alterations in gene expression, and that the strength of this method currently resides in its capacity to characterize patterns of gene expression rather than to quantify the expression of individual gene products. Thus, we performed quantitative analysis of gene expression with real-time RT-PCR, the accuracy of which to determine gene expression in human tissue has already been demonstrated elsewhere[2]

*Role of Inflammatory Chemokines in Dilated and Occlusive Atherosclerotic Diseases*

IL-8, a C-X-C chemokine which was upregulated in both AAA and CAS, is produced by various types of cells on stimulation with inflammatory stimuli[10–13] Low density lipoprotein (LDL)-deficient mice, irradiated and reconstituted with macrophages deficient in the murine IL-8 receptor homologue of human CXCR-2, show diminished macrophage recruitment to the lesion, suggesting a potential role for IL-8 in monocyte trafficking in vivo[14] Moreover, IL-8 may contribute to the development of atherosclerosis by stimulating angiogenesis[15] It exerts a variety of effects on leukocytes, particularly neutrophils;[16] and plays a critical role in the mobilization of stem cells through its induction of MMP-9[17] In terms of MMP synthesis in dilated and occlusive atherosclerotic diseases, we previously reported significant upregulation of the expression of MMP-1 and -3 in AAA tissues, and MMP-1, -3 and -9 in CAS tissues[2,3] MMP-9 is also upregulated in the infarct-related human coronary artery[18,19] Therefore, upregulation of IL-8 might be related to overexpression of these MMPs in human atherosclerotic tissues.

IL-8 is also known to inhibit the production of the tissue inhibitor of matrix metalloproteinases (TIMPs), which are potent antagonists of MMPs in vessel tissue[20] Our previous data indicate disproportional expression of MMPs/TIMPs in AAA as well as CAS[2,3] Therefore, in the clinical setting upregulation of IL-8 could be related to this disproportional TIMPs expression in both AAA and CAS, thus enhancing the development of dilated or occlusive manifestation of atherosclerosis probably through the activation of proteinase activity. It has been suggested that the chemokine receptor CXCR-2 enhances monocyte recruitment and disease progression[21] Indeed, the lack of CXCR-2 expression in bone marrow cells has been shown to be responsible for an almost 50% reduction in lesion development[22]

There was no statistical significance in the expression of MCP-1 and CCR-2 in AAA and CAS tissues, which may be partly explained by the relatively high gene expression in the adjacent control tissues that already had minimal atherosclerotic changes. One might speculate that the MCP-1 and CCR-2 system, which is upregulated in the early stage of atherosclerosis, could be relatively downregulated in the established stage, such as the significant dilated and/or stenotic lesions studied by us. This might result in an altered balance in the expression of the contributing and suppression genes, which leads to the severity of the disease process[1,4]

*Clinical Implications and Study Limitations*

Although the precise relationship between the inflammatory process of atherosclerosis and development of AAA and/or CAS, particularly in the established stage of the diseases, remains unclear, their frequent association and shared risk factors suggest common pathophysiologic mechanisms. This assertion is supported by the observation that both AAA and CAS exhibited increased expression of CXCR-2 as well as IL-8, both of which are thought to be important in inflammatory process of atherosclerosis, although we could not correlate the extent of disease severity with the levels of gene expression because of similar aneurysmal (>40 mm in diameter) and stenotic (>90%) lesions in the present cohort.

From the therapeutic point of view, it is interesting to note that the established anti-inflammatory pathways of HMG-CoA reductase inhibitors include the diminished expression of cytokines, such as IL-6 and IL-8, in cells implicated in atherogenesis or in human plasma[23,24] In addition, oxydized LDL-cholesterol enhances the upregulation of the expression of CXCR-2 in monocytes, contributing to disease progression associated with dilation or occlusion[25] Indeed, atorvastatin therapy, which suppresses the development of atherosclerosis as well as reduces the incidence of major adverse cardiac events[26,27] could decrease the spontaneous release of IL-8 in mononuclear cells of patients with coronary artery disease[28] Also, the effects of angiotensin-receptor inhibitors on suppression of atherosclerosis could be derived from inhibition of the overexpression of chemokines, such as IL-8, associated with reduction of macrophage accumulation in the lesion[29] Although it would be indeed intriguing to examine whether the expressions of IL-8 and CXCR-2 in AAA and CAS could be affected by these "anti-inflammatory" drugs in the present cohort, we could not correlate gene expression level to treatment because of the variety of drugs and their doses. A prospective study of intensive use of HMG-CoA reductase inhibitors and/or angiotensin-receptor inhibitors for AAA and CAS patients may demonstrate the effectiveness of these agents on the expression of IL-8 and CXCR-2 and whether this is protective or not in the established stage of dilated and/or stenotic lesions.

