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Effects of a 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitor, Fluvastatin, on Coronary Spasm After Withdrawal of Calcium-Channel Blockers

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Objectives The purpose of this study was to determine whether a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) suppresses coronary spasm.

Background Coronary spasm is associated with endothelial dysfunction. Statins have been shown to improve endothelial function.

Methods This was a prospective, randomized, open-label, end point study. Sixty-four patients who had no significant organic coronary stenosis and in whom coronary spasm was induced by intracoronary injection of acetylcholine (ACh) were randomly assigned to fluvastatin 30 mg/day plus the conventional calcium-channel blocker (CCB) therapy (31 patients, statin group) or the conventional CCB therapy (33 patients, nonstatin group). After 6 months of treatment, the intracoronary injection of ACh was repeated and the coronary spasm was assessed.

Results Coronary spasm was suppressed in 16 of the 31 patients (51.5%, $p < 0.0001$) of the statin group and in 7 of the 33 patients (21.2%, $p = 0.0110$) of the nonstatin group after 6 months of treatment. Thus, the number of patients with ACh-induced coronary spasm was significantly reduced in the statin group as compared with the nonstatin group (51.6% vs. 21.2%, $p = 0.0231$) after 6 months of treatment.

Conclusions The addition of fluvastatin 30 mg/day to the conventional CCB therapy for 6 months significantly reduced the number of patients with ACh-induced coronary spasm as compared with the conventional CCB therapy. Thus, a statin (fluvastatin) may possibly be a novel therapeutic drug for coronary spasm. (J Am Coll Cardiol 2008;51:1742–8) © 2008 by the American College of Cardiology Foundation

It is established that coronary spasm plays an important role not only in the pathogenesis of variant angina but also in ischemic heart disease in general, including resting angina, effort angina, acute myocardial infarction, and sudden death (1,2). Calcium-channel blockers (CCBs) have been shown to be highly effective in suppressing coronary spasm and are widely used as the standard therapy for coronary spasm (1–4). However, a substantial number of patients with coronary spasm are resistant to CCBs even in high doses, and lethal arrhythmias and/or sudden death occur in some of them (1). We have shown that endothelial nitric oxide (NO) activity is reduced and endothelial function impaired in the coronary arteries involved in spasm (1,5). There is increasing evidence that 3-hydroxy-3-methylglutaryl coen-

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zyme A reductase inhibitors (statins) improve endothelial dysfunction and reduce cardiovascular events in patients with coronary artery disease (6–9). Thus, it is possible that statins may also suppress coronary spasm and prove to be a novel therapy for coronary spasm. However, no studies have been done to test this hypothesis.

The present study was designed to examine whether addition of a statin to the conventional CCB therapy would result in greater reduction in coronary spasm as compared with the conventional CCB therapy.

Methods

Patients. The SCAST (Statin and Coronary Artery Spasm Trial) trial was a prospective randomized open-label end point study to examine the effect of a statin (fluvastatin) added to the conventional therapy on coronary spasm. We recruited 78 participants between January 2002 and December 2005 from 9 hospitals in Japan. Entry criteria were subjects who were 30 to 80 years of age who underwent coronary angiography because of chest pain and/or ischemic ECG changes on exercise and had no organic coronary stenosis (>50%) and in whom coronary spasm was induced by intracoronary injection of acetylcholine (ACh). Coronary spasm was defined as a total or subtotal obstruction or severe diffuse constriction of an epicardial coronary artery associated with transient myocardial ischemia as evidenced by ischemic ST-segment changes on ECG. Exclusion criteria included recent myocardial infarction, acute coronary syndrome, heart failure, liver disease, creatinine level >1.5 mg/dl, acute inflammation, malignant diseases, and cholesterol-lowering medication within a month. These 78 patients were registered and randomly assigned to either the statin group (fluvastatin 30 mg/day plus conventional therapy, n = 39) or the nonstatin group (conventional therapy, n = 39) by using a random number generating computer system. The conventional therapy consisted of CCBs (slow-release diltiazem 100 to 200 mg/day, or slow-release nifedipine 20 to 40 mg/day). The protocol of this study was approved by all site institutional review boards and each patient provided written informed consent.

Induction of coronary spasm. Coronary spasm was induced by intracoronary injection of ACh after diagnostic catheterization in the morning. The details of the method were previously reported (10). In brief, nitrates, CCBs, beta-adrenergic blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and other vasodilators or vasoconstrictors were withheld for >48 h. ACh was injected in incremental doses of 50 and 100 μg into the left coronary artery and then 50 μg into the right coronary artery under continuous monitoring of ECG and blood pressure. Coronary spasm induced by this method usually disappeared spontaneously within 1 to 2 min, and both the left and right coronary arteries could be examined separately unless severe spasm occurred in the left coronary artery and necessitated the prompt injection of isosorbide dinitrate into the artery. After the end of the test, isosorbide dinitrate (0.1 mg) was injected into the coronary artery and angiography was again performed.

Abbreviations and Acronyms

ACh = acetylcholine
CCB = calcium-channel blocker
ECG = electrocardiogram
LCA = left coronary artery
LDL = low-density lipoprotein
NO = nitric oxide
RCA = right coronary artery
ROCK = RhoA-associated kinase

Treatment and follow-up. Each patient was evaluated at 1, 3, and 6 months for assessment of angina episodes, drug compliance, ECG, lipid profile, and safety markers. At the 6-month follow-up, patients again underwent catheterization after withdrawal of CCBs for a week in both groups, but the statin was not withdrawn in the statin group. Care was taken to replicate angiographic views, tube height, catheter positions, and order of infusions used in the baseline study.

Assessment of coronary artery diameter and ECG changes in response to ACh injection. Severe coronary spasm to a residual lumen diameter <0.4 mm could not be accurately quantified because of technical limitations of the computer-assisted quantitative coronary angiography (11). However, the spasm sites at baseline were identified and the same segments and the nonspasm segments proximal to the spasm sites were evaluated quantitatively at the submaximal dose of ACh (50 μg) in the left coronary artery at baseline and follow-up at the core laboratory. Each segment was referenced to a specific anatomic landmark for identification and films from the baseline and follow-up were examined at the same time to ensure analysis of the identical portion of the vessel. The measurement was blinded to the ECG findings and the group assignment. An end-diastolic frame was digitized and the diameter of the index vessel was measured with CAAS II software (PIE Medical Imaging, Maastricht, Limburg, the Netherlands). Two or 3 sites of each segment were measured and the coronary response to ACh was expressed as the percentage change from baseline in mean lumen diameter and was compared at the same site of the same artery in the same patients before and after 6 months of treatment in each group. The ECG was examined in a blinded fashion as to the coronary angiographic findings and the group assignment at the core laboratory.

Laboratory methods. Fasting blood samples were drawn by venipuncture 1 to 2 days before coronary angiography and the hematological and biochemical analyses were done using standard laboratory procedures. Serum high sensitivity C-reactive protein was measured in duplicate by automated immunoturbidimetric assay using the Synchron LX20 Pro system (Beckman Coulter, Inc., Fullerton, California) (12).

Statistical analysis. The primary end point of the study was the ACh-induced coronary spasm 6 months after the treatment. We hypothesized that the induction rate of coronary spasm in the statin group would be different from that in the nonstatin group. The number of sample size

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(number of patients) (n = 76 to 78) was calculated based on a z test at the 2-tailed test 5% significance level and 80% power. A 30% treatment effect was considered to be clinically significant, assuming a recurrence rate of coronary spasm to be 80% to 90% in the nonstatin group. The secondary end point was the coronary artery diameter change in response to the submaximal dose (50 μg) of ACh. For continuous variables, differences between groups were evaluated by unpaired *t* test or Mann-Whitney rank-sum test, and those within groups by paired *t* test or Wilcoxon signed rank test. For discrete variables, differences were expressed as counts and percentages and were analyzed with chi-square (or Fisher exact) test between groups and with McNemar test or Fisher exact test within groups, as appropriate. A 2-tailed p value of <0.05 was considered to be statistically significant. Data were expressed as mean ± SD. However, when the variable was significantly skewed, the median (25th, 75th percentile) was reported.

This study was investigator initiated and the drug makers had no direct or indirect involvement in the design of the study, provision of the drug, data collection, or preparation of the manuscript.

Results

Clinical characteristics and adverse events. Of the 78 patients randomized, 14 patients were withdrawn (statin group: n = 8; nonstatin group: n = 6). In the statin group, 1 patient suffered from sudden death during an earthquake, 1 patient from drug allergy, and 1 patient underwent a cervical operation. In the nonstatin group, 1 patient could not undergo coronary angiography at 6-month follow-up because of trouble at catheterization. The remaining patients were withdrawn due to unwillingness to undergo the second catheterization (statin group: n = 5; nonstatin group: n = 5). Thus, a total of 64 patients (31 patients in the statin group and 33 patients in the nonstatin group) were available for analysis, and all of these patients had adhered to the protocol. The baseline characteristics of the 2 treatment groups are shown in Table 1.

