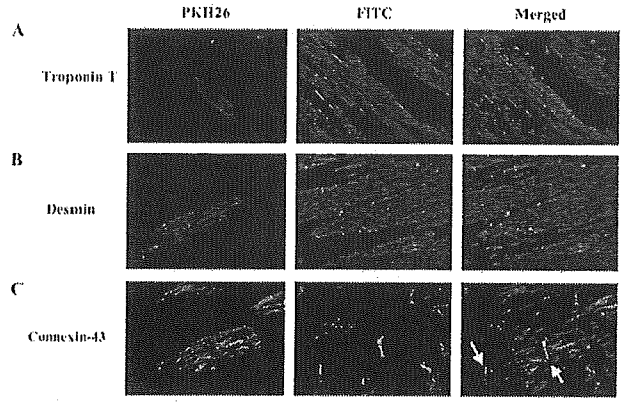

Figure 2. Differentiation of transplanted MSCs into cardiomyocytes. Transplanted MSCs were engrafted in the myocardium and stained for cardiac troponin T (A) and desmin (B). Engrafted MSCs also expressed connexin-43, a gap junction protein, at contact points with native cardiac myocytes (left arrow) and other transplanted cells (right arrow) (C). Magnification ×400.

Myogenesis and Angiogenesis Induced by MSCs

Red fluorescence–labeled MSCs were transplanted into the myocardium 5 weeks after immunization. Four weeks after transplantation, MSCs were engrafted into the myocardium (Figure 2). Immunofluorescence demonstrated that transplanted MSCs were positive for the cardiac markers cardiac troponin T and desmin (Figure 2). Transplanted MSCs also expressed connexin-43, a gap junction protein, at contact points with native cardiac myocytes as well as with MSCs. FACS analysis of isolated heart cells demonstrated that 8±1% of transplanted MSCs were double-positive for PKH26 and troponin T. These results suggest that a small number of transplanted MSCs can differentiate into cardiomyocytes.

Some transplanted MSCs formed vascular structures in the myocardium and were positive for von Willebrand factor (Figure 3A). Other MSCs were positive for SMA and participated in vessel formation as mural cells (Figure 3B). Alkaline phosphatase staining of the ischemic myocardium showed marked augmentation of neovascularization in the MSC-treated DCM group (Figures 4A–4C). Quantitative analysis demonstrated that capillary density was significantly

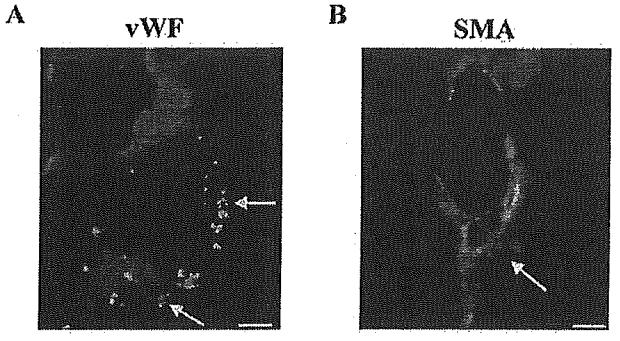

Figure 3. Differentiation of transplanted MSCs into vascular endothelial cells and smooth muscle cells. Some of the transplanted MSCs were positive for von Willebrand factor (vWF, A) and SMA (B) and formed vascular structures (A and B). Scale bars=10 μm.

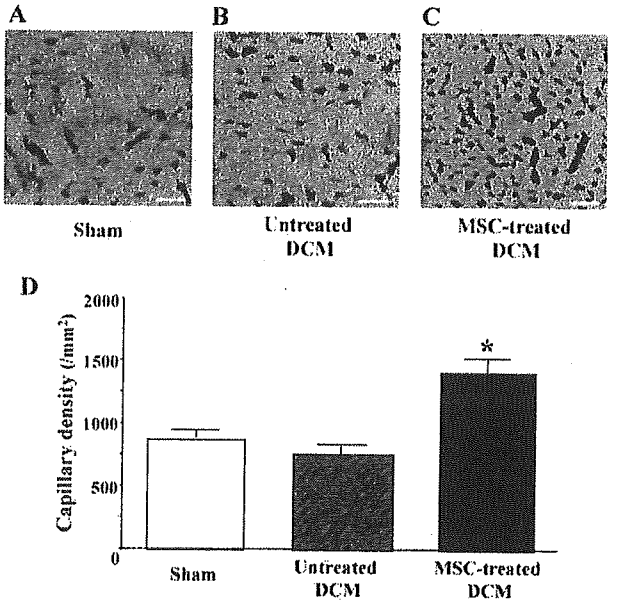

Figure 4. A–C, Representative samples of alkaline phosphatase staining of myocardium. Magnification, ×200. Scale bars=10 μm. D, Quantitative analysis of capillary density in the myocardium. Data are mean±SEM *P<0.05 vs untreated DCM group.

higher in the MSC-treated DCM group than in the untreated DCM group (Figure 4D).

Angiogenic, Antiapoptotic, and Mitogenic Factors Released From MSCs

After 24 hours of culture, MSCs secreted large amounts of angiogenic and antiapoptotic factors, including VEGF, HGF, and AM (Figure 5). Compared with MNCs that have commonly been used for regenerative therapy,[20–22] MSCs secreted 4-fold more VEGF and 5-fold more HGF. Similarly, MSCs secreted 6-fold more AM, an angiogenic and antiapoptotic peptide, compared with MNCs. MSCs also secreted a large amount, 10-fold greater than MNCs, of IGF-1, a growth hormone mediator for myocardial growth (Figure 5). Transplantation of MSCs significantly increased circulating VEGF (45.8±1.6 to 68.5±3.6 pg/mL, P<0.05), HGF (431.8±56.6 to 517.2±67.1 pg/mL, P<0.05), and AM (23.4±0.8 to 41.2±4.8 pg/mL, P<0.05) 24 hours after transplantation, although vehicle injection did not alter these parameters. Serum IGF-1 tended to increase after MSC transplantation (938.1±151.6 to 1063.5±116.9 pg/mL, P=NS), but this increase did not reach statistical significance.

Hemodynamic Effects of MSC Transplantation

Nine weeks after immunization, LV end-diastolic pressure showed a marked elevation in the untreated DCM group; this elevation was significantly attenuated in the MSC-treated DCM group (Figure 6A). LV maximum *dP/dt* was significantly lower in the untreated DCM group than in the sham group (Figure 6B). However, LV maximum *dP/dt* was significantly improved 4 weeks after MSC transplantation. There was no significant difference in heart rate or mean arterial pressure among the 3 groups (the Table). Echocardiographic studies demonstrated LV dysfunction and dilation

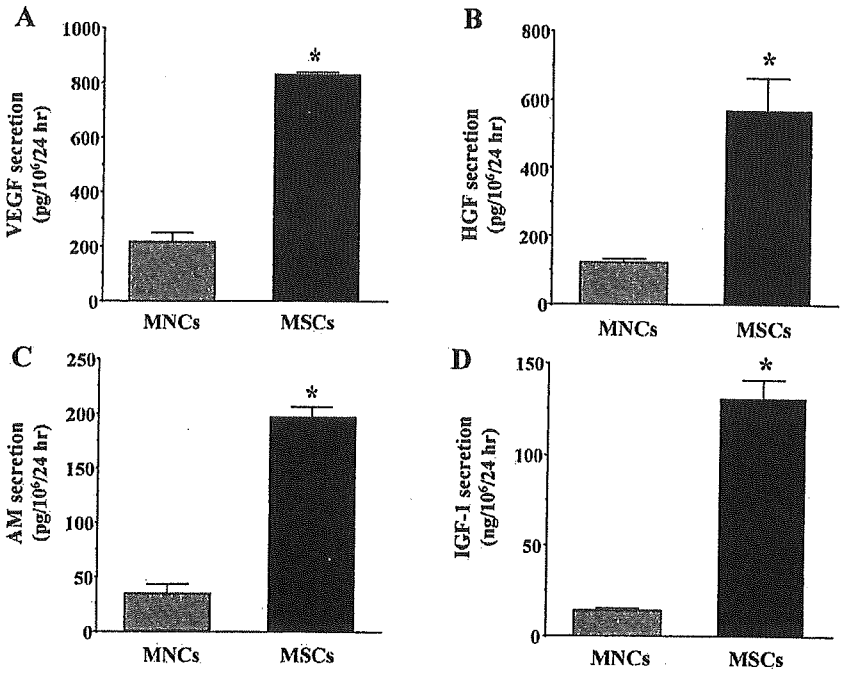

Figure 5. A–D, Angiogenic, antiapoptotic, and mitogenic factors produced by MSCs and bone marrow–derived MNCs). Compared with MNCs, MSCs secreted large amounts of VEGF, HGF, AM, and IGF-1. *$P<0.05$ vs MNCs.

in the untreated DCM group, as indicated by a decrease in percent fractional shortening and an increase in LV diastolic dimension (Figure 6C and 6D). However, MSC transplantation increased percent fractional shortening and inhibited the increase in LV diastolic dimension.

Reduction of Myocardial Fibrosis by MSC Transplantation

Masson's trichrome staining demonstrated modest myocardial fibrosis in the untreated DCM group (Figure 7A). However, MSC transplantation significantly attenuated the development of myocardial fibrosis. Quantitative analysis also demonstrated that the collagen volume fraction in the MSC-treated DCM group was significantly smaller than that in the untreated DCM group (Figure 7B). Western blot analysis showed that myocardial contents of MMP-2 and MMP-9 in the untreated DCM were significantly increased compared with those in the sham group (Figure 7C–E). However, the increases in MMP-2 and MMP-9 levels were attenuated by MSC transplantation, although the change in MMP-9 did not reach statistical significance.