One of the most important limitations in the present study is that we used the adjacent tissues, which were already involved in the disease, as the control. Therefore, the present study was not done with truly normal tissue. However, both control tissues did not show any dilated or occlusive lesions and might be considered to be in the "preaneurymal" or "preocclusive" state of the disease.

Even under these conditions, upregulation of IL-8 and associated CXCR-2 may have a crucial role in the development of manifested atherosclerotic disease.

## Conclusions

We used a membrane-based cDNA macroarray and real-time RT-PCR to characterize the cytokine-related gene expression in AAA and CAS. Although the functional significance of the individual gene products that were altered in AAA and CAS will require further investigation, this study demonstrates the potential of cDNA expression array and real-time RT-PCR in elucidating the molecular mechanisms responsible for the development of AAA and CAS.

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## References


1. Libby P. Inflammation in atherosclerosis. *Nature* 2002; **420:** 868–874.
2. Higashikata T, Yamagishi M, Sasaki H, Minatoya K, Ogino H, Ishibashi-Ueda H, et al. Application of real-time RT-PCR to quantifying gene expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human abdominal aortic aneurysm. *Atherosclerosis* 2004; **177:** 353–360.
3. Higashikata T, Yamagishi M, Higashi T, Nagata I, Iihara K, Miyamoto S, et al. Altered expression balance of matrix metalloproteinases and their inhibitors in human carotid plaque disruption: Results of quantitative tissue analysis using real-time RT-PCR method. *Atherosclerosis* 2005 July 20; [Epub ahead of print].
4. Boring L, Gosling J, Cleary M. Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature* 1998; **394:** 894–897.
5. Ito T, Ikeda U. Inflammatory cytokines and cardiovascular disease. *Curr Drug Targets Inflamm Allergy* 2003; **2:** 257–265.
6. Kusamo KF, Nakamura K, Kusano H, Nishii N, Banba K, Ikeda T, et al. Significance of the level of monocyte chemoattractant protein-1 in human atherosclerosis. *Circ J* 2004; **68:** 671–676.
7. Koch AE, Kunkel SL, Pearce WH, Shah MR, Parikh D, Evanoff HL, et al. Enhanced production of the chemotactic cytokines interleukin-8 and monocyte chemoattractant protein-1 in human abdominal aortic aneurysms. *Am J Pathol* 1993; **142:** 1423–1431.
8. Yamagishi M, Higashikata T, Higashi T, Nagata I, Ishibashi-Ueda H, Tomoike H, et al. Sustained upregulation of chemokine and its receptor genes associated with matrix metalloproteinase overexpression in human carotid plaque rupture: Results from a quantitative study with real-time RT-PCR method (abstract). *J Am Coll Cardiol* 2004; **43**(Suppl A): 497A.
9. Faber BC, Cleutjens KB, Niessen RL, Aarts PL, Boon W, Greenberg AS, et al. Identification of genes potentially involved in rupture of human atherosclerotic plaques. *Circ Res* 2001; **89:** 547–554.
10. Matsushima K, Oppenheim JJ. Interleukin 8 and MCAF: Novel inflammatory cytokines inducible by IL-1 and TNF. *Cytokine* 1989; **1:** 2–13.
11. Boisvert WA, Curtiss LK, Terkeltaub RA. Interleukin-8 and its receptor CXCR2 in atherosclerosis. *Immunol Res* 2000; **21:** 129–137.
12. Yound JL, Libby P, Schonbeck U. Cytokines in the pathogenesis of atherosclerosis. *Thromb Haemost* 2002; **88:** 554–567.
13. Hansson GK, Libby P, Schonbeck U, Yan ZQ. Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ Res* 2002; **91:** 281–291.
14. Boisvert WA, Santiago R, Curtiss LK. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 1998; **101:** 353–363.