Twenty-eight patients of the statin group and 27 patients of the nonstatin group had episodes of chest discomfort before the entry. Twenty-one of the patients (75.0%, $p < 0.0001$) in the statin group and 19 of the patients (70.4%, $p < 0.001$) in the nonstatin group became asymptomatic during 6 months of treatment. Thus, CCBs were

Table 1 Clinical Characteristics of the Study Subjects

Variables	Statin Group (n = 31)	Nonstatin Group (n = 33)	p Value
Age (yrs)	63.4 ± 12.5	61.8 ± 10.2	0.6091
Gender (male/female)	21/10	18/15	0.2795
Body mass index (kg/m^2)	23.6 ± 3.4	24.5 ± 3.7	0.3022
Hypertension	10/31	14/33	0.4012
Diabetes mellitus	6/31	6/33	0.9044
Current smoker	15/31	12/33	0.3304
Leukocyte (/μl)	6,425 ± 1,582	6,228 ± 2,049	0.6689
Hemoglobin (g/dl)	13.4 ± 1.7	13.6 ± 1.7	0.6382
Platelet ($\times 10^4$/μl)	22.8 ± 6.0	22.2 ± 8.3	0.7546
CRP (mg/l)*	1.94 (0.79, 5.04)	1.36 (0.38, 2.45)	0.3502
Total protein (g/dl)	6.8 ± 0.5	6.8 ± 0.4	0.8798
Albumin (g/dl)	3.98 ± 0.41	3.97 ± 0.33	0.8989
Fast blood sugar (mg/dl)	104.3 ± 22.7	111.2 ± 39.8	0.4082
AST (U/l)	25.3 ± 11.1	24.9 ± 9.3	0.8818
ALT (U/l)	25.6 ± 14.7	25.7 ± 17.1	0.7874
CPK (U/l)	113.0 ± 75.4	101.5 ± 63.2	0.5154
Total cholesterol (mg/dl)	193.8 ± 36.3	193.9 ± 44.2	0.9950
LDL cholesterol (mg/dl)	114.9 ± 33.2	119.7 ± 27.0	0.5366
HDL cholesterol (mg/dl)	55.5 ± 13.8	53.3 ± 15.1	0.5656
Triglyceride (mg/dl)	130.2 ± 67.0	134.3 ± 64.7	0.8021
Medications			
Ca-channel blockers	31/31	33/33	0.9642
Diltiazem/nifedipine	20/11	26/7	0.3778
Aspirin	11/31	13/33	0.7468
ACE inhibitor	3/31	2/33	0.6673
ARB	6/31	10/33	0.3121
Nitrate	4/31	7/33	0.5119
Beta-blocker	1/31	3/33	0.6136
Fibrate	1/31	0/33	0.4844

*Median (25th, 75th percentile).
ACE = angiotensin-converting enzyme; ALT = alanine aminotransferase; ARB = angiotensin II receptor blocker; AST = aspartate aminotransferase; CPK = creatine phosphate kinase; CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

highly effective in suppressing symptomatic coronary spasm in both groups in agreement with the results of previous studies (1–4), and there was no significant difference ($p = 0.924$) in the incidence of subjective symptoms during the treatment period between the 2 groups with these sample sizes. Twenty-five patients in the statin group and 22 patients in the nonstatin group underwent 24-h Holter monitoring on entry, and ischemic ECG changes were detected in 6 patients (24.0%) of the statin group and in 5 patients (22.7%) of the nonstatin group. After 6 months of treatment, ischemic ECG changes on Holter monitoring were detected in none of the statin group and in 2 patients of the nonstatin group. No adverse effects were detected in either group during the follow-up period.

Coronary angiographic and ECG changes in response to ACh at baseline and after 6 months of treatment. At the registration, spasm was induced at 26 left coronary artery (LCA) and at 19 right coronary artery (RCA) in the statin group, and at 27 LCA and at 16 RCA in the nonstatin group, accompanied by ischemic ECG changes as shown in Table 2. After 6 months of treatment and after withdrawal of CCBs for 1 week, spasm was induced at 12 LCA and at 10 RCA in the statin group, and at 21 LCA and at 13 RCA in the nonstatin group, accompanied by ST-segment changes as shown in Table 2. The ACh-induced coronary spasm after 6 months of treatment was similar to that of the baseline in location and type (total or subtotal obstruction, or severe diffuse narrowing).

Coronary spasm was suppressed in 16 out of 31 patients (51.6%, $p < 0.0001$) of the statin group and in 7 out of 33 patients (21.2%, $p = 0.0110$) of the nonstatin group after 6 months of treatment. Thus, the number of patients with ACh-induced coronary spasm was significantly reduced in the statin group as compared with the nonstatin group (51.6% vs. 21.2%, $p = 0.0231$) after 6 months of treatment (Fig. 1). The results also revealed that coronary spasm was induced in a high proportion of the patients after withdrawal of CCBs after 6 months of treatment.

Quantitative angiographic analysis showed that vasoconstrictor response (percent change in luminal diameter) to 50 μg ACh of the LCA at the same spasm segment of the same patient was significantly reduced in both groups after 6 month as compared with at baseline (from $-35.5 \pm 20.1\%$ to $-21.3 \pm 16.9\%$, $p < 0.0001$ in the statin group, and from $-36.8 \pm 21.6\%$ to $-30.1 \pm 26.3\%$, $p = 0.0221$ in the nonstatin group). There was thus a significant reduction in the constrictor response to ACh in the statin group as compared with the nonstatin group ($-21.3 \pm 16.9\%$ vs. $-30.1 \pm 26.3\%$, $p = 0.0087$) after 6 months of treatment (Fig. 2 left). However, there was no significant difference in the response at the nonspasm segments between the 2 groups ($-6.6 \pm 12.6\%$ in the statin group vs. $-10.3 \pm 12.8\%$ in the nonstatin group, $p = 0.1029$) after 6 months of treatment, although the response was significantly reduced in the statin group ($p = 0.0337$) after 6 months of treatment (Fig. 2, right).

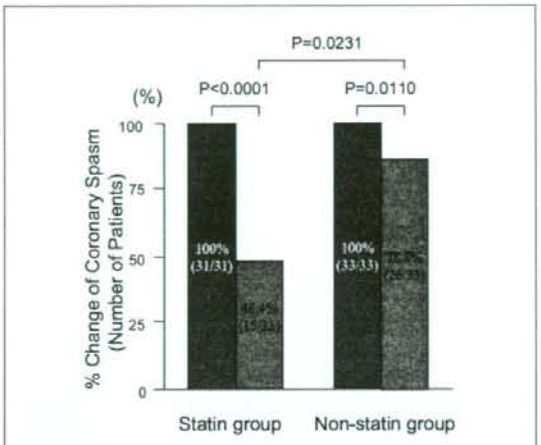

Figure 1 **Statin and ACh-Induced Coronary Spasm**

Number of patients with acetylcholine (Ach)-induced coronary spasm at baseline **(blue bars)** and after 6 months **(orange bars)** of treatment in the statin group and nonstatin group.

Lipid profile and other laboratory data. The results of lipid and other laboratory data are shown in Table 3. The levels of low-density lipoprotein (LDL) cholesterol and C-reactive protein decreased significantly in the statin group (from 114.9 ± 33.2 mg/dl to 86.4 ± 27.6 mg/dl, $p < 0.0001$, and from 1.94 [0.79, 5.04] mg/l to 0.60 [0.25, 2.20] mg/l, $p = 0.0077$, respectively), whereas there were no differences in these levels in the nonstatin group, after 6 months of treatment.

Table 2 **Ischemic ECG Changes Accompanying ACh-Induced Coronary Spasm at Baseline and After 6 Months of Treatment**

		Statin Group			Nonstatin Group		
	ECG Changes	Baseline	6 Months	p Value	Baseline	6 Months	p Value
LCA	ST-segment elevation	11/26 (42.3%)	3/26 (11.5%)		9/27 (33.3%)	6/27 (22.2%)	
	ST-segment depression	15/26 (57.7%)	9/26 (34.6%)		18/27 (66.7%)	15/27 (55.6%)	
	Total	26/26 (100%)	12/26 (46.2%)	< 0.0001	27/27 (100%)	21/27 (77.8%)	0.0229
RCA	ST-segment elevation	10/19 (52.6%)	5/19 (26.3%)		6/16 (37.5%)	5/16 (31.3%)	
	ST-segment depression	9/19 (47.4%)	5/19 (26.3%)		10/16 (62.5%)	8/16 (50.0%)	
	Total	19/19 (100%)	10/19 (52.6%)	< 0.0001	16/16 (100%)	13/16 (81.3%)	0.2258

ACh = acetylcholine; ECG = electrocardiogram; LCA = left coronary artery; RCA = right coronary artery.

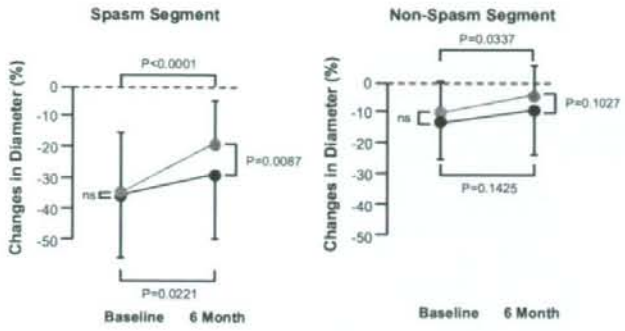

Figure 2 Coronary Artery Diameter Change to ACh Injection

Response of coronary artery diameter to intracoronary injection of acetylcholine (ACh) (50 μg) at baseline and after 6 months of treatment at the spasm segment (**left**) and the nonspasm segment (**right**) in the statin group (**orange**; n = 63) and nonstatin (**blue**; n = 66) group.