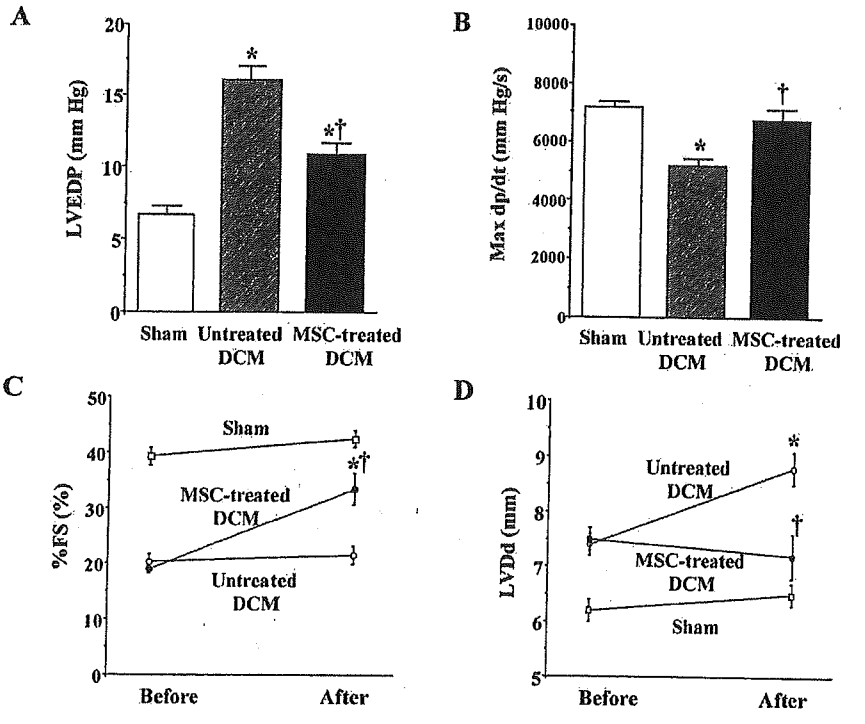

Figure 6. A and B, Effects of MSC transplantation on hemodynamic parameters. LVEDP indicates LV end-diastolic pressure; Max *dP/dt*, LV maximum *dP/dt*. Data are mean±SEM. *$P<0.05$ vs sham group; †$P<0.05$ vs untreated DCM group. C and D, Changes in echocardiographic parameters induced by MSC transplantation. %FS indicates LV fractional shortening. Data are mean±SEM *$P<0.05$ vs before transplantation; †$P<0.05$ vs the time-matched untreated DCM group.

Physiological Profiles of the 3 Experimental Groups

	Sham	Untreated DCM	MSC-Treated DCM
n	10	10	10
Body wt, g	421±8	372±4*	389±5*
LV wt/body wt, g/kg	1.91±0.05	2.18±0.06*	2.05±0.05
RV wt/body wt, g/kg	0.55±0.01	0.68±0.02*	0.60±0.03†
Heart rate, bpm	403±10	432±15	417±12
Mean arterial pressure, mm Hg	134±2	123±3	132±5

wt indicates weight; RV, right ventricle. Sham-operated rats were given vehicle only. The untreated DCM group included DCM rats treated with vehicle. The MSC-treated DCM group included DCM rats treated with MSCs. Data are mean±SEM.

*$P<0.05$ vs sham group; †$P<0.05$ vs untreated DCM group.

Discussion

In the present study, we have demonstrated the following effects of MSC transplantation in a rat model of DCM: (1) induction of myogenesis and angiogenesis; (2) differentiation of transplanted MSCs into cardiomyocytes, vascular endothelial cells, and smooth muscle cells; (3) secretion of large amounts of VEGF, HGF, AM, and IGF-1; (4) improvement of cardiac function and inhibition of ventricular remodeling; and (5) decrease in collagen volume fraction in the myocardium.

Earlier studies have shown that transplantation of MSCs improves cardiac function in experimental models of ischemic heart disease.[9,23] However, little information is available about the therapeutic potential of MSCs for chronic heart failure due to DCM. Previous studies have shown that porcine cardiac myosin-induced myocarditis progresses to a chronic phase resembling DCM.[13,14] Thus, we used this model 5 weeks after immunization as an example of experimental DCM.

In the present study, transplanted MSCs were engrafted into the myocardium in a rat model of DCM. Four weeks after transplantation, some of the engrafted MSCs were positively stained for cardiac troponin T and desmin. Transplanted MSCs also expressed connexin-43, a gap junction protein, at contact points with native cardiac myocytes as well as with MSCs. These results suggest that MSCs differentiate into cardiomyocytes in the myocardium and form connections with native cardiomyocytes in rats with DCM. Unlike earlier studies that have used a model of myocardial infarction,[7,9,23] we used a rat model of DCM to demonstrate the engraftment and cardiogenic differentiation of MSCs. Importantly, MSC transplantation improved cardiac function in these rats, as indicated by a significant decrease in LV end-diastolic pressure and an increase in LV dP/dt_{max}. Thus, the improvement in cardiac function may be a result of MSC-induced myocardial regeneration; however, further studies are necessary to investigate the mechanisms by which MSCs develop into cardiac myocyte–like cells.

Some of the transplanted MSCs were positive for a vascular endothelial cell marker and participated in vessel formation. MSC transplantation significantly increased capillary density in the myocardium. SMA staining revealed that MSCs differentiated into vascular smooth muscle cells, which play an important role in vessel maturation. Earlier studies have shown that transplantation of MNCs induces therapeutic angiogenesis in patients with limb ischemia or ischemic heart disease.[20–22] The angiogenic potential of MNCs is mediated at least in part by production by the cells of a variety of angiogenic factors.[24] Although MSCs have also been shown to produce VEGF,[10,25] there has been no study to compare their production between MSCs and MNCs. The present study demonstrated that MSCs secreted ≈4-fold more VEGF compared with MNCs. Furthermore, MSCs secreted large amounts of HGF and AM, potent angiogenic factors.[26–30] Taking these findings together, MSCs may contribute to neovascularization in the myocardium not only through their ability to generate capillary-like structures but also through growth factor–mediated paracrine regulation. Myocardial blood flow abnormalities have been documented in patients with heart failure caused by DCM.[12] Thus, it is possible that MSC-induced neovascularization contributes to improvement in cardiac function.

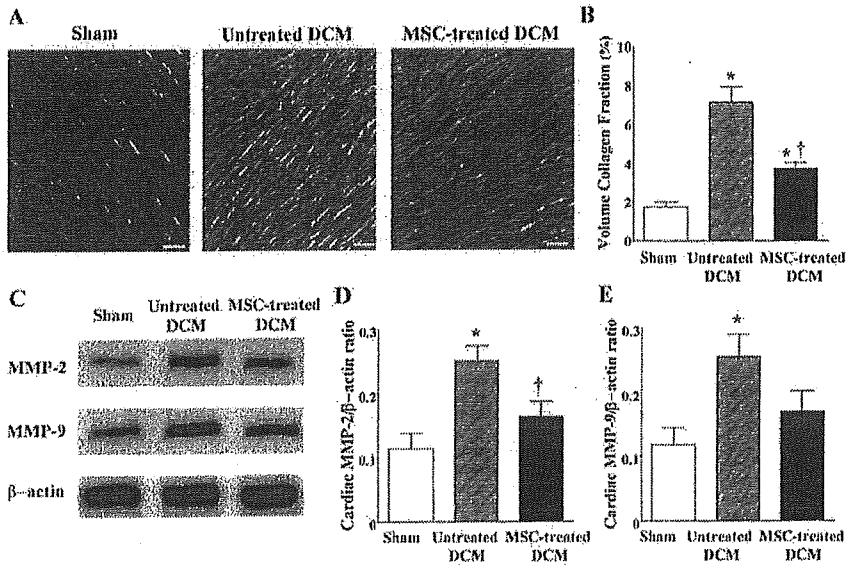

Figure 7. Effects of MSC transplantation on myocardial fibrosis. A, Photomicrographs show representative myocardial sections stained with Masson's trichrome. Scale bars=10 μm. B, Quantitative analysis demonstrated that the collagen volume fraction in the MSC-treated DCM group was significantly smaller than that in the untreated DCM group. C, Representative Western blots for MMPs-2 and -9 and β-actin in the heart. D and E, Quantitative analysis of cardiac tissue contents of MMP-2 and -9. Data are mean±SEM *$P<0.05$ vs sham group; †$P<0.05$ vs untreated DCM group.

HGF has not only angiogenic but also cardioprotective effects, including antiapoptotic, mitogenic, and antifibrotic activities.[26,27] *HGF* gene transfer into the myocardium improves myocardial function and geometry.[28] In particular, the antifibrotic effects of HGF through inhibition of transforming growth factor-β expression is beneficial for heart failure. Cultured MSCs secreted a large amount of HGF. In vivo, transplantation of MSCs slightly increased plasma HGF in rats. It significantly attenuated the development of myocardial fibrosis in a rat model of DCM. These results suggest that MSC-derived HGF may contribute to improvements in cardiac function partly through its antifibrotic effects.