15. Simonini A, Moscucci M, Muller DW, Bates ER, Pagani FD, Burdick MD. IL-8 is an angiogenic factor in human coronary atherectomy tissue. *Circulation* 2000; **101:** 1519–1526.
16. Chakrabarti S, Patel KD. Regulation of matrix metalloproteinase-9 release from IL-8-stimulated human neutrophils. *J Leukoc Biol* 2005; **78:** 279–288.
17. Pruijt JF, Verzaal P, van Os R, de Kruijf EJ, van Schie ML, Mantovani A, et al. Neutrophils are indispensable for hematopoietic stem cell mobilization induced by interleukin-8 in mice. *Proc Natl Acad Sci USA* 2002; **99:** 6228–6233.
18. Funayama H, Ishikawa SE, Kubo N, Katayama T, Yasu T, Saito M, et al. Increases in interleukin-6 and matrix metalloproteinase-9 in the infarct-related coronary artery of acute myocardial infarction. *Circ J* 2004; **68:** 451–454.
19. Higo S, Uematsu M, Yamagishi M, Ishibashi-Ueda H, Awata M, Morozumi T, et al. Elevation of plasma matrix metalloproteinase-9 in the culprit coronary artery in patients with acute myocardial infarction: Clinical evidence from distal protection. *Circ J* 2005; **69:** 1180–1185.
20. Moreau M, Brocheriou I, Petit L, Ninio E, Chapman MJ, Rouis M. Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: Relevance to stability of atherosclerotic plaque. *Circulation* 1999; **99:** 420–426.
21. Holm T, Damas JK, Holven K, Nordoy I, Brosstad FR, Ueland T, et al. CXC-chemokines in coronary artery disease: Possible pathogenic role of interactions between oxidized low-density lipoprotein, platelets and peripheral blood mononuclear cell. *J Thromb Haemost* 2003; **1:** 257–262.
22. Huo Y, Weber C, Forlow SB, Sperandio M, Thatte J, Mack M, et al. The chemokine KC, but not monocyte chemoattractant protein-1, triggers monocyte arrest on early atherosclerotic endothelium. *J Clin Invest* 2001; **108:** 1307–1314.
23. Takata M, Urakaze M, Temaru R, Yamazaki K, Nakamura N, Nobata Y. Pravastatin suppresses the interleukin-8 production induced by thrombin in human aortic endothelial cells cultured with high glucose by inhibiting the p44/42 mitogen activated protein kinase. *Br J Pharmacol* 2001; **134:** 753–762.
24. Ito T, Ikeda U, Yamamoto K, Shimada K. Regulation of interleukin-8 expression by HMG-CoA reductase inhibitors in human vascular smooth muscle cells. *Atherosclerosis* 2002; **165:** 51–55.
25. Lei ZB, Zhang Z, Jing Q, Qin YW, Pei G, Cao BZ, et al. OxLDL upregulates CXCR2 expression in monocytes via scavenger receptors and activation of p38 mitogen-activated protein kinase. *Cardiovasc Res* 2002; **53:** 524–532.
26. Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: A randomized controlled trial. *JAMA.* 2004; **291:** 1071–1080.
27. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med* 2004; **350:** 1495–1504.
28. Wahre T, Damas JK, Gullestad L, Holm AM, Pedersen TR, Arnesen KE, et al. Hydromethylglutaryl coenzyme A reductase inhibitors down-regulate chemokines and chemokine receptors in patients with coronary artery disease. *J Am Coll Cardiol* 2003; **41:** 1460–1467.
29. Dol F, Martin G, Staels B, Mares AM, Cazaubon C, Nisato D, et al. Angiotensin AT1 receptor antagonist Irbesrtan decreases lesion size, chemokine expression, and macrophage accumulation in apolipoprotein E-deficient mice. *J Cardiovasc Pharmacol* 2001; **38:** 395–405.

# Acute hyperglycemia is associated with adverse outcome after acute myocardial infarction in the coronary intervention era

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**Purpose** This study was undertaken to assess the association between acute hyperglycemia and inhospital outcome after acute myocardial infarction (AMI) in the percutaneous coronary intervention (PCI) era. We also assessed outcome of patients with a history of diabetes mellitus in the PCI era.