Discussion

Calcium-channel blockers are established as the standard therapy for coronary spasm (1,3,4). However, coronary spasm may not be completely controlled even with high doses of CCBs in a substantial number of patients (1,3,4). Moreover, it is not known how long the drugs should be administered for control of coronary spasm (13,14). Coronary spasm occurs most often from midnight to early morning and is often silent and is usually not induced by exercise in the daytime (1,14). Moreover, there are daily, weekly, and monthly, as well as circadian, variations in the frequency of coronary spasm (1,15,16) and the episodes of coronary spasm may not be detected even with 24-h ambulatory ECG monitoring as shown in this as well as previous studies (1,15). Accordingly, we examined the effect of a statin (fluvastatin) on the coronary spasm induced by the intracoronary injection of ACh in the present study.

The study showed that the addition of fluvastatin to the conventional therapy for 6 months significantly reduced the occurrence of coronary spasm as compared with the conventional therapy. The quantitative angiographic analysis also showed that the constrictor response to a submaximal dose of ACh at the same site of the same spasm segment was reduced significantly in the statin group as compared with the nonstatin group, but there was no significant difference in the response at the same site of the nonspasm segment between the 2 groups after 6 months of follow-up. Thus, the present study reveals that the spasm segment was specifically responsive to a statin (fluvastatin) as compared with the nonspasm segment. Fluvastatin significantly reduced the serum level of LDL cholesterol. However, it is not known whether the suppression of coronary spasm was directly caused by the lowering of LDL cholesterol. Our previous study shows that elevation of LDL cholesterol is not a risk factor for coronary spasm (17). Recent experimental and clinical evidence indicates that statins improve endothelial dysfunction and suppress inflammation independently of cholesterol lowering or through "pleiotropic" effects (8,9,18,19). We have shown that endothelial NO bioactivity was reduced and levels of markers of inflammation were increased in patients with coronary spasm (1,5,15,20). In the present study, the serum level of C-reactive protein, a marker of inflammation was reduced significantly in the statin group, whereas the level remained unchanged in the nonstatin group in agreement with the results of previous studies (8,9,21,22).

Table 3 Lipid Profile and Other Laboratory Data at Baseline and After 6 Months of Treatment

	Statin Group (n = 31)			Nonstatin Group (n = 33)		
Variables	Baseline	6 Months	p Value	Baseline	6 Months	p Value
Total-cholesterol (mg/dl)	193.8 ± 36.3	167.7 ± 35.3	<0.0001	193.9 ± 44.2	206.9 ± 26.5	0.0767
LDL cholesterol (mg/dl)	114.9 ± 33.2	86.4 ± 27.6	<0.0001	119.7 ± 27.0	117.1 ± 29.5	0.6124
HDL cholesterol (mg/dl)	55.5 ± 13.8	59.6 ± 13.9	0.0371	53.3 ± 15.1	54.6 ± 17.7	0.8151
Triglyceride (mg/dl)	130.2 ± 67.0	113.5 ± 63.1	0.1155	134.3 ± 64.7	150.3 ± 124.7	0.4491
CRP (mg/l)*	1.94 (0.79, 5.04)	0.60 (0.25, 2.20)	0.0077	1.18 (0.38, 2.45)	0.61 (0.20, 1.40)	0.4503
Platelet ($\times 10^4/\mu l$)	22.8 ± 6.0	21.6 ± 5.6	0.0363	22.2 ± 8.3	23.8 ± 8.8	0.2190

*Median (25th, 75th percentile).
Abbreviations as in Table 1.

In addition to inhibiting cholesterol synthesis, statins are shown to block the synthesis of isoprenoid intermediates of the cholesterol biosynthetic pathway, thereby preventing translocation and activation of RhoA (8,19). Emerging evidence indicates that inhibition of RhoA and its downstream RhoA-associated kinase (ROCK) pathway leads to the elevation of endothelial NO synthase expression and NO activity in conjunction with reduction of inflammation, as well as proliferation of vascular smooth muscle (8,18,19).

Coronary spasm may be regarded as an abnormal hypercontraction of coronary vascular smooth muscle and accumulating evidence indicates that hypercontraction of vascular smooth muscle is mainly caused by the enhanced Ca^{2+} sensitization through the activation of the RhoA/ROCK pathway (23–25).

Accordingly, it is reasonable to postulate that the Rho/ROCK pathway plays a key role in the pathogenesis of coronary spasm (24,25) and statins, including fluvastatin are likely to suppress coronary spasm by inhibiting the Rho/ROCK pathway, thereby improving endothelial function, enhancing NO activity, and suppressing inflammation and Ca^{2+} sensitivity of coronary smooth muscle. Indeed, we and others (18,26–28) have shown that statins enhance the expression of endothelial NO synthase gene in human endothelial cells. The present study thus suggested that a statin may be a novel disease-modifying drug for coronary spasm based on the underlying pathogenesis and improve the overall prognosis for the patients. On the other hand, coronary spasm was again induced in the majority of the patients on withdrawal of CCBs after 6 months of treatment without a statin. The results indicated that calcium-channel blockade for 6 months might not substantially modify the underlying pathogenesis of coronary spasm in the majority of the patients (15) and revealed that CCBs should not be withdrawn for at least 6 months.

One previous study reported that a statin reduced coronary vasoconstrictor response to ACh, reflecting improved endothelial function in patients with stable coronary artery disease after 5.5 months of treatment (29). However, other studies reported that 6 months of statins therapy had no significant effect on coronary endothelial vasomotor function in patients with stable coronary artery disease (30,31). We studied the spasm segment as well as nonspasm segment in patients with coronary spasm, because no previous studies examined the effect of a statin on coronary spasm. It is interesting to note that the effect of a statin appears within 1 to 3 months of treatment in patients with unstable coronary syndrome, whereas it is apparent only after 1 to 2 years in those with stable coronary artery disease (8,9,32). Different pathophysiological mechanisms of coronary disease may thus respond differently to a statin.

Study limitations. Although the present study reveals that an addition of fluvastatin to the conventional therapy suppresses coronary spasm, the duration of the study period was short (6 months) and the number of the study subjects was small because of the invasive nature of the study for demonstrating coronary spasm. A larger number of patients and longer periods of follow-up would be required to determine the long-term efficacy and safety of a statin (fluvastatin) for the treatment of coronary spasm by using a noninvasive and more sensitive method for detection of coronary spasm. This study thus provides a rationale for the use of a statin for the treatment of coronary spasm. It is not yet known whether these combination therapies will prove to be cost-effective and safe for long-term use in patients with coronary spasm. We used fluvastatin because the risk for rhabdomyolysis and the possible drug interaction with a CCB were reported to be lowest and the possible vascular effect was highest among the statins clinically available at the time of initiating this study (33). It thus remains to be determined whether statins other than fluvastatin may have the similar effects on coronary spasm.

Conclusions

The present study showed that an addition of fluvastatin 30 mg/day to the conventional CCB therapy for 6 months significantly reduced coronary spasm induced by intracoronary injection of ACh as compared with the conventional therapy. The quantitative angiographic analysis revealed that fluvastatin was specifically effective in suppressing the vasoconstrictor response of the spasm segments as compared with the nonspasm segments.

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Low-Dose Aspirin for Primary Prevention of Atherosclerotic Events in Patients With Type 2 Diabetes: A Randomized Controlled Trial

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Low-Dose Aspirin for Primary Prevention of Atherosclerotic Events in Patients With Type 2 Diabetes

A Randomized Controlled Trial

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Context Previous trials have investigated the effects of low-dose aspirin on primary prevention of cardiovascular events, but not in patients with type 2 diabetes.

Objective To examine the efficacy of low-dose aspirin for the primary prevention of atherosclerotic events in patients with type 2 diabetes.

Design, Setting, and Participants Multicenter, prospective, randomized, open-label, blinded, end-point trial conducted from December 2002 through April 2008 at 163 institutions throughout Japan, which enrolled 2539 patients with type 2 diabetes without a history of atherosclerotic disease and had a median follow-up of 4.37 years.

Interventions Patients were assigned to the low-dose aspirin group (81 or 100 mg per day) or the nonaspirin group.

Main Outcome Measures Primary end points were atherosclerotic events, including fatal or nonfatal ischemic heart disease, fatal or nonfatal stroke, and peripheral arterial disease. Secondary end points included each primary end point and combinations of primary end points as well as death from any cause.

Results A total of 154 atherosclerotic events occurred: 68 in the aspirin group (13.6 per 1000 person-years) and 86 in the nonaspirin group (17.0 per 1000 person-years) (hazard ratio [HR], 0.80; 95% confidence interval [CI], 0.58-1.10; log-rank test, $P=.16$). The combined end point of fatal coronary events and fatal cerebrovascular events occurred in 1 patient (stroke) in the aspirin group and 10 patients (5 fatal myocardial infarctions and 5 fatal strokes) in the nonaspirin group (HR, 0.10; 95% CI, 0.01-0.79; $P=.0037$). A total of 34 patients in the aspirin group and 38 patients in the nonaspirin group died from any cause (HR, 0.90; 95% CI, 0.57-1.14; log-rank test, $P=.67$). The composite of hemorrhagic stroke and significant gastrointestinal bleeding was not significantly different between the aspirin and nonaspirin groups.

Conclusion In this study of patients with type 2 diabetes, low-dose aspirin as primary prevention did not reduce the risk of cardiovascular events.

