using Sandostatin (octreotide acetate; Novartis, Basel, Switzerland) after an overnight fasting for at least 12 h. Sandostatin (9.8 pmol in bolus followed by a constant infusion of 73.5 pmol/h) and Novolin R insulin (Novo Nordisk S/A, Tokyo, Japan, 45 pmol/kg [7.5 mU/kg] in a bolus followed by a constant infusion at a rate of 4.62 pmol/kg/min [0.77 mU/kg/min]) were infused intravenously for 120 min.

Glucose in a final 12% solution containing KCl (0.5 μmol/kg/min) were infused at a rate of 0.033 mmol/kg/min [6 mg/kg/min] through an antecubital vein via a constant infusion pump. Blood samples were drawn routinely at 0 and 120 min (9:00 and 11:00 a.m.) for determination of glucose and insulin. Value of glucose at 120 min (SSPG) was used as a marker of insulin sensitivity to glucose utilization. High SSPG levels indicate peripheral insulin resistance. At 120 min SSPG was rapidly measured using a Glucometer (Bayer Corporation, Osaka, Japan) separate from the usual measurement of glucose and insulin. When rapidly measured, if SSPG was found to be lower than 250 mg/dl, oral glucose intake was necessary to prevent hypoglycemia after the insulin sensitivity test. The subjects should have lunch within 30 min after the insulin sensitivity test to prevent hypoglycemia. Homeostasis model assessment (HOMA-IR) was calculated from fasting glucose and insulin concentrations during insulin sensitivity test as follows: HOMA-IR = fasting glucose (mg/dl) × fasting insulin (μU/ml)/405.

2.3. Statistical analysis

Values are expressed as mean ± S.E. A probability value of <0.05 was considered to indicate statistical significance. The strength of the correlation between FMD and GTN with respect to risk factors was assessed by Pearson's linear correlation and multiple regression analysis. The effects of pioglitazone on each clinical parameter were assessed by paired *t*-test and Pearson's linear correlation.

3. Results

3.1. Association between endothelial dysfunction and each parameter in 48 subjects

A significant inverse correlation was observed between FMD and SSPG ($r = -0.462$, $p < 0.001$; Fig. 1). There was no relation between FMD and HbA_{1c} ($p = 0.856$). We also observed a significant inverse correlation between FMD and systolic blood pressure ($r = -0.360$, $p < 0.013$). No significant correlation was found between FMD and diabetic duration, diastolic blood pressure, total cholesterol, HDL cholesterol, triglyceride, age or BMI. There was no relationship between FMD and HOMA-IR ($p = 0.097$).

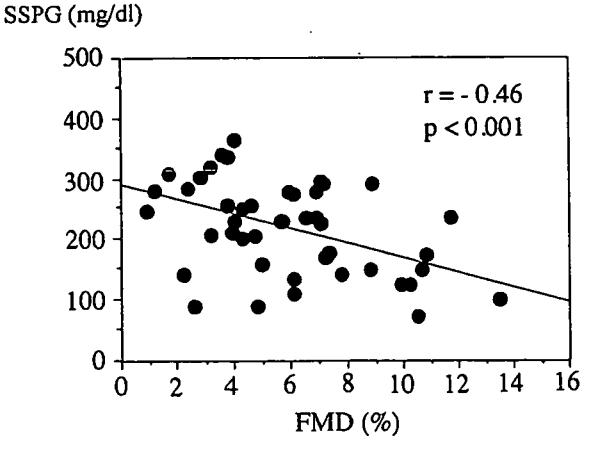

Fig. 1. Relationship between FMD and SSPG in subjects with type 2 diabetes. FMD, flow-mediated vasodilation; SSPG, steady state plasma glucose.

We performed multiple regression analysis to evaluate the independent influence of risk factors including SSPG, systolic blood pressure, HbA_{1c}, total cholesterol, BMI and age on FMD. FMD was independently related to SSPG (regression coefficient: $\beta = -0.419$, $p = 0.0086$) but not to systolic blood pressure ($\beta = -0.254$, $p = 0.0782$), HbA_{1c} ($\beta = -0.090$, $p = 0.5616$), total cholesterol ($\beta = -0.067$, $p = 0.6336$), BMI ($\beta = -0.258$, $p = 0.0863$) or age ($\beta = -0.085$, $p = 0.5650$).