**Methods** Between January 2001 and December 2001, 1253 patients were admitted to the hospitals within 48 hours after the onset of AMI. Plasma glucose was measured at hospital admission. Acute hyperglycemia was defined as plasma glucose of >11 mmol/L (198 mg/dL), regardless of the diabetic status. Primary PCI was performed in 898 (72%) patients.

**Results** The inhospital mortality rate was significantly higher in patients with acute hyperglycemia than in patients without (16% vs 6%, $P < .001$). However, there was no significant difference in mortality between diabetic and nondiabetic patients (8% vs 9%, $P = .54$). Acute hyperglycemia was associated with a higher inhospital mortality rate both in nondiabetic patients (24% vs 6%, $P < .001$) and in diabetic patients (10% vs 5%, $P = .039$). Acute hyperglycemia was associated with a higher incidence of no reflow during PCI (21% vs 12%, $P < .001$), but diabetes was not (14% vs 15%, $P = .71$).

**Conclusion** Acute hyperglycemia, but not diabetes, was a predictor for inhospital mortality after AMI in the PCI era. No reflow occurred more frequently during PCI in patients with acute hyperglycemia, suggesting that microvascular dysfunction might have contributed to adverse outcome of these patients. (Am Heart J 2005;150:814-820.)

An increase of plasma glucose concentration is often observed during early hours after the onset of acute myocardial infarction (AMI) not only in patients with diabetes mellitus but also in patients without diabetes mellitus.[1] It has been reported that both acute hyperglycemia and diabetes mellitus are independently associated with adverse outcomes after AMI in the prereperfusion era and in the thrombolytic era.[2-7] Primary percutaneous coronary intervention (PCI) has been shown to be more effective than thrombolytic therapy for the treatment of AMI.[8] Recent progress in treatment of AMI might have changed the association between acute hyperglycemia and outcome after AMI. This study was undertaken to assess the association between acute hyperglycemia and inhospital outcome after AMI in the contemporary PCI era. In addition, because acute hyperglycemia was often confused with chronic hyperglycemia, the association between diabetes mellitus and outcome after AMI in the PCI era was also investigated.

Despite the recent progress in PCI, it has been shown that coronary stent has no benefit in terms of reducing no-reflow phenomenon.[9] No-reflow phenomenon is associated with adverse outcome after AMI.[10,11] It has been reported that hyperglycemia impairs microvascular function and may cause no-reflow phenomenon.[12]

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**See Appendices.*

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**Table I.** Baseline characteristics of patients with and those without acute hyperglycemia

| | Acute hyperglycemia | | |
|---|---|---|---|
| | Present (n = 378) | Absent (n = 875) | P value |
| Age (y) | 70 ± 12 | 67 ± 12 | .007 |
| Men | 236 (62%) | 648 (74%) | <.001 |
| Hypertension | 209 (55%) | 489 (56%) | .85 |
| Previous angina | 115 (30%) | 327 (37%) | .02 |
| Previous infarction | 59 (16%) | 107 (12%) | .11 |
| Diabetes mellitus | 231 (61%) | 166 (19%) | <.001 |
| Time to admission (h) | 5.7 ± 8.0 | 7.1 ± 9.3 | .01 |
| Killip classes 2 to 4 | 133 (35%) | 143 (16%) | <.001 |
| ST elevation | 308 (81%) | 745 (85%) | .11 |
| Medication before infarction | | | |
| Aspirin | 43 (11%) | 88 (10%) | .49 |
| ACE inhibitor | 25 (7%) | 54 (6%) | .77 |
| ARB | 10 (3%) | 21 (2%) | .80 |
| β-Blocker | 18 (5%) | 45 (5%) | .78 |
| Ca channel blocker | 89 (24%) | 219 (25%) | .57 |
| Nicorandil | 13 (3%) | 33 (4%) | .77 |
| Statin | 32 (8%) | 54 (6%) | .15 |
| Any of above medications | 132 (35%) | 283 (32%) | .37 |
| Oral hypoglycemic drug | 114 (30%) | 58 (7%) | <.001 |
| Insulin | 49 (13%) | 17 (2%) | <.001 |
| Reperfusion therapy | | | |
| Thrombolysis | 40 (11%) | 64 (7%) | .06 |
| Balloon angioplasty | 43 (11%) | 130 (15%) | .10 |
| Stent | 219 (58%) | 506 (58%) | .97 |
| Bypass surgery | 9 (2%) | 9 (1%) | .08 |
| Neither | 67 (18%) | 168 (19%) | .54 |

Acute hyperglycemia was defined as plasma glucose of >11 mmol/L at admission, regardless of the diabetic status. *ACE*, Angiotensin-converting enzyme; *ARB*, angiotensin receptor blocker.