Trial Registration clinicaltrials.gov Identifier: NCT00110448

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DIABETES MELLITUS IS A POWERful risk factor for cardiovascular events. The Framingham Heart Study reported that diabetes was associated with odds ratios for coronary heart disease of 1.5 and 1.8 for men and women, respectively, and relative risks for stroke of 1.4 and 1.7 for men and women, respectively.[1-3] Individuals with diabetes have a 2- to 4-fold increased risk of developing cardiovascular events than those without diabetes.[6]

Several earlier investigations have shown that aspirin therapy is established as a secondary prevention strategy for cardiovascular events.[7-9] Clinical guidelines have recommended that individuals with risk factors for coronary heart disease should take aspirin for primary prevention and for secondary prevention; in particular, those with diabetes were considered good candidates for aspirin except for those with contraindications.[10-15] The American Diabetes Association recommends use of aspirin as a primary prevention strategy in patients with diabetes who are at increased cardiovascular risk, including those who are older than 40 years or who have additional risk factors, such as family history of coronary heart disease, hypertension, smoking, dyslipidemia, or albuminuria.[16] Nonetheless, the clinical trial data for aspirin in primary preven-

For editorial comment see p 2180.

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tion are limited. Several large trials of aspirin for primary prevention have examined its effects in subgroups with diabetes; these subgroup analyses did not demonstrate a significant effect on reducing vascular events because they were underpowered.[17-21] Thus, a primary prevention trial of aspirin for diabetic patients is needed.

The Japanese Primary Prevention of Atherosclerosis With Aspirin for Diabetes (JPAD) trial was undertaken to examine the efficacy of low-dose aspirin therapy for the primary prevention of atherosclerotic events in patients with type 2 diabetes.

METHODS

The JPAD trial was a prospective, randomized, open-label, controlled trial with blinded end-point assessment. Patient enrollment started in December 2002 and was completed in May 2005; patients were followed up until April 2008. Patients were enrolled and followed up at 163 institutions throughout Japan. The institutional review board at each participating hospital approved this trial, and written informed consent was obtained from each patient.

Trial Population

The inclusion criteria were diagnosis of type 2 diabetes mellitus, age between 30 and 85 years, and ability to provide informed consent. The exclusion criteria were electrocardiographic changes consisting of ischemic ST-segment depression, ST-segment elevation, or pathologic Q waves; a history of coronary heart disease confirmed by coronary angiography; a history of cerebrovascular disease consisting of cerebral infarction, cerebral hemorrhage, subarachnoid hemorrhage, and transient ischemic attack; a history of arteriosclerotic disease necessitating medical treatment; atrial fibrillation; pregnancy; use of antiplatelet or antithrombotic therapy, defined as aspirin, ticlopidine, cilostazol, dipyridamole, trapidil, warfarin, and argatroban; a history of severe gastric or duodenal ulcer; severe liver dysfunction; severe renal dysfunction, and allergy to aspirin.

Trial Protocol

Enrolled patients were randomly assigned to the aspirin group or the nonaspirin group. The randomization was performed as nonstratified randomization from a random number table. The study center prepared the sealed envelopes with random assignments and distributed them by mail to the physicians in charge at the study sites. Patients in the aspirin group were assigned to take 81 mg or 100 mg of aspirin once daily. Patients were followed up at each hospital visit or by telephone if necessary. Follow-up visits were scheduled every 2 weeks for patients seen in a clinic setting and every 4 weeks for patients seen in a hospital setting. Data for patients who were lost to follow-up were included at the day of last follow-up. Patients were allowed to use any concurrent treatment. Patients in the nonaspirin group were also allowed to use antiplatelet/thrombotic therapy, including aspirin, if needed and vice versa.

End Points

The primary end point was any atherosclerotic event, which was a composite of sudden death; death from coronary, cerebrovascular, and aortic causes; nonfatal acute myocardial infarction; unstable angina; newly developed exertional angina; nonfatal ischemic and hemorrhagic stroke; transient ischemic attack; or nonfatal aortic and peripheral vascular disease (arteriosclerosis obliterans, aortic dissection, mesenteric arterial thrombosis) during the follow-up period. Key secondary end points were each primary end point and combinations of primary end points and death from any cause. Adverse events analyzed included gastrointestinal (GI) events and any hemorrhagic events other than hemorrhagic stroke. All potential primary end points, secondary end points, and adverse events were adjudicated by an independent committee on validation of data and events that was unaware of the group assignments.

Sample Size Calculation

For sample size calculation, we first estimated the incidences of cardiovascular and cerebrovascular events among Japanese diabetic patients. The incidence of cardiovascular death, myocardial infarction, and cerebrovascular events were 7.5, 7.5, and 8.0 events per 1000 Japanese diabetic patients per year, respectively, according to the Hisayama-cho study[22] and Funagata study.[23] The total incidence of the atherosclerotic events, including peripheral arterial disease, was suggested to be 3 times the aforementioned number by the Hypertension Optimal Treatment (HOT) study.[24] Because the recent incidence of atherosclerotic events among Japanese individuals seemed relatively lower than that previously reported in Japan, we discounted 25% of the estimated 69 events that were expected to occur and estimated that 52 events per 1000 Japanese diabetic patients would occur annually.

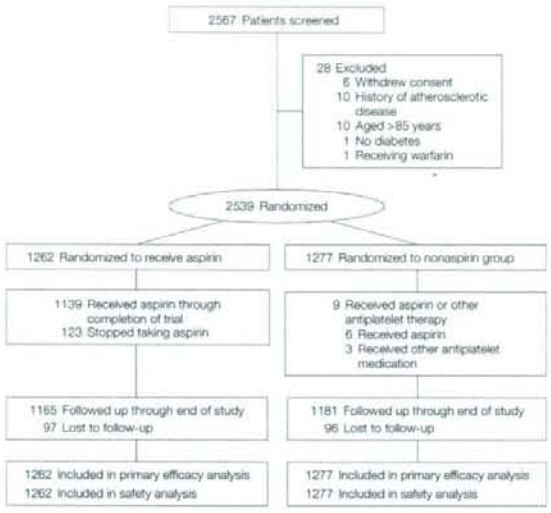
Based on a 2-sided α level of .05, a power of 0.95, an enrollment period of 2 years, and a follow-up period of 3 years after the last enrollment, we estimated that 2450 patients would need to be enrolled to detect a 30% relative risk reduction for an occurrence of atherosclerotic disease by aspirin.[19]

Statistical Analyses

Efficacy comparisons were performed on the basis of time to the first event, according to the intention-to-treat principle, including all patients in the group to which they were randomized with patients lost to follow-up censored at the day of the last visit. Safety analyses were performed on data from all enrolled patients. Following the descriptive statistics, cumulative incidences of primary and secondary end points were estimated by the Kaplan-Meier method and differences between groups were assessed with the log-rank test. We used the Cox proportional hazards model to estimate hazard ratios (HRs) of aspirin use along with 95% confidence intervals (CIs). We used the χ^2 test or Fisher exact test to evaluate adverse events.

We also conducted subgroup analyses for predetermined subgroups: sex (men, women); age (younger than 65 years, 65 years or older); hypertensive

Figure 1. Participation in Japanese Primary Prevention of Atherosclerosis With Aspirin for Diabetes (JPAD) Trial



status (hypertensive, normotensive); smoking status (current or past smoker, nonsmoker); and lipid status (hyperlipidemia, normolipidemia). Using the Cox proportional hazard model, proportional hazard assumptions were assessed on the plots of log (time) vs log [−log(survival)] stratified by index variables. Patients with missing values for any selected variable were excluded from the analyses that used the variable.

All statistical analyses were conducted using SAS version 9.1 (SAS Institute Inc, Cary, North Carolina) and S-Plus version 7.0 (Insightful Corp, Seattle, Washington). *P* values of less than .05 were considered statistically significant. An independent safety monitoring board monitored the safety and efficacy of the study after 2 years of follow-up for an interim assessment and at the end of the study.

RESULTS

Study Population

The study screened 2567 patients with type 2 diabetes mellitus without a history of atherosclerotic disease, including cardiovascular disease, stroke, and peripheral vascular disease, from December 2002 to May 2005 in 163 institutions (FIGURE 1). Six patients who withdrew their informed consent were excluded. Twenty-two patients met exclusion criteria. We randomly assigned 2539 patients as follows: 1262 patients in the aspirin group and 1277 patients in the nonaspirin group. Patients were followed up until April 2008. The median follow-up period was 4.37 years (95% CI, 4.35-4.39). A total of 193 patients were lost to follow-up, and data for those patients were censored at the day of last follow-up.

Baseline Clinical Characteristics

Baseline clinical characteristics, including treatments for diabetes, hypertension, and dyslipidemia and diabetic microvascular complications, were similar between the 2 groups (TABLE 1). Overall mean (SD) age was 65 (10) years; 55% of patients were men. Median duration of diabetes was 7.3 years in the aspirin group and 6.7 years in the nonaspirin group. Diabetes was well controlled in both groups: mean (SD) levels of glycated hemoglobin were 7.1% (1.4%) in the aspirin group and 7.0% (1.2%) in the nonaspirin group. The prevalence of hypertension and dyslipidemia was 58% and 53%, respectively. Blood pressure was well controlled in both groups: mean (SD) systolic pressure, 136 (15) mm Hg; mean (SD) diastolic pressure, 77 (9) mm Hg in the aspirin group and mean (SD) systolic pressure, 134 (15) mm Hg; mean (SD) diastolic pressure, 76 (9) mm Hg in the nonaspirin group.

By the end of the study, 123 patients (10%) in the aspirin group had stopped taking the study medication. Since aspirin therapy was allowed in the nonaspirin group, 6 patients (0.5%) had taken aspirin and 3 patients (0.2%) had taken other antiplatelet medication.