MSCs also produced AM, a potent vasodilator and cardioprotective peptide.[29] We have shown that AM prevents cardiomyocyte apoptosis through the phosphatidylinositol 3-kinase/Akt–dependent pathway[16] and that it has potent angiogenic effects.[30] AM inhibits proliferation of cardiac fibroblasts through the cAMP-dependent pathway.[31] Administration of AM inhibits LV remodeling and improves cardiac function in heart failure.[32–34] In the present study, cultured MSCs secreted a large amount of AM in vitro. In vivo, transplantation of MSCs markedly increased plasma AM level. Taken together, these findings suggest that MSCs may exert their cardioprotective effects through AM-mediated paracrine regulation.

IGF-1, a growth hormone mediator, plays an important role in myocardial and skeletal muscle growth.[35,36] Administration of IGF-1 improves cardiac function after myocardial infarction through enhancement of myocardial growth.[37] Its protective and antiapoptotic properties have been demonstrated in different models of myocardial ischemia.[38] Furthermore, IGF-1 exerts Ca^{2+}-dependent, positive inotropic effects through a phosphatidylinositol 3-kinase–dependent pathway.[39] Interestingly, the present study demonstrated that MSCs secreted significant amounts of IGF-1 in vitro, 10-fold greater than MNCs. These findings raise the possibility that MSC-derived IGF-1 may participate in myocardial growth and enhancement of myocardial contractility in a rat model of DCM.

MMPs also play a crucial role in extracellular remodeling in heart failure.[40] In fact, pharmacological inhibition of MMP activities prevents progressive LV remodeling in an animal model of heart failure.[41] In the present study, cardiac MMP-2 and MMP-9 were increased in rats with DCM, which is consistent with recent findings in patients with heart failure.[40,42] Interestingly, MSC transplantation attenuated the increases in cardiac MMP-2 and MMP-9 in a rat model of DCM. Although the underlying mechanisms remain unclear, MSC transplantation may influence extracellular remodeling in heart failure.

The present study has some limitations. First, immunohistochemical evidence suggests differentiation of MSCs into cardiomyocytes, vascular endothelial cells, and smooth muscle cells. However, further studies are necessary to convincingly demonstrate differentiation of MSCs into a specific cell type. Second, the model of DCM used in this study was an injury model, and the effects of treatment may be related to attenuation of the injury rather than to the established cardiomyopathy. Nonetheless, the experiment was performed 5 to 9 weeks after myosin injection, by which time inflammatory changes were hardly observed and had been replaced by fibrosis.[43]

Conclusions

MSC transplantation improved cardiac function in a rat model of DCM, possibly through induction of myogenesis and angiogenesis, as well as by inhibition of myocardial fibrosis. The beneficial effects of MSCs may be mediated at least in part by their differentiation into cardiomyocytes and vascular cells and by their ability to supply large amounts of angiogenic, antiapoptotic, and mitogenic factors. Thus, MSC transplantation has potential as a new therapeutic strategy for the treatment of DCM.

Acknowledgments

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CLINICAL PERSPECTIVE

Transplantation of stem or progenitor cells has the potential to improve and restore cardiac function. To date, experimenters investigating the possible therapeutic effects of stem cells in the heart have used models of infarction, and little information is available about the therapeutic potential of cell transplantation for heart failure due to dilated cardiomyopathy. In the present study, we demonstrated that transplantation of stem cells improved cardiac function in a model of myocarditis. We found evidence that stem cells may work to improve heart function by both myogenesis and angiogenesis while inhibiting myocardial fibrosis. Based on our data, part of the mechanism for this improvement may occur through the action of stem cells as a source of growth factors and cytokines in the heart. This study supports the overall notion that mesenchymal stem cells transplanted into the failing heart have potential as a new therapeutic strategy for the treatment of dilated cardiomyopathy.

Prognostic Usefulness of Serum Uric Acid After Acute Myocardial Infarction (The Japanese Acute Coronary Syndrome Study)

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Serum uric acid (UA) levels reflect circulating xanthine oxidase activity and oxidative stress production. Hyperuricemia has been identified in patients who have congestive heart failure and is a marker of poor prognosis in such patients. We investigated the relation between serum UA levels and Killip's classification suggestive of the severity of heart failure and whether hyperuricemia influences mortality of patients who have acute myocardial infarction (AMI). Using the Japanese Acute Coronary Syndrome Study database, we evaluated 1,124 consecutive patients who were hospitalized within 48 hours of onset of symptoms of AMI from January to December 2002. There was a close relation between serum UA concentration and Killip's classification. Patients who developed short-term adverse events had high UA concentrations. Serum UA levels, Killip's class, age, and peak creatine phosphokinase level were significant predictors of long-term mortality. The hazard ratio for patients in the highest quartile of UA was 3.7 compared with those in the lowest quartile for death after AMI after adjustment for independent factors that were related to mortality. The combination of the best UA cutoff (447 μmol/L) for predicting survival based on receiver-operating characteristics analysis and Killip's class significantly predicted the prognosis of acute and long-term AMI-related complications. In conclusion, our results suggest that hyperuricemia after AMI is associated with the development of heart failure. Serum UA level is a suitable marker for predicting AMI-related future adverse events, and the combination of Killip's class and serum UA level after AMI is a good predictor of mortality in patients who have AMI. **(Am J Cardiol 2005;96:489–495)**

Previous studies have reported that a high concentration of uric acid (UA) is a strong marker of an unfavorable prognosis of moderate to severe heart failure and cardiovascular disease.[1,2] Therefore, we hypothesized that serum UA concentrations would correlate with Killip's classification suggestive of the severity of left ventricular failure and that hyperuricemia was related to mortality in patients who had acute myocardial infarction (AMI).

Methods

Data sources: The Japanese Acute Coronary Syndrome Study (JACSS) is a retrospective and multicenter observational study that is being conducted at 35 medical institutions in Japan. The JACSS database includes information on 1,124 consecutive patients who were hospitalized at participating institutions within 48 hours after onset of symptoms of AMI from January to December 2002 and whose UA

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concentrations were measured on admission. AMI was defined as increased myocardial enzyme concentrations with typical chest pain persisting >30 minutes or electrocardiographic changes (including ischemic ST-segment depression, ST-segment elevation, or pathologic Q waves). Increased enzyme concentrations were defined as peak creatine phosphokinase levels >2 times the upper limit of normal. The study protocol was reviewed and approved by the ethical committee of each participating institution.

Patients: Patients who were treated with antihypertensive drugs or those whose baseline blood pressure was ≥140/90 mm Hg were considered to have hypertension. Diabetes mellitus was diagnosed according to criteria of the World Health Organization.[3] Hyperlipidemia was defined as a total cholesterol level ≥220 mg/dl and/or a triglyceride level ≥150 mg/dl. Cigarette smoking was defined as active smoking.

Serum UA concentrations and other laboratory data: Blood samples for measurements of serum UA concentrations and other biochemical assessments were obtained intravenously immediately after admission. UA and other biochemical variables were measured according to standard techniques adopted by the participating institutions, and serum creatinine and UA concentrations were expressed as micromoles per liter. UA was treated as a continuous variable and as a categorical variable, and variables were divided into quartiles according to serum UA concentrations.

Coronary angiography and reperfusion therapy: Allocation of coronary angiography and reperfusion therapy was determined by physicians. The perfusion grade of the infarct-related artery was assessed according to the Thrombolysis in Myocardial Infarction (TIMI) study classification.[4] The final TIMI flow grade was assessed on the final shot of emergency coronary angiography.

Short- and long-term morbidities and mortalities: The primary end point was mortality from any cause. Major adverse cardiac events, including cardiac death, reinfarction, unstable angina, heart failure, and stroke, were also assessed. We evaluated 30-day adverse events and mortality rates after AMI during long-term follow-up.

Statistical analysis: Subjects in the analysis were categorized into 4 quartiles according to their serum UA levels. Differences in frequencies were analyzed by the chi-square method. Continuous data were compared by 1-way analysis of variance followed by Scheffé's F test. Clinical characteristics considered to be associated with serum UA concentrations were included in the models. These characteristics included age, gender, background illness (hypertension, diabetes mellitus, or hyperlipidemia), smoking, admission characteristics (body mass index, serum creatinine levels, previous myocardial infarction, premedication, and preinfarction angina pectoris), time from symptom onset, coronary angiographic findings (multivessel involvement, culprit location, occlusion of the infarct-related coronary artery, and final TIMI flow grade in the acute phase), peak creatine phosphokinase levels, and left ventricular ejection fraction during the AMI and at follow-up. Killip's classes on hospital admission, depending on clinical manifestations of heart failure, were also assessed (class I = no heart failure; class II = S_3 and/or basal lung crepitations; class III = acute pulmonary edema; class IV = cardiac shock).[5]

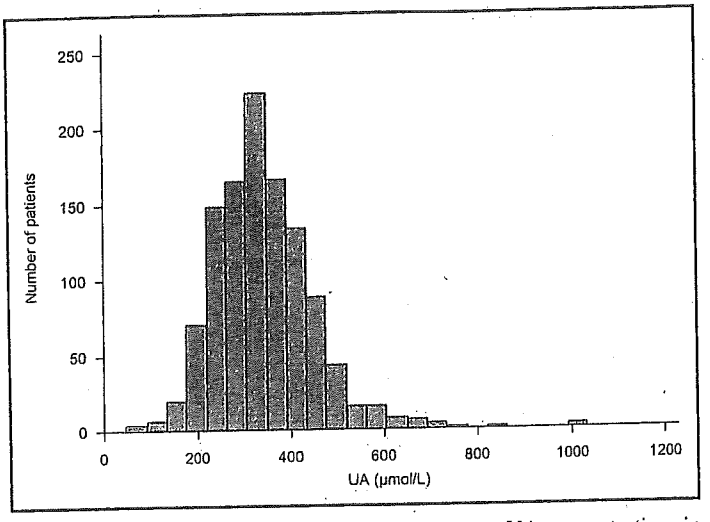

Figure 1. Histogram of distribution of baseline serum UA concentrations in 1,124 study patients.