**Table II.** Baseline characteristics of patients with and those without diabetes mellitus

| | Diabetes mellitus | | |
|---|---|---|---|
| | Present (n = 397) | Absent (n = 856) | P value |
| Age (y) | 67 ± 11 | 69 ± 13 | .007 |
| Men | 273 (69%) | 611 (71%) | .35 |
| Hypertension | 255 (64%) | 443 (52%) | <.001 |
| Previous angina | 128 (32%) | 314 (37%) | .12 |
| Previous infarction | 70 (18%) | 96 (11%) | .002 |
| Plasma glucose at admission (mmol/L) | 13.2 ± 5.6 | 8.7 ± 3.4 | <.001 |
| Time to admission (h) | 6.5 ± 8.9 | 6.8 ± 9.0 | .70 |
| Killip classes 2 to 4 | 102 (26%) | 174 (20%) | .03 |
| ST elevation | 322 (81%) | 731 (85%) | .06 |
| Medication before infarction | | | |
| Aspirin | 60 (15%) | 71 (8%) | <.001 |
| ACE inhibitor | 37 (9%) | 42 (5%) | .004 |
| ARB | 14 (4%) | 17 (2%) | .11 |
| β-Blocker | 22 (6%) | 41 (5%) | .57 |
| Calcium-channel blocker | 112 (28%) | 196 (23%) | .04 |
| Nicorandil | 21 (5%) | 25 (3%) | .04 |
| Statin | 45 (11%) | 41 (5%) | <.001 |
| Any of above medications | 157 (40%) | 258 (30%) | .001 |
| Oral hypoglycemic drug | 172 (43%) | 0 (0%) | <.001 |
| Insulin | 66 (17%) | 0 (0%) | <.001 |
| Reperfusion therapy | | | |
| Thrombolysis | 116 (29%) | 216 (25%) | .14 |
| Balloon angioplasty | 59 (15%) | 114 (13%) | .46 |
| Stent | 206 (52%) | 519 (61%) | .004 |
| Bypass surgery | 9 (2%) | 9 (1%) | .10 |
| Neither | 77 (19%) | 158 (18%) | .69 |

Diabetes mellitus was defined as previous or current diagnosis of diabetes mellitus regardless of the glycemic status at admission.

A second objective of this study was to ascertain whether acute hyperglycemia was associated with no-reflow phenomenon during PCI for AMI.

# Methods

## Patients

The JACSS is a retrospective observational multicenter study conducted at 35 medical institutions.[13] Between January 2001 and December 2001, 1640 consecutive patients who were admitted to the participating institutions within 48 hours after the onset of AMI were enrolled in the JACSS. Plasma glucose was measured at the time of hospital admission in 1253 (76%) patients, who constituted the current study group. Acute myocardial infarction was defined by a combination of 2 of the following 3 characteristics: chest pain consistent with ongoing myocardial ischemia persisting longer than 30 minutes, ischemic electrocardiographic changes, and peak creatine kinase value more than twice the normal upper limit.

Acute hyperglycemia was defined as plasma glucose of >11 mmol/L (198 mg/dL) at admission, regardless of the diabetic status. Patients were thought to have diabetes mellitus if they had previous or current diagnosis of diabetes mellitus, regardless of the glycemic status at admission. The study protocol was reviewed and approved by the ethical committee of each participating institution.

## Coronary angiography and PCI

Percutaneous coronary intervention was performed as reperfusion therapy in 898 (72%) patients: coronary stent in 725 (58%) patients and conventional balloon angioplasty in 17 (14%) patients. The allocation of coronary angiography and reperfusion therapy was determined by physician's decision. The perfusion status of the infarct artery was assessed in accordance with the TIMI study classification.[14] Angiographic no-reflow was thought to be present if the perfusion of the infarct artery was TIMI-0 to TIMI-2 flow during PCI, despite th absence of stenosis of >50%, flow-limiting coronary dissection or hypotension. Treatment of no-reflow, including intracoronary infusion of vasodilators, depended on the physician's decision.[15] Final TIMI flow grade was assessed on the final shc of the acute angiography.