Efficacy Analysis

A total of 154 atherosclerotic events occurred (TABLE 2). The incidence of the primary end point of any atherosclerotic event, a composite of sudden death, death from cardiovascular or aortic causes, nonfatal acute myocardial infarction, unstable angina, exertional angina, nonfatal ischemic and hemorrhagic stroke, transient ischemic attack, and nonfatal aortic and peripheral vascular disease (arteriosclerosis obliterans, aortic dissection, mesenteric arterial thrombosis), was not significantly different in the aspirin group (68 events, 5.4%) than in the nonaspirin group (86 events, 6.7%) (HR, 0.80; 95% CI, 0.58-1.10; log-rank test, $P=.16$) (Table 2 and FIGURE 2).

The combined end point of fatal coronary events and fatal cerebrovascular events occurred in 1 patient (stroke) in the aspirin group and 10 patients (5 fatal myocardial infarctions and 5 fatal strokes) in the nonaspirin group (HR, 0.10; 95% CI, 0.01-0.79; $P=.0037$). Other secondary coronary, cerebrovascular, and peripheral vascular disease end points are shown in Table 2; there were no significant differences between the aspirin group and the nonaspirin group in these end points. There were 2 deaths due to aortic dissection, both in the low-dose aspirin group, and 1 nonfatal aortic dissection in the nonaspirin group. A total of 13 hemorrhagic strokes occurred; the incidences in each group were similar (6 in the aspirin group and 7 in the

nonaspirin group). There was 1 fatal hemorrhagic stroke in the aspirin group and 4 in the nonaspirin group.

Death from causes other than cardiovascular events were as follows for the aspirin group and nonaspirin group, respectively: there were 15 and 19 deaths due to malignancy, 2 and 5 due to infection, 3 and 0 due to suicide, 2 and 0 due to traffic crashes, and 1 and 1 due to liver cirrhosis. Therefore, 23 patients in the aspirin group and 25 patients in the nonaspirin group died from causes other than cardiovascular events. Eight patients in the aspirin group and 3 patients in the nonaspirin group died from unknown causes. A total of 34 patients in the aspirin group and 38 patients in the nonaspirin group died from any cause (HR, 0.90; 95% CI, 0.57-1.14; log-rank test, $P = .67$).

Subgroup Analyses

In the 1363 patients aged 65 years or older (719 in the aspirin group and 644 in the nonaspirin group), the incidence of atherosclerotic events was significantly lower in the aspirin group (45 events, 6.3%) than in the nonaspirin group (59 events, 9.2%) (HR, 0.68; 95% CI, 0.46-0.99; $P = .047$). In the 1176 patients younger than age 65 years, there were 23 events in the aspirin group (4.2%) and 27 events in the nonaspirin group (4.3%), a difference that was not significant (HR, 1.0; 95% CI, 0.57-1.70; $P = .98$). A formal test of interaction with age did not show a significant result ($P = .27$). There were no significant differences between the aspirin group and nonaspirin group in other subgroup analyses, including men, women, hypertensive, normotensive, current or past smokers, nonsmokers, dyslipidemia, and normolipidemia (FIGURE 3).

Safety

The prespecified analysis of adverse events is shown in TABLE 3. The hemorrhagic events consisted of GI bleeding in 12 patients in the aspirin group and 4 in the nonaspirin group and retinal hemorrhage in 8 patients in the aspirin group and 4 in the nonaspirin group. In the aspirin group, 4 patients had serious adverse events that needed a transfusion; no patients in the non-

Table 1. Baseline Clinical Characteristics

Characteristic	No. (%) Aspirin Group (n = 1262)	No. (%) Nonaspirin Group (n = 1277)
Age, mean (SD), y	65 (10)	64 (10)
Male	706 (56)	681 (53)
Current smoker	289 (23)	248 (19)
Past smoker	545 (43)	482 (38)
Body mass index, mean (SD)[a]	24 (4)	24 (4)
Hypertension	742 (59)	731 (57)
Dyslipidemia	680 (54)	665 (52)
Systolic blood pressure, mean (SD), mm Hg	136 (15)	134 (15)
Diastolic blood pressure, mean (SD), mm Hg	77 (9)	76 (9)
Duration of diabetes, median (IQR), y	7.3 (2.8-12.3)	6.7 (3.0-12.5)
Diabetic microvascular complication		
Diabetic retinopathy	187 (15)	178 (14)
Diabetic nephropathy	169 (13)	153 (12)
Proteinuria, ≥15 mg/dL	222 (18)	224 (18)
Diabetic neuropathy	163 (13)	137 (11)
Dermal ulcer	6 (0.5)	6 (0.5)
Treatment for diabetes		
Sulfonylureas	737 (58)	710 (56)
α-Glucosidase inhibitors	422 (33)	414 (32)
Biguanides	168 (13)	186 (15)
Insulin	166 (13)	160 (13)
Thiazolidines	63 (5)	65 (5)
Treatment for hypertension and dyslipidemia		
Calcium channel blockers	436 (35)	440 (34)
Angiotensin-II receptor antagonists	269 (21)	266 (21)
Angiotensin-converting enzyme inhibitors	178 (14)	195 (15)
β-Blockers	75 (6)	87 (7)
α-Blockers	53 (4)	38 (3)
Statins	322 (26)	328 (26)
Family history		
Type 2 diabetes mellitus	526 (42)	513 (40)
Ischemic heart disease	147 (12)	143 (11)
Stroke	275 (22)	251 (20)
Patient medical history		
Peptic ulcer	83 (7)	96 (8)
Clinical laboratory measurements, mean (SD)		
Hemoglobin A_{1c} level, %	7.1 (1.4)	7.0 (1.2)
Fasting plasma glucose level, mg/dL	148 (50)	146 (48)
Total cholesterol level, mg/dL	202 (34)	200 (34)
Fasting triglyceride level, mg/dL	135 (88)	134 (89)
HDL cholesterol level, mg/dL	55 (15)	55 (15)
Blood urea nitrogen level, mg/dL	16 (5)	16 (5)
Serum creatinine level, mg/dL	0.8 (0.3)	0.8 (0.2)
Red blood cells, $\times 10^6$/mL	45.2 (4.7)	45.0 (4.8)
White blood cells, $\times 10^3$/mL	6.2 (1.6)	6.1 (1.7)
Hemoglobin level, g/dL	14.0 (1.5)	14.0 (1.5)

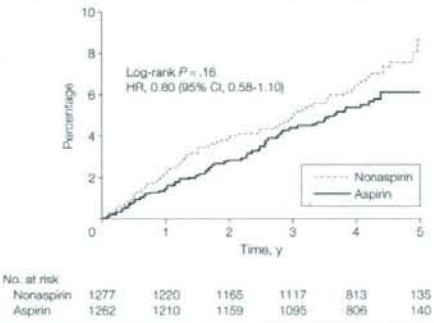
Abbreviations: HDL, high-density lipoprotein; IQR, interquartile range.
SI conversion factors: To convert glucose to mmol/L, multiply by 0.0555; to convert total and HDL cholesterol to mmol/L, multiply by 0.0259; to convert triglyceride to mmol/L, multiply by 0.0113; to convert urea nitrogen to mmol/L, multiply by 0.357; to convert creatinine to μmol/L, multiply by 88.4.
[a] Calculated as weight in kilograms divided by height in meters squared.

Table 2. Atherosclerotic Events

	Aspirin Group		Nonaspirin Group			
	No. (%)	No. per 1000 Person-Years	No. (%)	No. per 1000 Person-Years	Hazard Ratio (95% CI)	*P* Value
Primary end point: all atherosclerotic events	68 (5.4)	13.6	86 (6.7)	17.0	0.80 (0.58-1.10)	.16
Coronary and cerebrovascular mortality	1 (0.08)	0.2	10 (0.8)	2.0	0.10 (0.01-0.79)	.0037
CHD events (fatal + nonfatal)	28 (2.2)	5.6	35 (2.7)	6.9	0.81 (0.49-1.33)	.40
Fatal MI	0	0	5 (0.4)	1.0		
Nonfatal MI	12 (1.0)	2.4	9 (0.7)	1.8	1.34 (0.57-3.19)	.50
Unstable angina	4 (0.3)	0.8	10 (0.8)	2.0	0.40 (0.13-1.29)	.13
Stable angina	12 (1.0)	2.4	11 (0.9)	2.2	1.10 (0.49-2.50)	.82
Cerebrovascular disease (fatal + nonfatal)	28 (2.2)	5.6	32 (2.5)	6.3	0.84 (0.53-1.32)	.44
Fatal stroke	1 (0.08)	0.2	5 (0.4)	1.0	0.20 (0.024-1.74)	.15
Nonfatal stroke						
Ischemic	22 (1.7)	4.4	24 (1.9)	4.6	0.93 (0.52-1.66)	.80
Hemorrhagic	5 (0.4)	1.0	3 (0.2)	0.6	1.68 (0.40-7.04)	.48
Transient ischemic attack	5 (0.4)	1.0	8 (0.6)	1.6	0.63 (0.21-1.93)	.42
Peripheral artery disease[a]	7 (0.6)	1.4	11 (0.9)	2.2	0.64 (0.25-1.65)	.35

Abbreviations: CHD, coronary heart disease; CI, confidence interval; MI, myocardial infarction.
[a] Arteriosclerosis obliterans (5 in aspirin group and 8 in nonaspirin group); aortic dissection (2 fatal in the aspirin group and 1 nonfatal in the nonaspirin group); mesenteric artery thrombosis (1 in the nonaspirin group), and retinal artery thrombosis (1 in the nonaspirin group).