Odds ratios and 95% confidence intervals of 30-day adverse events and long-term mortality were calculated using logistic regression analysis. Cox's proportional hazards analysis was performed to assess risk of mortality. Cumulative event curves were plotted using the Kaplan-Meier survival method and differences between curves were tested for statistical significance by the log-rank analysis. We performed multivariate analysis as the need arose (StatView 5.0, Abacus Concept, Inc., Berkeley, California). A p value <0.05 was considered statistically significant in all analyses. All data were expressed as mean ± SD unless otherwise indicated.

To compare different predictive values at a particular time point, areas under the curve for sensitivity and specificity were constructed. The best prognostic cutoff for predicting mortality at the respective times was defined as that which yielded the highest product of sensitivity and specificity.[6] To contrast prognostic accuracy, a receiver-operating characteristic curve was generated using LABROC5 (http://www.radiology.vehicago.edu/k-l/roc_soft.htm), which was provided by Metz et al.[7]

Results

Patients' clinical background and angiographic demographics: Figure 1 shows the distribution of serum UA levels. Serum UA concentrations ranged from 48 to 1,035 μmol/L (0.8 to 17.4 mg/dl). The median UA concentration was 333 μmol/L (5.6 mg/dl) and the interquartile interval was 274 to 399 μmol/L (4.6 to 6.7 mg/dl). Coronary reper-

Table 1
Clinical and angiographic characteristics of patients with acute myocardial infarction by quartiles of serum uric acid concentration

Variables	UA Quartile (μmol/L)			
	1 (<274) (n = 273)	2 (274–333) (n = 299)	3 (333–399) (n = 276)	4 (>399) (n = 276)
Age (yrs)	69 ± 11	67 ± 13	67 ± 12	68 ± 13
Men	55%	75%*	78%*	76%*
Hypertension	52%	52%	58%	65%*†
Diabetes mellitus	34%	29%	29%	33%
Hyperlipidemia	32%	35%	39%	38%
Body mass index (kg/m²)	22.8 ± 3.3	23.8 ± 3.3*	23.8 ± 3.1*	24.2 ± 3.4*
Current smoker	38%	51%*	55%*	43%*
Serum creatinine (μmol/L)	65.0 ± 29.2	80.5 ± 84.6	94.4 ± 104.5*	112.7 ± 85.9*†
Previous myocardial infarction	7%	11%	13%*	17%*†
Premedication				
Antiplatelet use	12%	14%	17%	18%
Statin use	8%	11%	9%	7%
Angiotensin-1 receptor blocker use	3%	5%	7%	7%
Preinfarction angina pectoris	37%	40%	34%	31%
Time from symptom onset (h)	6.5 ± 8.3	6.8 ± 9.3	5.5 ± 7.1	6.2 ± 8.8
Killip's class III–IV	5%	6%	11%*	24%*†‡
Peak creatine phosphokinase (IU/L)	2,577 ± 2,032	3,088 ± 2,995	3,056 ± 2,709	3,643 ± 4,876*
Coronary multivessel involvement	42%	46%	45%	45%
Culprit LAD location	47%	40%	42%	42%
Occlusion of IRCA on acute phase	62%	61%	59%	61%
Final TIMI grade 3 flow	89%	88%	86%	87%
Left ventricular ejection fraction				
Acute phase (n = 360)	51 ± 13%	53 ± 9%	49 ± 12%	48 ± 13%†
Discharge (n = 539)	55 ± 12%	53 ± 11%	52 ± 14%	54 ± 12%

* $p < 0.05$ versus quartile 1; † $p < 0.05$ versus quartile 2; ‡ $p < 0.05$ versus quartile 3, by chi-square test between quartiles or 1-way analysis of variance followed by Scheffé's F test.
IRCA = infarct-related coronary artery; LAD = left anterior descending.

fusion therapy was performed in 943 patients (84%) immediately after admission: coronary stent implantation in 743 patients (66%), conventional balloon angioplasty in 146 patients (13%), and intracoronary thrombolysis or intravenous thrombolysis in 54 patients (5%). Patients were followed for an average period of 454 ± 159 days (maximum 699).

Greater proportions of men, smokers, and those who had previous myocardial infarction were seen in the higher UA quartiles. In addition, there was a graded relation between increasing body mass index and creatinine and higher UA quartiles. High Killip's class and high peak levels of creatine phosphokinase were also observed in the high UA quartile (Table 1).

Table 2
Multiple regression analysis for serum uric acid levels on admission

Independent Variables	Regression Coefficients	SE	95% CI	p Value
Men	0.602	0.119	0.369–0.835	<0.0001
Body mass index	0.089	0.016	0.058–0.121	<0.0001
Killip's classification	0.293	0.066	0.165–0.422	<0.0001
Serum creatinine	0.377	0.058	0.263–0.492	<0.0001
Previous myocardial infarction	0.520	0.160	0.207–0.833	0.0012
Hypertension	0.301	0.108	0.090–0.513	0.0052

The p values reflect multiple regression analysis after stepwise regression, including all variables listed in Table 1.
CI = confidence interval.

Clinical determinants of serum UA concentrations: To assess determinants of serum UA concentrations, multiple regression analysis was performed after a stepwise regression that included all clinical variables listed in Table 1. Results showed that serum UA concentrations were significantly correlated with male gender, body mass index, Killip's class, serum creatinine, previous myocardial infarction, and hypertension ($r = 0.3659$, $p < 0.0001$; Table 2).

Short-term adverse events and long-term mortality: Patients who developed short-term adverse events had high UA concentrations. Patients in quartile 4 were >5 times more likely to show a 30-day all-cause mortality compared with those in quartile 1 (Table 3). Multivariate analysis that included all variables that were significantly associated with all-cause mortality in univariate analysis showed that UA concentrations, in addition to Killip's class, age, and peak creatine phosphokinase, closely correlated with all-cause mortality (Table 4). Figure 2 shows survival without all-cause mortality in patients after AMI based on UA quartiles. The hazard ratio for patients in quartile 4 was 3.7 compared with that of patients in quartile 1 for death after AMI after nonadjustment and adjustment for independent factors that were closely associated with all-cause mortality (Table 5).

Table 3
Thirty-day adverse events by quartiles of serum uric acid concentrations

UA Quartile (μmol/L)	30-Day Cardiac Death				30-Day MACE				30-Day Total Death			
		OR	95% CI	p Value		OR	95% CI	p Value		OR	95% CI	p Value
1 (<274; n = 273)	5 (2%)	1.000	—	—	15 (5%)	1.000	—	—	6 (2%)	1.000	—	—
2 (274–333; n = 299)	7 (2%)	1.285	0.403–4.097	0.6727	15 (5%)	0.908	0.435–1.895	0.7980	10 (3%)	1.540	0.552–4.295	0.4095
3 (333–399; n = 276)	8 (3%)	1.600	0.517–4.954	0.4150	15 (5%)	0.989	0.473–2.064	0.9754	8 (3%)	1.328	0.455–3.881	0.6037
4 (>399; n = 276)	30 (11%)	6.537	2.496–17.115	0.0001	39 (14%)	2.830	1.521–5.267	0.0010	31 (11%)	5.631	2.309–13.729	0.0001

OR values are based on logistic regression analysis regarding 30-day adverse events (cardiac death, MACE, and total death).
MACE = major adverse cardiac events; OR = odds ratio; other abbreviation as in Table 2.

Best UA cut-off concentration, Killip's classes, and long-term mortality: Receiver-operating characteristic areas under the curve (mean ± SEM) at 30 days, 6 months, and 1 year were 0.7072 ± 0.0455 (95% confidence interval 0.6125 to 0.7896), 0.7173 ± 0.0401 (95% confidence interval 0.6339 to 0.7902), and 0.7014 ± 0.0370 (95% confidence interval 0.6252 to 0.7695), respectively, and the receiver-operating characteristic area under the curve at 6 months was the largest. The sensitivity and specificity for prediction of mortality using the best cut-off value for serum UA levels at 6 months (447 μmol/L, 7.5 mg/dl) were 64% and 66%, respectively. Killip's classification and serum UA concentrations independently and significantly predicted poor prognosis; therefore, application of the best UA cut-off value to Killip's classes that were divided into 2 groups (low = Killip's classes I and II; high = Killip's classes III and IV) improved the positive and negative discriminatory powers for survival prediction (Figure 3). The hazard ratio of patients who were in a high Killip's class and had a serum UA concentration >447 μmol/L was ~22 compared with those who were in a low Killip's class and had a serum UA level ≤447 μmol/L for death after AMI, after adjusting for age and peak creatine phosphokinase level, which were closely associated with all-cause mortality (Table 6).

In patients who were in a high Killip's class and had a serum UA concentration ≥447 μmol/L (n = 57), TIMI grade 3 flow was associated with better 30-day and long-term survival rates compared with TIMI perfusion grade 0, 1, and 2 flows, although it was statistically insignificant (70% vs 55%, p = 0.2490, and 65% vs 45%, p = 0.1471, respectively).

Discussion

In the present study, we found a close relation between serum UA concentrations and Killip's classification suggestive of left ventricular failure. High UA concentrations on admission were strongly associated with adverse clinical outcome in patients who had AMI. The total mortality rate of patients whose serum UA concentrations were in the highest quartile was ~3.7 times higher than that in those whose UA concentrations were in the lowest quartile. Further, adding Killip's class to serum UA concentration improved its prognostic power.