## End points

The primary end point was all-cause inhospital mortality. Other important clinical outcomes, including cardiac death, reinfarction, unstable angina, heart failure, and stroke, were also assessed during hospitalization. In patients who under-

**Table III.** The incidence of inhospital mortality and MACE

| | Acute hyperglycemia | | | Diabetes mellitus | | |
|---|---|---|---|---|---|---|
| | Present (n = 378) | Absent (n = 875) | P value | Present (n = 397) | Absent (n = 856) | P value |
| Death | 60 (16%) | 50 (6%) | <.001 | 32 (8%) | 78 (9%) | .54 (ns) |
| MACE | 76 (20%) | 84 (10%) | <.001 | 56 (14%) | 104 (12%) | .34 (ns) |

*MACE*, Major adverse cardiac events including cardiac death, reinfarction, unstable angina, heart failure, and stroke; *ns*, not significant.

went PCI as reperfusion therapy, appearance of angiographic no-reflow during PCI was reported.

### Data analysis

Statistical analysis was performed with the $\chi^2$ test for categorical variables. The *t* test and analysis of variance were used for continuous variables. To assess the relationship between plasma glucose level and mortality, Cox proportional hazards regression model was used, and odds ratio (OR) and 95% CI were obtained. In this analysis, plasma glucose was used as a continuous variable. Multivariate analysis was performed adjusting diabetes mellitus, age, sex, hypertension, previous angina, previous infarction, time to admission, Killip class, ST elevation, use of cardiovascular medication before AMI, and PCI as reperfusion therapy. Differences were considered significant if the *P* value was <.05.

## Results

### Patient characteristics

Acute hyperglycemia was associated with older age, more women, more diabetes mellitus, more Killip class ≥2, less previous angina, and shorter time from the onset of AMI to admission (Table I). Diabetes mellitus was associated with younger age, more hypertension, more Killip class ≥2, more previous infarction, higher plasma glucose on admission, and less stent implantation (Table II).

There was no significant difference in medications before AMI between patients with acute hyperglycemia and patients without, except for more use of oral hypoglycemic drugs and insulin in patients with acute hyperglycemia. The use of cardiovascular medications was significantly more frequent in diabetic patients than in nondiabetic patients.

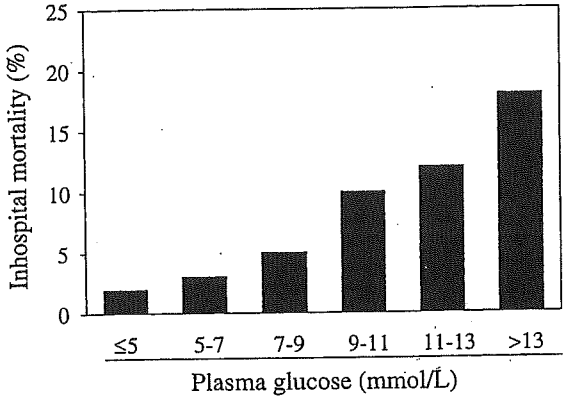
Hemoglobin A1c was measured during hospitalization in 561 (45%) patients. Hemoglobin A1c was 5.4% ± 0.5% in nondiabetic patients without acute hyperglycemia, 5.7% ± 0.8% in nondiabetic patients with acute hyperglycemia, 6.4% ± 1.1% in diabetic patients without acute hyperglycemia, and 8.0% ± 1.7% in diabetic patients with acute hyperglycemia ($P < .001$).

### Inhospital outcomes

The inhospital mortality rate was significantly higher in patients with acute hyperglycemia than in patients without (Table III). Major adverse cardiovascular events, including cardiac death, reinfarction, unstable angina, heart failure and stroke, occurred more frequently in patients with acute hyperglycemia. The inhospital mortality increased as plasma glucose increased (Figure 1). An increase of 1 mmol/L (18 mg/dL) in plasma glucose was associated with an increase in mortality risk of 12% in univariate analysis (OR 1.12, 95% CI [1.08-1.16], $P < .001$) and 10% in multivariate analysis (OR 1.10, 95% CI [1.05-1.15], $P < .001$). On the contrary, there was no significant difference in the inhospital mortality rate and the major adverse cardiovascular events rate between diabetic and nondiabetic patients. Inhospital mortality of patients with acute hyperglycemia was twice as high as mortality of patients with diabetes mellitus (16% vs 8%). Acute hyperglycemia was associated with a higher inhospital mortality rate both in nondiabetic patients (24% vs 6%, $P < .001$) and in diabetic patients (10% vs 5%, $P = .039$).