Figure 2. Total Percentage of Atherosclerotic Events According to Treatment Group



CI indicates confidence interval; HR, hazard ratio.

aspirin group required transfusion. Another 13 patients in the aspirin group had minor bleeding. There was no significant difference in the composite of hemorrhagic stroke and severe GI bleeding, which occurred in 10 patients in the aspirin group and in 7 patients in the nonaspirin group.

COMMENT

Myocardial infarction and ischemic stroke are leading causes of mortality and morbidity in patients with type 2 diabetes.[25] Given the rapid increase in the number of patients with type 2 diabetes worldwide and especially in Asia, establishing effective means of primary prevention of coronary and cerebrovascular events is an important public health priority.[26] In the JPAD primary prevention trial of 2539 type 2 diabetic patients without documented cardiovascular disease, the incidence of the primary end point of total atherosclerotic events, consisting of coronary, cerebrovascular, and peripheral vascular events, was not significantly different in the group that received prophylactic aspirin (81 or 100 mg once daily) than in the nonaspirin group. With the exception of fatal coronary and cerebrovascular events, none of the prespecified secondary end points were reduced significantly in the low-dose aspirin group. The incidence of fatal coronary and cerebrovascular events, a prespecified secondary end point, was significantly reduced in the low-dose aspirin group ($P = .0037$). A benefit of low-dose aspirin on the primary end point also was suggested in the subgroup of patients aged 65 years or older, which had a significant 32% relative reduction in total atherosclerotic events ($P = .047$). The cardiovascular mortality benefit was achieved with a small increase in cases of serious GI bleeding (4 patients in the aspirin group had bleeding that required transfusion), but no excess of fatal GI or cerebral hemorrhages.

The JPAD trial enrolled 2539 diabetic patients without documented coronary or cerebrovascular complications; the sample size was the largest among the previous primary prevention studies in respect to the number of diabetic patients enrolled. However, no difference was found in the effect of aspirin on the primary end point or most secondary end points.

The interpretation of these results is challenging because the overall event rates were low: 17 in 1000 Japanese diabetic patients. This is one-third of the event rate anticipated in our sample-size calculations, which were based on the Hisayama-cho[22] and Funagata[23] epidemiologic studies conducted in Japan in the 1990s. Current treatment of cardiovascular risk factors in patients with type 2 diabetes has improved since the 1990s and may have ac-

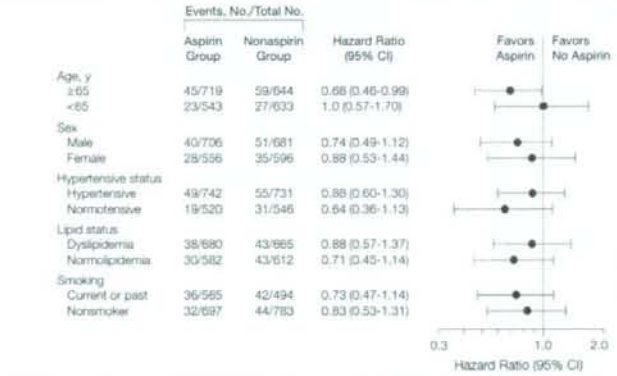
counted for the lower event rates: there is better control of glucose, blood pressure, and lipid levels in clinical practice. The baseline characteristics of patients in the JPAD trial were similar to those in previous studies except that body mass index was relatively lower in the JPAD trial than that in the previous studies, although similar to that in other studies of Japanese diabetics.[4,6,19-21,27,28]

A meta-analysis of primary prevention trials that included the British Doctors' Trial, the Physicians' Health Study, the Thrombosis Prevention Trial, the Hypertension Optimal Treatment (HOT) study, the Primary Prevention Project (PPP) trial, and the Women's Health Study showed that aspirin therapy significantly reduced the risk of total coronary heart disease, nonfatal myocardial infarction, and total cardiovascular events with a nonsignificant trend for decreased risk of stroke, cardiovascular mortality, and all-cause mortality.[29] However, the evidence for aspirin in prevention of cardiovascular events in diabetic patients has been surprisingly scant. Previous studies investigating the effects of low-dose aspirin on primary prevention of cardiovascular events did not enroll solely diabetic patients but enrolled patients with hypertension in the HOT study; patients with 1 or more cardiovascular risk factors in the Thrombosis Prevention Trial and the PPP trial; and a healthy population in the British Doctors' Trial, the Physicians' Health Study, and the Women's Health Study.

Several large primary prevention trials have included subgroup analyses of patients with diabetes. The Physicians' Health Study of 22 071 healthy men randomized to receive 325 mg of aspirin every other day or placebo showed a significant reduction in myocardial infarction for the entire population, but there was no significant difference for the small number of individuals with diabetes in the 2 treatment groups (11/275 in the aspirin group and 26/258 in the placebo group).[18] The Antithrombotic Trialists' Collaboration meta-analysis of 287 randomized trials reported effects of antiplatelet therapy (mainly aspirin) vs control in 135 000 patients and showed a nonsignificant 7% reduction in the odds for serious vascular events for the subgroup of 5126 patients with diabetes.[19]

Sacco et al[20] described the effects of aspirin on atherosclerotic disease in patients with diabetes as a subgroup of the PPP trial, which investigated the effects of aspirin and vitamin E in a 2-by-2 factorial trial of 4495 patients with at least 1 known major cardiovascular risk factor.[21] The original study was stopped on ethical grounds after a mean follow-up of 3.6 years because aspirin was associated with a lower risk of atherosclerotic disease in the overall group. The results of a subgroup analysis of 1031 diabetic patients did not

Figure 3. Subgroup Analysis of Incidence of Atherosclerotic Events



CI indicates confidence interval (shown as error bars in the plot).

Table 3. Adverse Effects

	No.	
	Aspirin Group	Nonaspirin Group
Bleeding, gastrointestinal[a]		
Hemorrhagic gastric ulcer	5	3
Bleeding from esophageal varices	1	0
Bleeding from colon diverticula	2	0
Gastrointestinal bleeding due to cancer	2	0
Hemorrhoid bleeding	1	0
Gastrointestinal bleeding (cause unknown)	1	1
Bleeding, other		
Retinal bleeding	8	4
Bleeding after tooth extraction	1	0
Subcutaneous hemorrhage	3	0
Hematuria	2	1
Nose bleeding	6	1
Chronic subdural hematoma	2	0
Nonbleeding gastrointestinal event		
Nonhemorrhagic gastritis	3	0
Nonhemorrhagic gastric ulcer	17	3
Nonhemorrhagic duodenal ulcer	1	1
Only gastrointestinal symptom	26	0
Other		
Anemia	4	0
Asthma	1	0

[a] In the aspirin group, 4 cases of severe gastrointestinal bleeding required transfusion.

reach statistical significance, possibly because of the early stopping of the trial and the subgroup size.[20] In addition, medication adherence was poor in the PPP trial: 28.2% of subjects assigned to aspirin had stopped this therapy by the conclusion of the trial. In the JPAD study, only 10% of patients in the aspirin group stopped this therapy by the end of the mean 4.37 years of follow-up.

Because of the low event rate in JPAD, our study was underpowered for demonstrating that aspirin had a significant effect on reducing total atherosclerotic events. However, the observation in the JPAD trial of an effect of aspirin on the secondary outcome of fatal cardiovascular events was also seen in the PPP trial. Aspirin did not reduce cardiovascular mortality in the HOT study, and it did not reduce fatal stroke in the Women's Health Study. The reason for the discrepancy in the preventive effect of aspirin on fatal cardiovascular events is not clear at present. The total number of fatal events was small (ranging from 13 to 49) in the JPAD trial as well as the PPP trial and in the subgroup population with diabetes in the HOT study. A larger trial is needed to determine the efficacy of low-dose aspirin on mortality.

The JPAD trial composite primary end point also included hemorrhagic stroke. The finding that aspirin did not increase the risk of hemorrhagic stroke was consistent with findings from prior reports,[21,24,30,31] although the population studied was patients with diabetes. The finding of no increase in hemorrhagic stroke in the JPAD trial is of particular clinical importance because hemorrhagic stroke is more common in Japanese populations than in the West.[32,33] Moreover, there was no fatality due to hemorrhagic events except for hemorrhagic stroke; however, the hemorrhagic events that required surgical interventions or transfusion were observed in 4 patients in aspirin group.

The study design may be considered a limitation of the JPAD trial (prospective, randomized, open-label, controlled trial with blinded end-point assessment), as it did not have the advantages of a double-blind, randomized trial. The Japanese Pharmaceutical Affairs Law limits the use of placebo in physician-initiated studies because it is an unapproved medicine. However, the end-point classification was conducted by a blinded, independent committee on validation of data and events that was unaware of the group assignments.

Previous clinical studies indicate that a cardiovascular risk reduction is difficult to achieve by aggressively controlling plasma glucose levels in diabetic patients.[34-37] These studies suggested that the contribution of lowering glucose levels to the reduction of macrovascular events appears to be minimal, at least in the first few years of treatment. Although improved glucose control can protect against the development of microvascular complications, the absence of a reduction in macrovascular events implicates an additive effect of nonglycemic risk factors that often accompany diabetes, such as hypertension, hyperlipidemia, and hypercoagulability. Additional medications such as angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor blockers, statins, and antiplatelet agents may be needed in patients with type 2 diabetes mellitus. The JPAD trial indicates that among these medications, aspirin is well tolerated for primary prevention and may provide an additional low-cost option.