With respect to GTN, no significant correlation was observed between GTN and SSPG or other parameters, including HbA_{1c}, diabetic duration, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, triglyceride, age or BMI.

3.2. Effects of pioglitazone treatment on endothelial function and insulin resistance

The effects of treatment with pioglitazone were assessed in 10 male subjects with type 2 diabetes (a subgroup of 48 subjects). Table 1 shows the clinical parameters of the 10 subjects before and after pioglitazone treatment. SSPG, HbA_{1c} and fasting plasma glucose decreased and FMD increased significantly due to pioglitazone treatment. However, BMI, total cholesterol, HDL-cholesterol, triglyceride, systolic blood pressure and diastolic blood pressure did not

Table 1
Clinical characteristics of the subjects with type 2 diabetes treated with pioglitazone

	Before Tx	After Tx
Number	10	
Age (years)	65 ± 2	
SSPG (mg/dl)	230 ± 13	185 ± 17*
FMD (%)	4.5 ± 1.1	8.1 ± 1.5***
Body mass index (kg/m^2)	24.4 ± 0.4	24.7 ± 0.4
Fasting plasma glucose (mg/dl)	162 ± 11	133 ± 8*
HbA_{1c} (%)	8.4 ± 0.4	7.0 ± 0.3**
Total cholesterol (mg/dl)	199 ± 8	206 ± 7
HDL cholesterol (mg/dl)	47 ± 4	50 ± 4
Triglyceride (mg/dl)	120 ± 15	129 ± 13
Systolic blood pressure (mmHg)	137 ± 5	137 ± 2
Diastolic blood pressure (mmHg)	78 ± 5	79 ± 1

Values are mean ± S.E. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. before Tx. Tx, Treatments with pioglytazone.

significantly change. GTN was also not significantly altered.

The change in FMD before and after administration of pioglitazone was not significantly correlated with the change in HbA_{1c} ($p = 0.314$) or fasting plasma glucose ($p = 0.717$). The increase in FMD, that is, the improvement in endothelial function, was significantly correlated with the decrease in SSPG ($r = -0.649$, $p < 0.05$).

4. Discussion

In this study we found that vascular endothelial dysfunction was associated with insulin resistance in type 2 diabetes. This result was supported by the effects of the insulin sensitizer, pioglitazone, which improved both endothelial dysfunction and insulin resistance in patients with type 2 diabetes.

The close association between insulin resistance and endothelial dysfunction is our main interest. In a study by Hogikyan et al. [3], insulin resistance as measured by the insulin sensitivity index (minimal model: S_I), was not found to be correlated with endothelial dysfunction in subjects with type 2 diabetes. They measured the forearm blood flow (FABF) using venous occlusion plethysmography and used the FABF response to acetylcholine as an index of endothelial function. The narrow range of S_I values among the subjects might have led to the lack of a relationship between S_I and endothelial dysfunction. In addition, the sensitivity of the techniques using plethysmography might have been low.

Balletshofer et al. [12] reported a significant association between endothelial dysfunction and insulin resistance, as measured by the glucose clamp method, in young normotensive and normoglycemic first-degree relatives of patients with type 2 diabetes. Therefore, this association was observed in a non-diabetic population at future risk of type 2 diabetes.

Insulin causes endothelium-derived nitric oxide (NO)-dependent vasodilation [13]. It is suggested that this insulin action occurs via the phosphatidylinositol 3-kinase and Akt pathway [14,15]. As for insulin action, phosphatidylinositol 3-kinase activation is critical for insulin-mediated glucose uptake into skeletal muscle [16]. Therefore, insulin resistance due to a systemic defect in the phosphatidylinositol 3-kinase pathway might cause a combined defect in insulin-mediated glucose uptake and insulin-mediated endothelial vasodilation.

Among the risk factors for atherosclerosis, insulin resistance was found to be the sole predictor of endothelium dependent vasodilation by multiple regression analysis in the present study. We observed no relationship between FMD and HbA_{1c}. Bagg et al. found that a short-term reduction of HbA_{1c} levels did not appear to affect endothelial function in patients with type 2 diabetes [17]. Furthermore, Mather et al. reported that insulin resistance was the sole predictor of endothelial dysfunction following metformin treatment in type 2 diabetes in stepwise multivariate analysis, and HbA_{1c} and glucose levels were not significant predictors of endothelial dysfunction [18].