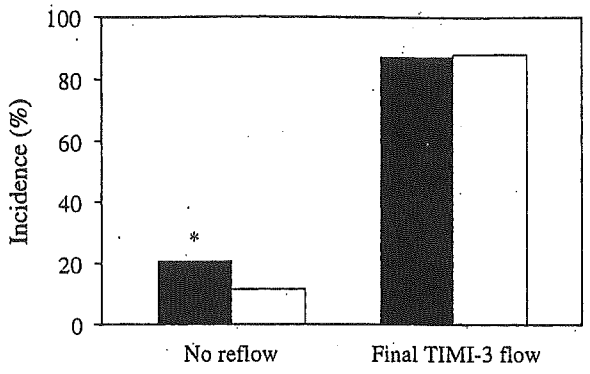
Peak creatine kinase was obtained in 1187 (94%) patients. Peak creatine kinase was significantly higher in

**Figure 1**



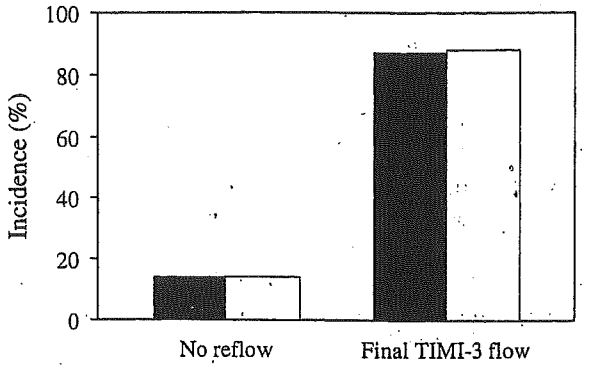

The inhospital mortality rate increased as plasma glucose increased (2% in patients with plasma glucose ≤5 mmol/L, 3% in patients with plasma glucose 5 to 7 mmol/L, 5% in patients with plasma glucose 7 to 9 mmol/L, 10% in patients with plasma glucose 9 to 11 mmol/L, 12% in patients with plasma glucose 11 to 13 mmol/L, and 18% in patients with plasma glucose >13 mmol/L; $P < .001$).

**Figure 2**




During coronary intervention, the incidence of angiographic no-reflow was more frequent in patients with acute hyperglycemia (black bars) than in patients without acute hyperglycemia (white bars). However, the incidence of final TIMI-3 was not different, *$P < .001$.

**Figure 3**




There was no significant difference in the incidence of angiographic no-reflow and final TIMI-3 flow between patients with diabetes mellitus (black bars) and patients without diabetes mellitus (white bars).

patients with acute hyperglycemia than in patients without (3176 ± 2945 vs 2698 ± 2557 IU/L, $P = .005$). However, there was no significant difference in peak creatine kinase between diabetic and nondiabetic patients (2695 ± 2580 vs 2905 ± 2730 IU/L, $P = .21$).

### Angiographic no-reflow during PCI

See Figures 2 and 3. Among 898 patients who underwent PCI, angiographic no-reflow occurred in 128 (14%) patients during the procedure. Angiographic no-reflow was associated with a higher inhospital mortality rate (12% vs 5%, $P = .01$), a higher major adverse cardiovascular events rate (18% vs 9%, $P = .003$), and higher peak creatine kinase (4094 ± 3575 vs 2915 ± 2569 IU/L, $P < .001$). The incidence of angiographic no-reflow was significantly higher in patients with acute hyperglycemia than in patients without (21% vs 12%, $P < .001$) but was not different between diabetic and nondiabetic patients (14% vs 15%, $P = .71$). Acute hyperglycemia was associated with angiographic no-reflow both in nondiabetic patients (26% vs 12%, $P < .001$) and in diabetic patients (17% vs 9%, $P = .036$). The incidence of final TIMI-3 flow was not different regardless of the presence or absence of acute hyperglycemia (87% vs 88%, $P = .84$) or diabetes mellitus (87% vs 88%, $P = .73$).