In summary, in the JPAD trial, the first prospectively designed trial to evaluate low-dose aspirin in patients with type 2 diabetes without previous cardiovascular disease, low-dose aspirin as primary prevention did not reduce the risk of cardiovascular events. Despite a large sample size, the event rate in the study was lower than anticipated. Aspirin was well tolerated in these patients, as there was no increase in hemorrhagic strokes and a small increase in serious GI hemorrhagic events (4 patients required transfusion). These findings should be interpreted in context with the low incidence of atherosclerotic disease in Japan and the current management practice for cardiovascular risk factors and suggest the need to conduct additional studies of aspirin for primary prevention of cardiovascular disease in diabetic patients.

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ORIGINAL ARTICLE

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Acute myocardial infarction as a systemic prothrombotic condition evidenced by increased von Willebrand factor protein over ADAMTS13 activity in coronary and systemic circulation

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Abstract The aim of the present study is to clarify the roles of circulating ADAMTS13 and von Willebrand factor (VWF) in the formation of coronary artery thrombi in acute myocardial infarction (AMI). Twenty-six AMI patients, 37 age-matched healthy controls, and 20 young controls were studied. Plasma ADAMTS13 activity and levels of VWF antigen (VWF:Ag) and unusually large VWF multimer (UL-VWFM) were measured in the femoral vein (FV), aortic root (Ao), and coronary sinus (Cs) immediately before percutaneous coronary intervention (PCI) during the acute phase of AMI, as well as 6 months later. During the acute phase of AMI, plasma levels of VWF:Ag were similar in FV, Ao, and Cs, and were higher than those of age-matched control. In contrast, ADAMTS13 activity in three sampling points in AMI patients was similar to that of age-matched controls. Thus, the ratio of VWF:Ag to ADAMTS13 activity in the acute phase of AMI was significantly higher in all three sampled sites than that of age-matched controls. In the chronic phase, plasma levels of VWF:Ag, ADAMTS13 activity, and the ratio of VWF:Ag to ADAMTS13 activity were similar to those of age-matched controls. UL-VWFM was detected in the acute phase of AMI but not in the chronic phase. The present study showed that the plasma VWF:Ag levels are increased and ADAMTS13 activity is relatively decreased in both systemic and coronary circulation during the acute phase of AMI, suggesting that an imbalance between the enzyme and its substrate may play a role in the formation of occlusive thrombi in a coronary artery.

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Introduction

The rapid closure of the coronary artery by acutely formed arterial thrombi, which are composed of platelets, fibrin, and inflammatory cells, is the major cause of acute myocardial infarction (AMI).[1,2] Although the exact mechanism of coronary thrombus formation is not fully understood, the binding of von Willebrand factor (VWF) to glycoproteins Ibα and IIb/IIIa on the surface of platelets is known to lead to platelet activation and subsequent aggregation, which is an initial step toward formation of coronary thrombi.[3,4] Earlier reports have shown that circulating levels of VWF antigen (VWF:Ag) is elevated in patients during the acute phase of AMI,[5,6] and increased levels of plasma VWF:Ag can predict primary and secondary coronary events.[7–9] Thus, VWF appears to be involved in the formation of coronary thrombi as a cause of AMI, although blocking of VWF function has not yet been clinically proven to prevent the onset of AMI. It is not clear, however where and how VWF is produced during AMI.

Von Willebrand factor is synthesized in vascular endothelial cells and then released into the plasma as unusually large VWF multimer (UL-VWFM),[4] which has most potent biological activities interacted with platelet, and is rapidly degraded into smaller VWF multimers by ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13),[4,9] a metalloproteinase that specifically cleaves multimeric VWF between Tyr1605 and Met1606 within the VWF A2 domain.[9] Loss-of-function mutation of ADAMTS13 leads to Upshaw–Schulman syndrome, a form of congenital thrombotic thrombocytopenic purpura. Reduction of ADAMTS13 activity keeps circulating UL-VWFM levels high, which leads to platelet clumping and formation of platelet-rich thrombi. Recently, Sakai et al.[6] reported that UL-VWFM was detected in plasma drawn from peripheral veins in patients with AMI. To understand

the mechanism for the formation of coronary arterial thrombi in AMI, we measured plasma ADAMTS13 activity together with circulating levels of its substrate, VWF:Ag, in three sites: the aorta (Ao) near the ostium of the infarction-related coronary artery, the coronary sinus (Cs), and the femoral vein (FV). Samples were taken immediately before the percutaneous transluminal coronary intervention (PCI) during the acute phase of AMI and compared with those taken during the chronic phase.

Materials and methods

Patients

We studied 26 Japanese patients with AMI (5 women and 21 men; mean age 67.8 ± 11.6 years; range 38–89 years) admitted to the Nara Medical University Hospital between August 2004 and February 2005. The diagnosis of AMI was based on sustained chest pain of typical character and location, electrocardiographic ST-T elevation in two or more leads, disrupted regional wall motion on echocardiograms, and plasma levels of cardiac enzymes, including creatine phosphokinase (CK) and its MB fraction, that were greater than twice the normal upper limit. Of the 26 patients, 18 had hypertension, 21 had dyslipidemia, 13 had diabetes mellitus, 5 were obese, and 19 smoked. All of the patients received emergency coronary angiography and PCI within 24 h from the onset of AMI (the first symptoms). Clinical characteristics and drugs used are summarized in Table 1. The culprit lesions were in the right coronary artery in 6 patients, the left anterior descending coronary artery in 18, and the left circumflex coronary artery in 2. The peak CK level in AMI patients averaged 2960 IU/l and ranged from 344 to 12930 IU/l. All of the patients received intracoronary stents, implanted at the culprit lesions, and were subsequently given aspirin (81 mg/day, per os) and ticlopidine (200 mg/day, per os) or cilostazol (200 mg/day, per os) as antiplatelet therapy. An angiotensin-converting enzyme inhibitor and/or angiotensin-II receptor blocker were also administered to all patients. In addition, 10 patients received a β-blocker, 6 a calcium channel blocker, 7 a diuretic, and 15 a statin. Six months after the first onset of AMI, coronary angiography was again carried out in all of the patients. Written informed consent was obtained from all patients and control subjects participating in the study. The protocol was approved by the institutional review board of Nara Medical University (#2002-009).

Young and age-matched healthy control subjects

Study participants included both young and age-matched healthy control subjects. Young healthy subjects consisted of 30 volunteers (15 women and 15 men) aged from 20 to 39 years with a mean age of 30 ± 12.0 years, and age-matched healthy subjects consisted of 37 healthy volunteers (19 women and 18 men) aged from 39 to 93 years with a mean age of 64.2 ± 14.0 years. Both groups had no history of angina, myocardial infarction, coronary artery bypass graft surgery, PCI, or any electrocardiographic abnormalities. Blood samples were collected from the antecubital vein early in the morning, before breakfast. Nine of the age-matched controls (4 women and 5 men, mean age 48.1 ± 4.8 years, range 41–52 years) were also studied to evaluate the circadian variation of VWF:Ag and ADAMTS13 activity in plasma. In those subjects, blood samples were collected from the antecubital vein in the morning (09:30) and in the evening (20:00).

Table 1. Characteristics of patients with acute myocardial infarction

	Patient	Age-matched control subjects	*P* value
Age (years)	67.8 (38–89)	64.2 (39–93)	0.29
Sex (female/male)	5/21	19/18	<0.01
Coronary risk factor (yes/no)			
Hypertension	8/18	0/37	<0.01
Dyslipidemia	21/5	2/35	<0.01
Diabetes mellitus	13/13	3/34	<0.01
Obesity	5/21	7/30	0.41
Smoking	19/7	7/30	<0.01
Peak CK (IU/l) (mean)	2960 (344–12930)		
Location of AMI			
RCA/LAD/LCx	6/18/2		
Medication (yes/no)			
Aspirin	26/0		
Ticlopidine or Cilostazol	26/0		
ACE-I or ARB	26/0		
β-Blocker	10/16		
Calcium-antagonist	6/20		
Diurea	7/19		
Statin	15/11		

Values in parentheses indicate range
CK, creatine phosphokinase; AMI, acute myocardial infarction; RCA, right coronary artery; LAD, left anterior descending artery; LCx, left circumflex artery; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin-II receptor blocker

Blood sampling

In the AMI patients, emergency cardiac catheterization was performed within 90 min of their arrival in our hospital. Blood samples were collected using a 7-F sheath inserted into the patient's femoral vein (FV), a 6-F Cs catheter placed in the Cs through an FV sheath, and a 4-F Judkins catheter placed at the Ao. Unfractionated heparin and contrast medium were not used before pre-PCI blood sampling. Blood was sampled at the femoral vein (FV), the aortic root near the ostium of the infarction-related coronary artery (Ao), and the coronary sinus vein (Cs) immediately before and after emergency PCI. Six months after the onset of AMI, all 26 patients underwent a second round of coronary angiography, at which time blood was again collected from the same three areas. In young healthy and age-matched control subjects, blood samples were drawn from the antecubital vein. Preliminary experiments showed that there was no difference in plasma levels of VWF:Ag and ADAMTS13 activity among the antecubital vein, the FV, and the right atrium.

Blood was collected into plastic tubes with 1/10th volume of 3.8% sodium citrate. Platelet-poor plasma was prepared by centrifugation at 3000× *g* at 4°C for 15 min and stored in aliquots at –80°C until analysis.