Treatment with HMG-CoA inhibitors (statins) has been shown to improve endothelial dysfunction [19–21]. Therefore, statin treatment may have affected the relationship between FMD and risk factors in the present study. In 48 diabetic subjects, 5 were treated with pravastatin and one with simvastatin. We performed statistical analysis in 42 subjects without statin treatment. There was a significant inverse correlation between SSPG and FMD ($r = -0.538$, $p < 0.001$). A significant inverse correlation was observed between FMD and systolic blood pressure ($r = -0.330$, $p < 0.05$). No significant correlation was found between FMD and HbA_{1c}, diabetic duration, diastolic blood pressure, total cholesterol, HDL cholesterol, triglyceride, age or BMI. On multiple regression analysis, FMD was independently related to SSPG (regression coefficient:$\beta = -0.500$, $p = 0.0032$) but not to systolic blood pressure, HbA_{1c}, total cholesterol, BMI or age.

Smoking is associated with endothelial dysfunction [22,23]. Smoking might interfere in the relationship between FMD and risk factors. In 48 diabetic subjects, 13 were smokers in the present study. Statistical analysis was performed in 35 non-smokers. A significant correlation was found between SSPG and FMD ($r = -0.582$, $p < 0.001$). There was a significant inverse correlation between FMD and systolic blood pressure ($r = -0.357$, $p < 0.05$). No significant correlation was observed between FMD and HbA_{1c}, diabetic duration, diastolic blood pressure, total cholesterol, HDL cholesterol, triglyceride, age or BMI. On multiple regression analysis, FMD was independently related to SSPG (regression coefficient:$\beta = -0.591$, $p = 0.0019$) but not to systolic blood pressure, HbA_{1c}, total cholesterol, BMI or age. In the present study, FMD did not correlate with HOMA-IR. SSPG is a more sensitive marker to measure insulin sensitivity than HOMA-IR.

Endothelial dysfunction and insulin resistance were improved by pioglitazone treatment in the present study. SSPG, HbA_{1c} and fasting plasma glucose were decreased and other risk factors were not changed by the treatment. It was reported that hyperglycemia itself inhibits endothelial NO synthase activity [24] and causes endothelial dysfunction [25]. On the other hand,

insulin resistance was also associated with endothelial dysfunction in 48 subjects with type 2 diabetes in this study. The change in FMD before and after treatment with pioglitazone was not significantly correlated with the change in HbA_{1c} or fasting plasma glucose, and the increase in FMD was significantly correlated with the decrease in SSPG in this study. Because of the small number of subjects ($n = 10$), we cannot exclude the possibility that the decreased plasma glucose level improved endothelial dysfunction. The decrease in plasma glucose level might be associated with improved endothelial function if the pioglitazone study was performed with more cases. It can at least be said that insulin resistance is an important factor affecting endothelial function. As previously described, a similar study [18] found that treatment with metformin improved both endothelial function and insulin resistance, and the glucose level and HbA_{1c} were not significant predictors of endothelial dysfunction. Considering generally than the above-mentioned points, it is suggested that increased insulin sensitivity plays an important role in the improvement of endothelial function by pioglitazone treatment.

Pistrosch et al. [26] demonstrated that treatment with rosiglitazone, another PPARγ activator, ameliorated insulin resistance measured by glucose clamp method, and improved endothelial function determined by venous occlusion plethysmography in patients with recently diagnosed type 2 diabetes. They performed a double-blind cross-over trial and treated with rosiglitazone and nateglinide in random order. Glycemic control was comparable under rosiglitazone and nateglinide. Only rosiglitazone improved insulin resistance and endothelial function in the study. Thus, they also showed the relation between insulin sensitivity and endothelial function independent of glucose level in type 2 diabetes.

In conclusion, in the present study we demonstrated significant association between vascular endothelial dysfunction and insulin resistance in type 2 diabetes, and pioglitazone treatment improved both endothelial dysfunction and insulin resistance with a statistical link. These data support the concept of the important role of insulin resistance in the pathogenesis of endothelial dysfunction in type 2 diabetes mellitus.