Epidemiologic studies have suggested that hyperuricemia is a risk factor for cardiovascular disease.[8,9] Our results showed that serum UA concentrations correlate significantly with male gender, body mass index, serum creatinine concentrations, and hypertension. These results are similar to those of previous studies.[10,11] We also demonstrated that serum UA concentrations are influenced by Killip's classi-

Table 4
Predictors of mortality

Variable	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	p Value	OR	95% CI	p Value
Killip's classification	2.608	2.173–3.131	<0.0001	2.125	1.711–2.639	<0.0001
Age (per yr)	1.067	1.042–1.092	<0.0001	1.059	1.029–1.090	<0.0001
Uric acid (per μmol/L)	1.005	1.003–1.006	<0.0001	1.004	1.002–1.006	<0.0001
Peak creatine phosphokinase (per IU/L)	1.000	1.000–1.000	<0.0001	1.000	1.000–1.000	0.0001
Serum creatinine (per μmol/L)	1.002	1.001–1.004	0.0004	—	—	—
Body mass index (per kg/m^2)	0.841	0.762–0.928	0.0006	—	—	—
Coronary vessel involvement	1.697	1.228–2.345	0.0014	—	—	—
Previous myocardial infarction	2.415	1.375–4.255	0.0022	—	—	—
Hyperlipidemia	2.550	1.363–4.771	0.0034	—	—	—
Final TIMI flow grade	0.738	0.549–0.994	0.0454	—	—	—

The p values in multivariate analysis as results of Cox's proportional hazards analysis after stepwise regression analysis including all variables that were significantly associated with all-cause mortality in univariate analysis.
Abbreviations as in Tables 2 and 3.

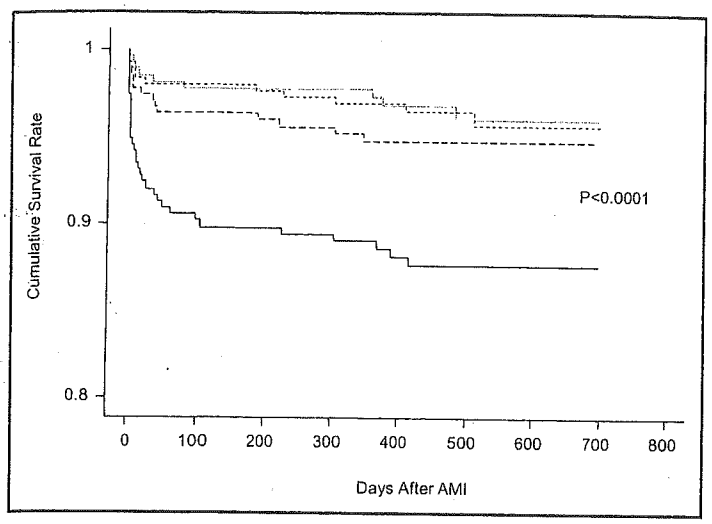

Figure 2. Survival without all-cause mortality in patients after AMI based on serum UA concentrations of <274 μmol/L in quartile 1 *(thin solid line)*, 274 to 333 μmol/L in quartile 2 *(short-dash line)*, 333 to 399 μmol/L in quartile 3 *(long-dash line)*, and >399 μmol/L in quartile 4 *(thick solid line)*.

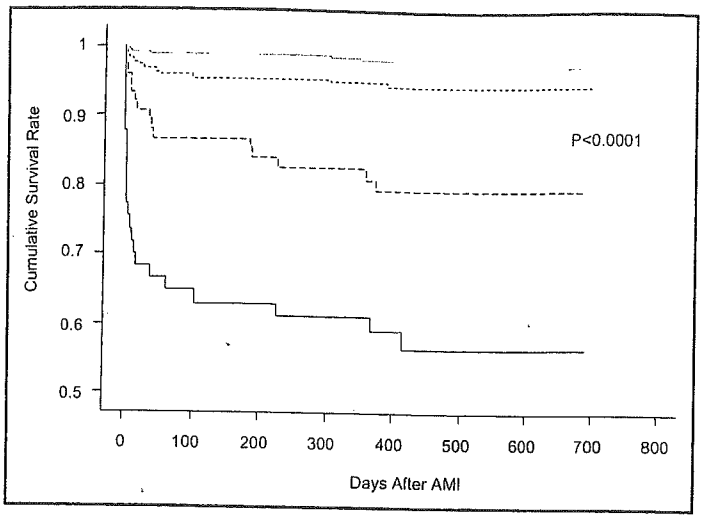

Figure 3. Survival without all-cause mortality in patients after AMI based on combinations of the best UA cut-off concentration of 447 μmol/L (7.5 mg/dl) for predicting survival and Killip's class: Killip's classes I and II plus a UA concentration ≤447 μmol/L *(thin solid line)*, Killip's classes I and II plus a UA concentration >447 μmol/L *(short-dash line)*, Killip's classes III and IV plus a UA concentration ≤447 μmol/L *(long-dash line)*, and Killip's classes III and IV plus a UA concentration >447 μmol/L *(thick solid line)*.

fication and previous myocardial infarction, which are considered to be deeply involved in decreased cardiac output that is caused by AMI.

Prompt restoration of myocardial blood flow is the therapeutic goal in AMI because early reperfusion decreases mortality rates.[12] In patients who had AMI, were in a high Killip's class, and had high UA concentrations, our results associated TIMI grade 3 flow with decreased short- and long-term mortality rates compared with TIMI perfusion grade 0, 1, and 2 flows; however, these differences were not significant in the present study. A failing heart due to AMI may cause tissue hypoperfusion and hypoxia, which trigger xanthine oxidase activation and oxidative stress production.[13,14] Xanthine oxidase and oxidative stress as reflected by UA may form a vicious cycle that promotes severe heart failure.[13,15] Therefore, UA may not be only a bystander marker but also a causative marker of mortality in patients who have AMI. In this regard, improvement of coronary reperfusion alone may be less effective in ameliorating heart failure and decreasing mortality rate in patients who have AMI and high UA level and are in a high Killip's class.

Adjunctive therapy designed to decrease xanthine oxidase activity and inhibit oxidative stress production is expected to sever the vicious cycle. The Losartan Intervention For Endpoint reduction in hypertension (LIFE) study demonstrated that lowering serum UA concentrations by losartan was associated with a beneficial effect on cardiovascular outcome.[16] The UA-lowering effect of atorvastatin may have contributed to the decrease in cardiovascular mortality in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) study.[17] Therefore, any drug interventions, such as therapy to decrease serum UA level in addition to coronary reperfusion, may have a favorable effect on mortality in patients who have AMI.

Our study is limited by being a retrospective study. However, it included all patients who had AMI and were entered in the 2002 database. All patients were followed after onset of AMI, so that the results of the present study should reflect the actual condition of patients in Japan who have AMI.

Table 5
Association between uric acid quartiles and all-cause mortality

UA Quartile (μmol/L)	Total Death	Unadjusted			Adjusted		
		OR	95% CI	p Value	OR	95% CI	p Value
1 (<274; n = 273)	9 (3%)	1.000	—	—	1.000	—	—
2 (274–333; n = 299)	11 (4%)	1.098	0.455–2.650	0.8351	1.618	0.541–4.836	0.3892
3 (333–399; n = 276)	14 (5%)	1.542	0.668–3.564	0.3105	1.503	0.511–4.425	0.4594
4 (>399; n = 276)	33 (12%)	3.753	1.795–7.843	0.0004	3.716	1.417–9.741	0.00765

Hazard ratios compared with quartile 1 with regard to long-term mortality after nonadjustment and adjustment for independent factors that were closely associated with all-cause mortality in multivariate analysis (Killip's classification, age, and peak creatine phosphokinase level).

Abbreviations as in Tables 2 and 3.

Table 6
Association between combination of best uric acid cut-off concentration with Killip's classes and all-cause mortality

Group	Total Death	Unadjusted			Adjusted		
		OR	95% CI	p Value	OR	95% CI	p Value
1 (n = 772)*	16 (2%)	1.000	—	—	1.000	—	—
2 (n = 219)†	12 (5%)	2.664	1.260–5.632	0.0103	3.465	1.555–7.720	0.0024
3 (n = 76)‡	15 (20%)	10.431	5.156–21.105	<0.0001	8.573	3.822–19.230	<0.0001
4 (n = 57)§	24 (42%)	27.005	14.328–50.896	<0.0001	22.473	10.802–46.754	<0.0001

Hazard ratios compared with quartile 1 with regard to long-term mortality after nonadjustment and adjustment for independent factors that were closely associated with all-cause mortality in multivariate analysis (age and peak creatine phosphokinase level).

* Killip's classes I and II plus serum UA levels ≤447 μmol/L.

† Killip's classes I and II plus serum UA levels >447 μmol/L.

‡ Killip's classes III and IV plus serum UA levels ≤447 μmol/L.

§ Killip's classes III and IV plus serum UA levels >447 μmol/L.