Assays of ADAMTS13 activity, VWF:Ag, and UL-VWFM

Plasma ADAMTS13 activity was determined using a highly sensitive enzyme-linked immunosorbent assay (ELISA) recently developed by our laboratory.[10] The assay system includes a recombinant GST-VWF73-His polypeptide as a substrate and a murine monoclonal antibody that specifically recognizes the Tyr1605 residue in the VWF-A2 domain exposed by ADAMTS13 cleavage; it does not recognize the uncleaved form of the peptide. Plasma VWF:Ag was measured by a sandwich enzyme immunoassay using rabbit anti-human VWF polyclonal antibody (Dako, Kyoto, Japan). Plasma ADAMTS13 activity and VWF:Ag levels were expressed as percentages of those of reference peripheral plasma obtained from 20 healthy volunteers aged 20–40 years. The lower detection limit of the ELISA for ADAMTS13 activity was 0.5% of the reference peripheral plasma activity. Plasma UL-VWFM was analyzed by sodium dodecyl sulfate – 0.9% agarose gel electrophoresis using 1 µl samples, after which VWF multimers were visualized by Western blotting and luminography, as described previously.[11]

Statistical analysis

The data are expressed as mean ± SD. Comparison between acute and chronic data was performed using the paired

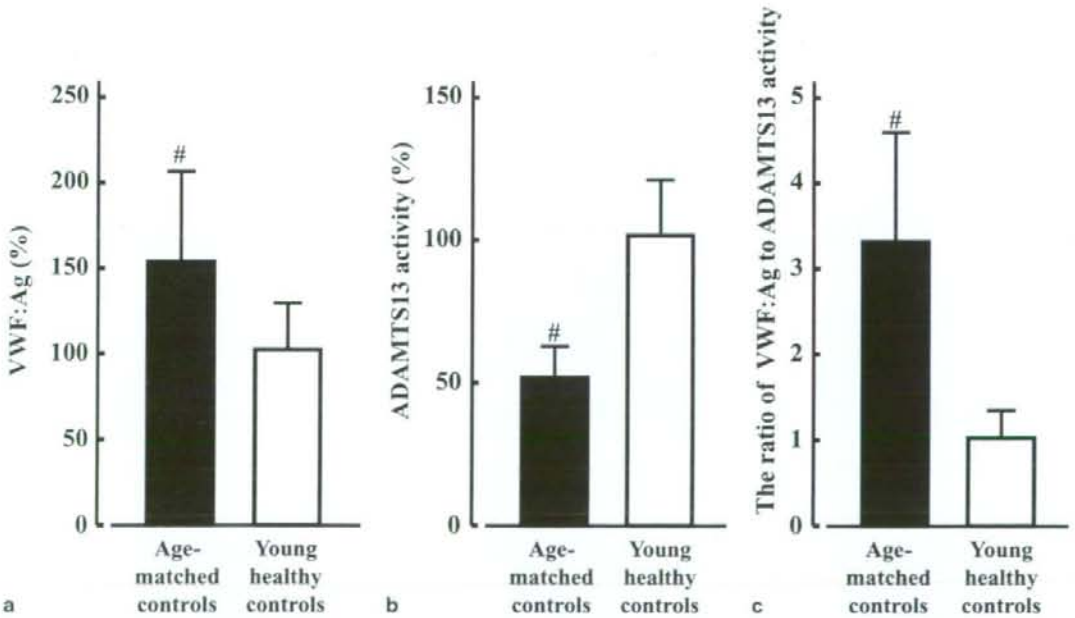

Fig. 1a–c. Comparison of plasma von Willebrand factor antigen (*VWF:Ag*) levels and ADAMTS13 activity between healthy young subjects and age-matched controls. **a** Plasma VWF:Ag levels. **b** Plasma ADAMTS13 activity. **c** Ratios of VWF:Ag to ADAMTS13 activity. Shown are mean ± SD; $^{\#}P < 0.001$ vs young subjects

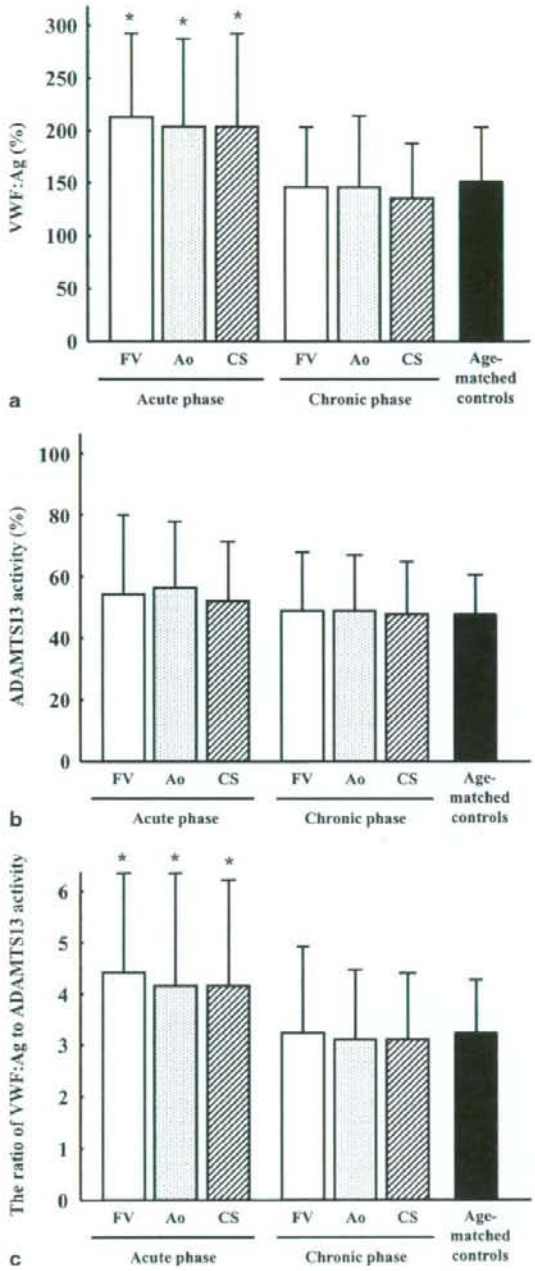

Fig. 2a–c. Plasma von Willebrand factor antigen (*VWF:Ag*) levels and ADAMTS13 activity and the ratio of VWF:Ag to plasma ADAMTS13 activity during the acute and chronic phases of AMI. **a** VWF:Ag levels, **b** ADAMTS13 activity, and **c** ratios of VWF:Ag to ADAMTS13 activity before percutaneous coronary intervention (PCI) during the acute phase and chronic phase of acute myocardial infarction (AMI). Measurements were made using plasma samples collected from the femoral vein (*FV*), aortic root (*Ao*), and coronary sinus (*Cs*) of the AMI patients and peripheral blood samples collected from control subjects. Shown are means ± SD; **P* < 0.05 vs age-matched controls

Student's *t*-test or Wilcoxon signed-rank test, when appropriate. Comparison among the three groups of subjects was performed by analysis of variance. The analyses were carried out using the statistical software Statview (version 5.0; SAS Institute, Cary, NC, USA). A *P* value of less than 0.05 was considered statistically significant.

Results

Differences between healthy young and age-matched controls

Plasma levels of VWF:Ag were significantly higher in healthy age-matched controls than in the young subjects (151% ± 58% vs 102% ± 33%, $P < 0.001$) (Fig. 1). Conversely, the plasma ADAMTS13 activity was lower in the age-matched controls than in the young subjects (51% ± 15% vs 104% ± 22%, $P < 0.001$), resulting in a three-fold higher ratio of VWF:Ag to ADAMTS13 activity in the age-matched controls than in young healthy controls (3.3 ± 1.4 vs 1.0 ± 0.3, $P < 0.001$) (Fig. 1).

VWF:Ag levels

During the acute phase of AMI before PCI, plasma VWF:Ag levels were significantly higher ($P < 0.01$) at the FV (211% ± 75%), Ao (204% ± 78%), and Cs (205% ± 90%) than in peripheral blood samples from the age-matched controls (151% ± 58%) (Fig. 2a). During the chronic phase, these values ($P < 0.05$) fell to levels similar to those seen in the age-matched controls (FV, 149% ± 69%; Ao, 148% ± 73%; and Cs, 133% ± 52%). There also were no differences in VWF:Ag levels among sampling sites (Fig. 2a).

ADAMTS13 activity

Plasma ADAMTS13 activity did not differ among blood samples collected from the FV, Ao, and Cs before PCI during the acute phase of AMI (FV, 55% ± 22%; Ao, 57% ± 22%; Cs, 54% ± 19%), or during the chronic phase of AMI (FV, 51% ± 19%; Ao, 52% ± 17%; Cs, 51% ± 22%). In fact, all of these values were similar to ADAMTS13 activity in peripheral blood from the age-matched controls (51% ± 15%) (Fig. 2b). Moreover, ADAMTS13 activity in the acute phase was similar to that in the chronic phase at each sampling point. There was no significant inverse correlation between ADAMTS13 activity and plasma level of VWF:Ag in the acute phase of AMI.

The ratio of VWF:Ag to ADAMTS13 activity

During the acute phase of AMI, the ratio of VWF:Ag to ADAMTS13 activity before PCI was significantly higher ($P < 0.05$) in the FV (4.5 ± 2.4), Ao (4.2 ± 2.5), and Cs (4.2 ± 2.4) than in the peripheral blood samples from age-matched