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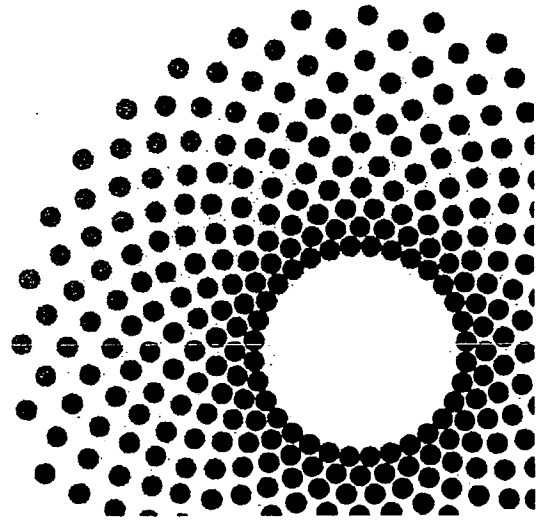

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Impaired flow-mediated vasodilatation and insulin resistance in type 2 diabetic patients with albuminuria

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ABSTRACT

An elevated urinary albumin excretion is associated with an increased risk of cardiovascular disease due to atherosclerosis, but the pathophysiological mechanism underlying this association is poorly understood. We studied 217 diabetic patients, that is, 121 normoalbuminuric patients, 71 microalbuminuric patients, and 25 macroalbuminuric patients. We evaluated flow-mediated dilatation of brachial artery (%FMD, one endothelial function marker associated with endogenous NO production), von Willebrand factor (vWF, endothelial activation marker), high-sensitive CRP (hsCRP, a low-grade inflammation marker), asymmetric dimethyl arginine (ADMA, an endogenous inhibitor of NO synthesis), and insulin sensitivity by steady-state plasma glucose method. %FMD was apparently decreased in microalbuminuric and macroalbuminuric patients compared with normoalbuminuric patients ($p < 0.001$). Moreover, %FMD was significantly correlated with the degree of albuminuria ($r = -0.38$, $p < 0.05$). On the other hand, vWF and hsCRP did not show significant difference between normoalbuminuric patients and microalbuminuric patients. In diabetic patients with macroalbuminuria, ADMA was significantly elevated compared to those with normoalbuminuria. Insulin sensitivity was significantly associated with urinary albumin excretion rate. These results suggested that endothelial dysfunction which may be due to impaired NO production and insulin resistance underlie the association between diabetic nephropathy and atherosclerosis in diabetic patients.

1. Introduction

Elevated urinary albumin excretion rate (UAER) is strongly associated with an increased risk of cardiovascular diseases, which is independent of conventional risk factors including hypertension, hyperlipidemia, and smoking, among individuals with and without type 2 diabetes [1,2]. This suggests that elevated UAER may be associated with atherosclerosis by the unidentified mechanism.

The endothelium plays a crucial role in the maintenance of vascular tone and structure, and endothelial dysfunction is a key feature of atherosclerosis. Nitric oxide (NO) is one of the important endothelium-derived vasoactive mediators. NO is involved in a wide variety of regulatory mechanisms of cardiovascular system, including vascular tone and vascular structure [3].

Flow-mediated endothelium-dependent vasodilatation (FMD) method is based on the endothelial stimulus of increased shear stress (the tangential force on the vessel wall exerted by flowing blood). Increased shear stress is caused by post-ischemic hyperemia and elicits a slow Ca^{+}-independent two to threefold increase in NO production [4,5]. Indeed,

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Celemajer et al. reported that flow mediate vasodilatation was mainly blocked by N-monomethyl-L-arginine (an inhibitor of endothelial NO synthetase) [6].

To clarify the contribution of impaired NO production in vascular endothelium to the association between atherosclerotic disease and diabetic nephropathy, we examined FMD by ultrasonography. In addition, we measured asymmetric dimethyl arginine (ADMA), an endogenous NO synthesis inhibitor [3]. Since low-grade inflammation is another key feature of the pathophysiology of atherosclerosis [7], we further examined high-sensitive CRP, which is an inflammation marker, to investigate whether this feature is involved in the association between atherosclerotic disease and diabetic nephropathy.

It has recently been indicated that microalbuminuria and atherosclerosis are closely associated with insulin resistance [8–10], implying that insulin resistance may underlie these pathophysiological conditions although the causative relationship remains unknown. In the present study, we further examined insulin sensitivity in the type 2 diabetic patients with different stage of albuminuria and analyzed the correlation between insulin sensitivity and FMD, to investigate whether elevated UAER and endothelial dysfunction may be associated with insulin resistance.