Appendix

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Beneficial Effect of Preinfarction Angina on In-Hospital Outcome is Preserved in Elderly Patients Undergoing Coronary Intervention for Anterior Acute Myocardial Infarction

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Background Preinfarction angina improves survival after acute myocardial infarction (AMI) in nonelderly but not elderly patients in the thrombolytic era. However, it remains unclear whether preinfarction angina has a beneficial effect on clinical outcome in elderly patients undergoing percutaneous coronary intervention (PCI).
Methods and Results The study group comprised 484 anterior AMI patients who were admitted within 24 h of onset and underwent emergency PCI. Patients were divided into 2 groups: those aged <70 years (nonelderly patients, n=290) and those aged ≥70 years (elderly patients, n=194). Angina within 24 h before AMI was present in 42% of nonelderly patients and in 37% of elderly patients. In nonelderly patients, preinfarction angina was associated with a lower in-hospital mortality rate (1% vs 7%, p=0.02). Similarly, in elderly patients, preinfarction angina was associated with a lower in-hospital mortality rate (6% vs 16%, p=0.03). Multivariate analysis showed that the absence of preinfarction angina was an independent predictor of in-hospital mortality in both nonelderly (odds ratio 4.20; 95% confidence interval (CI) 1.20–10.6; p=0.04) and elderly patients (odds ratio 3.04; 95%CI 1.06–18.1; p=0.04).
Conclusions Angina within the 24 h before AMI is associated with better in-hospital outcomes in elderly and nonelderly patients. (*Circ J* 2005; **69:** 630–635)

Key Words: Aging; Angina pectoris; Myocardial infarction; Reperfusion

Brief episodes of ischemia before sustained coronary artery occlusion protect the heart by delaying lethal injury and significantly limiting the size of the infarct, an effect known as ischemic preconditioning[1,2] Clinical studies have confirmed that angina shortly before the onset of acute myocardial infarction (AMI) is associated with a smaller infarct size and better short- and long-term outcomes[3–6] However, it has been reported that in the thrombolytic era preinfarction angina limits infarct size and improves clinical outcome in nonelderly, not elderly, patients with AMI[7,8] and it remains unclear whether preinfarction angina has a beneficial effect on clinical outcome in elderly patients undergoing percutaneous coronary intervention (PCI). In this study, we assessed the relation of preinfarction angina to in-hospital outcome in nonelderly and elderly patients with anterior AMI who underwent PCI.

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Methods

Patients

The Japanese Acute Coronary Syndrome Study (JACSS) is a retrospective, observational multicenter trial[9] involving 484 patients with anterior AMI who fulfilled the following inclusion criteria: (1) admission within 24 h of symptom onset; (2) coronary angiography performed immediately after admission; (3) emergency percutaneous transluminal coronary angioplasty, stenting or both of the left anterior descending coronary artery (LAD); and (4) availability of a detailed clinical history. The diagnosis of anterior AMI was based on typical chest pain lasting more than 30 min, ST-segment elevation of at least 1 mm in 2 contiguous precordial leads, and a subsequent increase in the serum creatine

Table 1 Clinical Characteristics and Coronary Angiographic Findings in Nonelderly (<70 Years Old) and Elderly (≥70 Years Old) Patients

	Nonelderly (n=290)	*Elderly (n=194)*	*p value*
Age (years)	*58±8*	*77±5*	*0.000*
Preinfarction angina (%)	*123 (42)*	*71 (37)*	*0.201*
Men (%)	*243 (84)*	*109 (56)*	*0.000*
Killip ≥2 on admission (%)	*41 (14)*	*54 (28)*	*0.000*
Risk factors			
Smoking (%)	*164 (57)*	*51 (26)*	*0.000*
Hyperlipidemia (%)	*109 (38)*	*49 (25)*	*0.005*
Diabetes mellitus (%)	*82 (28)*	*46 (24)*	*0.265*
Hypertension (%)	*139 (48)*	*127 (66)*	*0.000*
Prior infarction (%)	*29 (10)*	*25 (13)*	*0.323*
Time to admission (h)	*4.0±4.7*	*5.4±5.0*	*0.003*
Multivessel disease (%)	*91 (31)*	*81 (42)*	*0.019*
TIMI flow grade 0 at initial CAG (%)	*205 (71)*	*124 (64)*	*0.118*
Final TIMI flow grade ≥2 (%)	*280 (97)*	*190 (98)*	*0.372*
Final TIMI flow grade 3 (%)	*258 (89)*	*170 (88)*	*0.652*
Stent implantation (%)	*224 (77)*	*156 (80)*	*0.405*
Peak creatine kinase (IU/L)	*3,803±3,064*	*3,305±2,412*	*0.045*
In-hospital mortality (%)	*12 (4)*	*24 (12)*	*0.001*

Data are presented as mean±standard deviation or number (%) of patients.
TIMI, Thrombolysis in Myocardial Infarction; CAG, coronary angiography.

Table 2 Clinical Characteristics and Coronary Angiographic Findings in Nonelderly (<70 Years Old) Patients According to the Presence or Absence or Preinfarction Angina

	No angina (n=167)	*Angina (n=123)*	*p value*
Age (years)	*58±8*	*57±8*	*0.355*
Men (%)	*139 (83)*	*104 (85)*	*0.763*
Killip ≥2 on admission (%)	*26 (15)*	*15 (12)*	*0.415*
Risk factors			
Smoking (%)	*90 (54)*	*74 (60)*	*0.287*
Hyperlipidemia (%)	*62 (37)*	*47 (38)*	*0.850*
Diabetes mellitus (%)	*56 (34)*	*26 (21)*	*0.021*
Hypertension (%)	*83 (50)*	*56 (46)*	*0.482*
Prior infarction (%)	*19 (11)*	*10 (8)*	*0.362*
Time to admission (h)	*3.6±4.2*	*4.5±5.3*	*0.103*
Multivessel disease (%)	*52 (31)*	*39 (32)*	*0.918*
TIMI flow grade 0 at initial CAG (%)	*126 (75)*	*79 (64)*	*0.038*
Final TIMI flow grade ≥2 (%)	*160 (96)*	*120 (98)*	*0.419*
Final TIMI flow grade 3 (%)	*139 (83)*	*119 (97)*	*0.000*
Stent implantation (%)	*126 (75)*	*98 (80)*	*0.396*

Data are presented as mean±standard deviation or number (%) of patients.
TIMI, Thrombolysis in Myocardial Infarction; CAG, coronary angiography.

kinase concentration to more than twice the upper limit of normal. Preinfarction angina was defined as the presence of typical chest pain occurring at rest or during exercise and persisting for less than 30 min within 24 h before the onset of AMI[3,6] The study protocol was reviewed and approved by the ethics committee of each participating hospital.

Coronary Angiography and Coronary Intervention

Coronary angiography was performed immediately after admission to assess the perfusion status of the LAD according to the Thrombolysis in Myocardial Infarction (TIMI) study classification[10] The recanalization method was left to the attending physician's discretion. Final TIMI flow grade was assessed on the final angiograms. Multivessel disease was defined as ≥75% stenosis in 1 or more vessels remote from the LAD.

Statistical Analysis

Data are expressed as mean±SD. Categorical data were compared by chi-square analyses. Student's t-test was used to compare continuous variables. A probability value <0.05 was considered to indicate a statistically significant difference. Multiple logistic regression analysis was used to examine the determinants of in-hospital mortality. Variables used for analysis included age, sex, time to admission, prior infarction, Killip class on admission, preinfarction angina, initial occlusion status in the LAD, multivessel disease, stent implantation, final TIMI flow grade, hypertension, diabetes mellitus, hyperlipidemia, and smoking. Odds ratios and 95% confidence intervals were calculated. Analyses were done using SPSS PC software (Chicago, IL, USA).

Results

Patient Characteristics

There were 290 patients aged <70 years (nonelderly patients, mean age 58 years, range 29–69) and 194 patients

Table 3 Clinical Characteristics and Coronary Angiographic Findings in Elderly (≥70 Years Old) Patients According to the Presence or Absence of Preinfarction Angina

	No angina (n=123)	*Angina (n=71)*	*p value*
Age (years)	*77±5*	*78±6*	*0.519*
Men (%)	*75 (61)*	*34 (48)*	*0.077*
Killip ≥2 on admission (%)	*41 (33)*	*13 (18)*	*0.025*
Risk factors			
Smoking (%)	*32 (26)*	*19 (27)*	*0.910*
Hyperlipidemia (%)	*29 (24)*	*20 (28)*	*0.478*
Diabetes mellitus (%)	*29 (24)*	*17 (24)*	*0.954*
Hypertension (%)	*84 (68)*	*43 (61)*	*0.275*
Prior infarction (%)	*17 (14)*	*8 (11)*	*0.609*
Time to admission (h)	*5.1±4.6*	*5.8±5.6*	*0.392*
Multivessel disease (%)	*54 (44)*	*27 (38)*	*0.424*
TIMI flow grade 0 at initial CAG (%)	*78 (63)*	*46 (65)*	*0.848*
Final TIMI flow grade ≥2 (%)	*121 (98)*	*69 (97)*	*0.574*
Final TIMI flow grade 3 (%)	*107 (87)*	*63 (89)*	*0.723*
Stent implantation (%)	*100 (81)*	*56 (79)*	*0.682*

Data are presented as mean±standard deviation or number (%) of patients.
TIMI, Thrombolysis in Myocardial Infarction; CAG, coronary angiography.

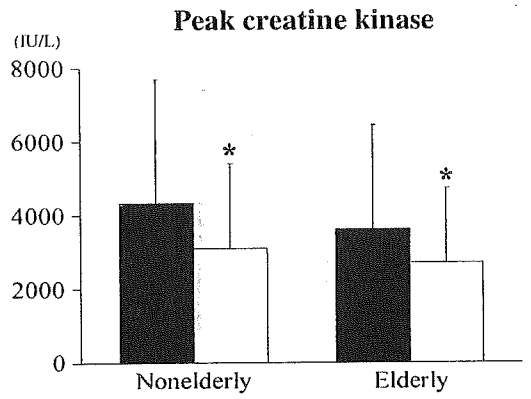

Fig 1. In both nonelderly (age <70 years) and elderly patients (age ≥70 years), the peak creatine kinase concentration was significantly lower in those with (white bar) than in those without (black bar) preinfarction angina. *p<0.05 vs patients without preinfarction angina.