2. Methods

2.1. Study subjects

We studied 217 patients with type 2 diabetes who were <75 years of age. Patients with a current acute illness (including clinically significant infectious disease) were excluded from this study. Twenty-four-hour urine collections were performed for two consecutive days to determine the stage of diabetic nephropathy. Creatinine clearance (Ccr) was calculated from the 24-h urine sample and serum creatinine levels. The patients were divided into three groups according to the UAER, as follows: normoalbuminuria (UAER <30 mg/day), microalbuminuria (30 ≤ UAER < 100 mg/day) and macroalbuminuria (UAER ≥ 300 mg/day). To exclude diabetic patients with nondiabetic kidney disease, we excluded patients with hematuria or abnormal urinary sediments. This study was conducted with the approval of National Cardiovascular Center Trust Ethics Committee, and patients gave written informed consent before participation.

2.2. Brachial artery flow-mediated dilatation

Using ultrasonography, arterial endothelium and smooth muscle function were measured by examining brachial artery responses to endothelium-dependent and endothelium-independent stimuli. Ultrasoundsonographic measurements were carried out according to the method described by Celemajer et al. [6]. Brachial artery diameter was measured from B-mode ultrasound images using 10-MHz liner array transducer (ProSound SSD-5500; Aloka, Japan) while an ECG trace was simultaneously recorded. The right brachial artery was scanned in longitudinal sections 1–10 cm above elbow, after at least 15 min of rest in the supine position, the skin surface was marked and the arm was kept in the same position during the study.

Baseline measurements of the diameter were carried out. Endothelium-dependent vasodilatation (flow-mediated dilatation) was determined by scans during reactive hyperemia. A pneumatic cuff placed around the forearm was inflated to 220 mmHg and was deflated after 4.5 min. The diameter of the brachial artery was scanned and recorded after dilation. After 10 min rest, the second control scan of the diameter was recorded. Then, sublingual glyceryl trinitrate spray (300 μg) was administered and 3.5 min later a final scan of the diameter was recorded.

Measurements of the vessel diameter were taken from the anterior to posterior "m" line (interface between the media and adventitia) at end-diastole, coincident with the R wave on a continuously recorded ECG. The diameters at four cardiac cycles were measured for each scan, and these results were averaged. Determinations of the FMD were carried out 45–60 s after the cuff release to measure a maximal diameter. Vasodilatation by reactive hyperemia or glyceryl trinitrate (NTG) was expressed as the percent change in diameter compared with the baseline values.

2.3. Insulin sensitivity test

Glucose utilization in response to insulin was evaluated with a newly modified steady-state plasma glucose (SSPG) method with octreotide acetate (Sandostatin; Novartis) after an overnight fasting period of 12 h [11]. Sandostatin (9.8-pmol bolus followed by a constant infusion of 73.5 pmol/h) and Humulin R insulin (45 pmol/kg bolus followed by a constant infusion at a rate of 4.62 pmol/(kg min); Eli Lilly) were infused intravenously for 120 min. Glucose in a final 12% solution containing KCl (0.5 μmol/(kg min)) was infused at a rate of 0.033 mmol/(kg min) (6 mg/(kg min)) through an antecubital vein via a constant infusion pump. Blood samples were drawn routinely at 0 and 120 min (9:00 and 11:00 a.m.) for the determination of glucose, insulin, and lipids. The value of glucose at 120 min (SSPG) was used as a marker of insulin sensitivity to glucose utilization. High SSPG levels showed peripheral insulin resistance.

Another marker of insulin resistance (IR) was estimated by calculating homeostasis model assessment (HOMA-IR) index ((fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l))/22.5) [12].

2.4. Measurement of vWF, hsCRP, and ADMA

vWF was determined in citrated plasma using a homemade enzyme-linked immunosorbent assay. Data are given as the percentage of pooled human plasma (set at 100%). Serum hsCRP concentration was determined by latex nephelometry method (SRL, Tokyo, Japan). Serum ADMA concentration was determined by high-performance liquid chromatography method (SRL, Tokyo, Japan).

2.5. Statistical analysis

Values are expressed as means ± S.D. Statistical analysis was performed by use of ANOVA followed by Scheffes' test. The