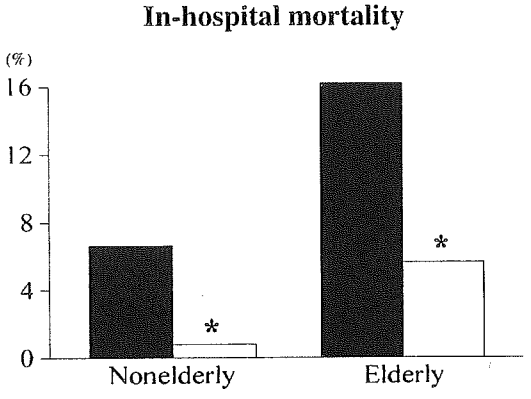

Fig 2. In both nonelderly patients (age <70 years) and elderly patients (age ≥70 years), in-hospital mortality was significantly lower in those with (white bar) than in those without (black bar) preinfarction angina. *p<0.05 vs patients without preinfarction angina.

aged ≥70 years (elderly patients, mean age 77 years, range 70–95). Overall, stent implantation was performed in 380 patients (79%). The final TIMI flow grade was ≥2 in 470 patients (97%) and 3 in 428 (89%). The baseline characteristics of all the patients are presented in Table 1. The nonelderly and elderly patient groups differed with regard to age, sex, Killip class on admission, smoking, hyperlipidemia, hypertension, time to admission, and multivessel disease. However, there were no differences in diabetes mellitus, prior infarction, the prevalence of initial or final TIMI flow grade, or stent implantation. Preinfarction angina was slightly but not significantly less frequent in elderly patients.

The baseline characteristics of the nonelderly patients with and without preinfarction angina are presented in Table 2. These groups were similar with regard to age, sex, Killip class on admission, coronary risk factors other than diabetes, prior infarction, time to admission, the prevalence of multivessel disease, final TIMI flow grade ≥2, and stent implantation. The prevalence of both diabetes mellitus and an initial TIMI flow grade of 0 was significantly lower and the prevalence of final TIMI flow grade 3 was significantly higher in patients with preinfarction angina.

The baseline characteristics of the elderly patients with and without preinfarction angina are presented in Table 3. These groups were similar with regard to age, sex, coronary risk factors, prior infarction, time to admission, the prevalence of multivessel disease, initial and final TIMI flow grades, and stent implantation. The prevalence of Killip class ≥2 on admission was significantly lower in patients with preinfarction angina.

Peak Creatine Kinase (CK) Concentration (Fig 1)

The peak CK concentration was significantly lower in elderly patients than in nonelderly patients, but in both groups, preinfarction angina was associated with a lower peak CK.

In-Hospital Mortality (Fig 2)

During hospitalization (mean 14 days), 36 patients (7.4%) died; 86% of in-hospital deaths were related to cardiac causes. In-hospital mortality was significantly higher in elderly patients than nonelderly patients. In both groups, preinfarction angina was associated with lower in-hospital mortality. Multivariate analysis revealed that the absence of preinfarction angina was an independent

Table 4 Multivariate Analysis of Factors Associated With In-Hospital Mortality in Nonelderly (<70 Years Old) and Elderly (≥70 Years Old) Patients According to the Presence or Absence of Preinfarction Angina

Variable	Nonelderly (<70 years old)		Elderly (≥70 years old)	
	Odds ratio (95%CI)	p value	Odds ratio (95%CI)	p value
Age	1.03 (0.93–1.13)	0.603	1.19 (1.04–1.37)	0.008
Female	0.84 (0.12–5.72)	0.858	1.70 (0.38–7.64)	0.489
Time to admission	0.95 (0.77–1.19)	0.673	0.96 (0.81–1.15)	0.680
Prior infarction	7.20 (1.44–36.0)	0.016	4.96 (1.02–24.2)	0.048
Killip class ≥2	4.82 (1.12–20.8)	0.035	32.2 (7.38–49.4)	<0.001
Absence of preinfarction angina	4.20 (1.20–10.6)	0.037	3.04 (1.06–18.1)	0.044
TIMI flow grade 0 at initial CAG	1.63 (0.34–7.75)	0.537	3.46 (0.66–18.1)	0.141
Multivessel disease	1.10 (0.19–2.00)	0.264	1.05 (0.21–1.68)	0.220
Stent implantation	1.58 (0.31–8.00)	0.584	0.61 (0.12–3.05)	0.551
Final TIMI flow grade	0.86 (0.36–2.10)	0.752	0.09 (0.02–0.38)	0.001
Hypertension	4.77 (0.96–22.6)	0.058	1.53 (0.37–6.34)	0.561
Diabetes mellitus	2.92 (0.76–11.3)	0.120	1.02 (0.16–1.77)	0.191
Hyperlipidemia	0.67 (0.16–2.72)	0.571	0.26 (0.08–1.74)	0.164
Smoking	1.01 (0.25–4.02)	0.995	0.58 (0.10–4.30)	0.592

95%CI, 95% confidence interval; TIMI, Thrombolysis in Myocardial Infarction; CAG, coronary angiography.

predictor of in-hospital death in both nonelderly and elderly patients (Table 4).

Discussion

In the present study preinfarction angina occurring within 24 h of the onset of anterior AMI was associated with a lower peak CK concentration and lower in-hospital mortality after PCI in elderly and nonelderly patients. Multivariate analysis showed that the absence of preinfarction angina was an independent predictor of in-hospital mortality in both groups of patients. These findings suggest that the beneficial effects of preinfarction angina on in-hospital outcome is preserved independenty of age in patients undergoing PCI for anterior AMI.

Preinfarction Angina

Clinical studies have reported that in the thrombolytic era the presence of preinfarction angina is associated with a smaller infarct and better survival[3–6] Andreotti et al have shown that thrombolytic therapy results in more rapid recanalization in patients with preinfarction angina than in those without it.[11] Ishihara et al found that after thrombolytic therapy, recanalization of an occluded infarct-related artery is more frequently achieved in patients with preinfarction angina than in those without it[6] Experimentally, brief antecedent ischemia has been shown to enhance recombinant tissue plasminogen activator-induced thrombolysis.[12] Taken together, these findings suggest that early implementation of thrombolytic therapy may partly contribute to better outcomes in patients with preinfarction angina who undergo this treatment. The beneficial effects of preinfarction angina may also be explained by other mechanisms, including ischemic preconditioning, collateral circulation, and intermittent occlusion.[13] Ischemic preconditioning is a cardioprotective phenomenon in which short periods of myocardial ischemia make the myocardium more resistant to subsequent episodes.[1,2] In the present study we showed that preinfarction angina per se, apart from the perfusion status of the infarct-related artery before and after recanalization, was related to improved in-hospital survival. These findings suggest that the beneficial effects of preinfarction angina on clinical outcome may be related to the cardioprotective effect of ischemic preconditioning.

Ischemic Preconditioning and Aging

Experimental studies have demonstrated that the effects of ischemic preconditioning are attenuated with age[14,15] and several mechanisms have been proposed for this phenomenon, including decreased adenosine triphosphate concentrations or superoxide dismutase activity, reduced production of stress-induced proteins, reductions in norepinephrine release and • -adrenergic receptor stimulation, increased intracellular calcium concentrations, increased vulnerability of myocardium to ischemia, and attenuated activation of the KATP channels.[14–18] On the other hand, Przyklenk et al have shown that ischemic preconditioning reduces infarct size in both middle-aged and old rabbits independently of morphologic and functional cardiovascular aging, characterized by myocyte hypertrophy, increased myocardial fibrosis, and attenuated responsiveness to • -• adrenergic stimulation.[19] These findings are supported by studies done by Loubani et al[20] demonstrating in experimental models that necrosis induced by severe ischemic insults to the human myocardium is not exacerbated by increasing age and that ischemic preconditioning equally protects the myocardium in both elderly and younger patients. Thus, the relation between aging and the implications of ischemic preconditioning remain a matter of debate.

Preinfarction Angina and Aging

In contrast to previous studies[7,8] our observational multicenter study found that the presence of angina within 24 h of infarction was associated with a smaller infarct and a better in-hospital outcome in elderly and nonelderly patients. Several reasons may account for inconsistencies with the results of prior studies. First, in the study by Abete et al[7] coronary angiography was not performed in most of the patients. Second, only 34% of elderly patients with preinfarction angina received thrombolytic therapy in their study, which might have contributed to a poorer outcome. Preinfarction angina has been shown to provide no benefit in the absence of reperfusion[21] In our study, a final TIMI flow of grade ≥2 was achieved in 97% of the patients. The study by Ishihara et al demonstrated that preinfarction angina is associated with better short- and long-term outcomes in nonelderly patients than in elderly patients who underwent emergency cardiac catheterization[8] Their study was performed between 1981 and 1994, whereas our study

period was in 2001. The recent improvements in cardiac catheterization including PCI,[22] treatment and patient care may partially explain the discrepancy between their findings and ours. Another likely reason for the inconsistent results is the definition of "elderly", which seems to have changed over time. Indeed, over the past 20 years, the definition of "elderly" in studies of outcome in patients undergoing cardiac surgery and related procedures has gradually increased from ≥65 years old to ≥80 years old.[23–25] Unspecified or unmeasured baseline characteristics of patients aged ≥70 years may also have differed our study and previous investigations. We limited our study group to patients undergoing emergency PCI, a decision that might be at least in part related to the patients' daily activities. Elderly patients in our study may have thus had a relatively high level of physical activity. Experimental studies have shown that exercise training restores the protective effect of ischemic preconditioning in the aging heart by increasing norepinephrine release.[26]

Study Limitations

This was a small, retrospective, observational, nonrandomized study. Furthermore, the subjects were limited to those with anterior AMI who underwent PCI because we recently showed that preinfarction angina improves in-hospital outcome after PCI in patients with anterior AMI, but not in those with nonanterior AMI.[27] The inclusion of these latter patients would have confounded assessment of the effect of preinfarction angina on in-hospital outcome. Another major limitation is the quantification of ischemic episodes. Because episodes of preinfarction angina were ascertained on the basis of patient history, silent ischemia was not taken into account. Silent ischemia has been shown to occur frequently in elderly patients.[28] In our study, preinfarction angina was slightly less frequent in elderly patients, but if we had taken silent ischemia into account, the benefits of preinfarction angina may have become clearer in elderly patients. Further prospective studies are needed to confirm whether the beneficial effects of preinfarction angina are preserved in elderly patients.

Conclusion

The presence of angina within 24 h of an anterior AMI is associated with a smaller infarct and better in-hospital outcome in elderly and nonelderly patients undergoing PCI.

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Appendix I

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Effects of Glucose Abnormalities on In-Hospital Outcome After Coronary Intervention for Acute Myocardial Infarction

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on behalf of the Japanese Acute Coronary Syndrome Study (JACSS) Investigators

Background The effects of glucose abnormalities on outcomes after percutaneous coronary intervention (PCI) remain unclear. We examined the association between glucose abnormalities and in-hospital outcome in patients undergoing PCI for acute myocardial infarction (AMI).

Methods and Results A total of 849 patients with AMI who were admitted within 12 h after symptom onset and underwent emergency PCI were classified according to the presence or absence of admission hyperglycemia, defined as a blood glucose level on admission of >11 mmol/L and whether they had a history of diabetes mellitus: group 1 (n=504), non-diabetic patients without admission hyperglycemia; group 2 (n=111), diabetic patients without admission hyperglycemia; group 3 (n=87), non-diabetic patients with admission hyperglycemia; and group 4 (n=147), diabetic patients with admission hyperglycemia. Among groups 1, 2, 3 and 4, in-hospital mortality was 2.6, 2.7, 11.5 and 8.8%, respectively ($p<0.01$). Multivariate analysis showed that compared with group 1 patients, the odds ratio (95%confidence interval) for in-hospital mortality among those in groups 2, 3, and 4 were 0.80 (0.24–2.60, p=0.708), 2.29 (1.10–5.49, p=0.039), and 2.14 (1.14–4.69, p=0.048), respectively.

Conclusions In-patients undergoing PCI for AMI, admission hyperglycemia, irrespective of the presence or absence of diabetes, is associated with increased in-hospital mortality, whereas diabetes without admission hyperglycemia is not. (*Circ J* 2005; **69:** 375–379)

Key Words: Glucose; Myocardial infarction; Reperfusion; Stent

Patients with diabetes have been established to have poorer outcomes after acute myocardial infarction (AMI) than non-diabetic patients[1,2] Furthermore, hyperglycemia itself on admission (admission hyperglycemia) is also associated with an increased risk of adverse events, including heart failure, cardiogenic shock, and death after AMI, irrespective of whether diabetes was previously diagnosed[3–6] Recently, Wahab et al report that diabetes, admission hyperglycemia, or both were associated with adverse outcomes after AMI during the thrombolytic era[6]

Thrombolytic therapy has been established to significantly reduce mortality among both diabetic and non-diabetic patients with AMI[7] Despite substantial benefits, thrombolytic therapy is less likely to be given to diabetic patients, which might contribute to their poorer outcome[6,8] Recently, percutaneous coronary intervention (PCI) is increasingly used for reperfusion therapy, improving the outcome of patients with AMI. In diabetic patients with AMI, primary angioplasty is associated with fewer and less severe adverse events than thrombolytic therapy[9] suggesting that PCI might have a beneficial effect on survival in diabetic patients. The aim of this study was to examine the relations of glucose abnormalities to infarct size and in-hospital mortality in patients with AMI who underwent PCI.

Methods

Study Population

The Japan Acute Coronary Syndrome Study (JACSS) was a retrospective, observational multicenter trial. Between January and December 2001, patients with AMI admitted to 35 participating hospitals in Japan were studied.

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Table 1 Baseline Characteristics

	Group 1 (n=504)	Group 2 (n=111)	Group 3 (n=87)	Group 4 (n=147)	p value
Age (years)	65±12	66±10	71±12	66±13	<0.01
Male	77%	79%	68%	65%	<0.01
Time from symptom onset to admission (h)	3.6±2.8	3.2±2.4	3.0±2.2	3.3±2.5	NS
Killip >1 on admision	14%	14%	25%	27%	<0.01
Previous infarction	10%	18%	10%	14%	NS
Previous angina	40%	40%	38%	29%	NS
Blood glucose level on admission (mmol/L)	7.5±1.7	8.4±1.7	13.9±3.2	16.1±4.6	<0.01
HbA_{1c} (%)†	5.3±0.6 (n=209)	6.9±1.2 (n=78)	5.7±0.9 (n=31)	8.1±1.8 (n=102)	<0.01
Diabetes mellitus	0	100%	0	100%	
Hyperlipidemia	28%	45%	23%	41%	<0.01
Hypertension	53%	63%	53%	62%	NS
Smoking	52%	55%	45%	46%	NS
Serum creatinine on admission (mg/d)	0.9±0.9	1.1±1.1	1.0±0.8	1.2±1.2	NS
Medication before AMI					
Oral hypoglycemic drug	0	35%	0	45%	<0.01
Insulim	0	9%	0	22%	<0.01
Aspirin	8%	16%	7%	15%	<0.01
β-blocker	4%	7%	6%	5%	NS
ACE inhibitor	5%	12%	6%	10%	<0.05
HMG CoA	4%	15%	7%	14%	<0.01
Anterior AMI	51%	40%	56%	43%	NS
ST-segment elevation	91%	90%	95%	91%	NS
3-vessel disease	10%	19%	12%	21%	<0.01
TIMI flow grade 0 at initial CAG	68%	61%	75%	63%	NS
Final TIMI flow grade ≥2	97%	97%	97%	95%	NS
Final TIMI flow grade 3	90%	87%	87%	88%	NS
Stent implantation	79%	73%	83%	78%	NS

AMI, acute myocardial infarction; ACE, angiotensin-converting enzyme; CAG, coronary angiography; HbA_{1c}, glycosylated hemoglobin; HMG CoA, hydroxymethylglutaryl-coenzyme A reductase inhibitors.
†HbA_{1c} was measured during hospitalization in only 420 patients.
Group 1, Non-diabetic patients without admission hyperglycemia; Group 2, Diabetic patients without admission hyperglycemia; Group 3, Non-diabetic patients with admission hyperglycemia; Group 4, Daibetic patients with admission hyperglycemia.
Data are presented as mean values±SD or percentages of patients.

A diagnosis of AMI required at least 2 of the following characteristics: typical chest pain persisting for 30 min or longer, ischemic electrocardiographic changes, and a peak creatine kinase level equivalent to more than twice the upper limit of normal. The study protocol was reviewed and approved by the ethical committee of each participating hospital. A total of 849 patients who met the following entry criteria were studied: (i) admission within 12 h from the onset of AMI; (ii) coronary angiography performed immediately after admission; (iii) percutaneous transluminal coronary angioplasty, stenting, or both of the infarct-related artery; (iv) measurement of blood glucose level on admission; and (v) availability of a detailed clinical history. Data from all subjects, excluding information that could be used to identify patients, such as names and identification numbers, were transmitted to a central data collection center, located in the Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, for analysis.

Coronary Angiography and Coronary Intervention

Written informed consent for coronary catheterization was obtained from all patients at each hospital. Coronary angiography was performed immediately after admission. The perfusion status of the infarct-related artery was assessed according to the Thrombolysis in Myocardial Infarction (TIMI) study classification.[10] The recanalization method was left to the physicians' discretion. Final TIMI flow grade was assessed on the basis of final angiograms obtained on admission.

Data Analysis

Previous angina was defined as the presence of typical chest pain occurring at rest or during exercise and persisting for less than 30 min, within 24 h before the onset of AMI. Diabetes mellitus was considered present if this diagnosis and antidiabetic treatment, including drugs or insulin, had been given to the patient, if the fasting glucose level was found to be=126 mg/dl (7.0 mmol/L) on the previous occasion or if the results of an oral glucose tolerance test were abnormal. Patients who did not meet these criteria were considered not to have diabetes mellitus. Blood samples for measurement of blood glucose level were obtained on admission. Admission hyperglycemia was defined as a blood glucose level on admission of >198 mg/dl (11 mmol/L)[6,11] Glycosylated hemoglobin (HbA_{1c}) was measured in 420 patients (49%) within 14 days after admission. Patients were classified into 4 groups, based on their history of diabetes and their blood glucose level on admission:

Group 1 (n=504): Non-diabetic patients without admission hyperglycemia;
Group 2 (n=111): Diabetic patients without admission hyperglycemia;
Group 3 (n=87): Non-diabetic patients with admission hyperglycemia;
Group 4 (n=147): Diabetic patients with admission hyperglycemia.