## Discussion

Although it has been demonstrated that increased plasma glucose at admission is associated with adverse outcome after AMI in the reperfusion era, most of these study patients were treated with thrombolysis, and there were few data on patients undergoing primary PCI.[6,7,16,17] Recently, Wahab et al[7] have reported that plasma glucose is an independent predictor of mortality after AMI in the thrombolytic era. However, only 34% of the study patients underwent thrombolytic therapy, and PCI was performed in <10% of the patients. JACSS is a contemporary multicenter study in which >70% of the patients underwent PCI as reperfusion therapy. We used the same definition of acute hyperglycemia as did Wahab et al, so that our result could be compared with previous findings. This study showed that acute hyperglycemia was associated with adverse inhospital outcome after AMI in the contemporary PCI era.

It remains controversial whether acute hyperglycemia predisposes to adverse outcome or is simply a consequence of large infarct size. A higher incidence of Killip class ≥2 suggests that acute hyperglycemia may reflect extensive myocardial damage. However, recent experimental studies have suggested that hyperglycemia per se exacerbates myocardial damage in AMI. Hyperglycemia increases interstitial fibrosis and myocyte apoptosis that exaggerate left ventricular remodeling.[18] Also, hyperglycemia abolishes the cardioprotective effect of ischemic preconditioning by closing $K_{ATP}$ channels.[19,20] A recent clinical study reported that acute hyperglycemia was associated with impaired predischarge left ventricular ejection fraction in patients with AMI, independently of acute left ventricular ejection fraction.[21]

Another potential mechanism for the association between acute hyperglycemia and adverse outcome is microvascular dysfunction. Experimental studies have reported that hyperglycemia aggravates platelet-dependent thrombosis, increases circulating adhesion molecules that augment capillary leukocyte plugging, attenuates endothelium-dependent vasodilation, and reduces collateral blood flow by adversely affecting nitric oxide availability.[22-25] These changes impair microvascular function. Recently, Iwakura et al[12]

reported that hyperglycemia was associated with no-reflow phenomenon on myocardial contrast echocardiography in patients with angiographically successful reperfusion after PCI. No-reflow phenomenon is a strong predictor for adverse outcome after AMI.[10,11] We also showed that angiographic no-reflow occurred more frequently in patients with acute hyperglycemia, suggesting that impaired microvascular function might have contributed to adverse outcome after AMI in patients with acute hyperglycemia.

Interestingly, there was no significant difference in inhospital outcome between diabetic and nondiabetic patients. Previous studies have demonstrated that diabetes mellitus is associated with adverse outcome after thrombolysis for AMI.[26,27] In several studies that used noninvasive indices, reperfusion was achieved less frequently after thrombolysis in patients with diabetes mellitus than in patients without.[28] Angeja et al[29] reported that diabetes mellitus was associated with less complete ST-segment resolution after thrombolysis, even in patients with TIMI-3 flow. However, the incidence of complete ST-segment resolution was similar between diabetic and nondiabetic patients after PCI. More effective reperfusion by PCI, as compared with thrombolysis, may improve outcome of diabetic patients. Recent studies have reported that diabetes mellitus did not increase short-term mortality after AMI,[30-32] especially in non-insulin-requiring patients with diabetes.[33] Lower incidence of non-insulin-requiring patients with diabetes in this study (83% of diabetic patients) may also account for relatively favorable outcome of patients with diabetes mellitus. In addition, the use of cardiovascular medications before admission for the index episode of AMI was more frequent in diabetic patients than in nondiabetic patients. Pharmacological cardiovascular prevention might have offset the adverse effect of diabetes mellitus on short-term outcome after AMI.[30]

In this study, mortality was higher in nondiabetic patients with acute hyperglycemia than in diabetics with acute hyperglycemia. Although experimental studies have reported that diabetic hearts are tolerant to ischemia in some conditions,[33] it is unclear whether it may occur in human beings. One possible interpretation of this data is that unrecognized diabetes mellitus is a marker of adverse outcome in patients with AMI. However, the mean value of hemoglobin A1c of nondiabetic patients with acute hyperglycemia was significantly lower than that of diabetic patients with acute hyperglycemia ($P < .001$). Recent studies have reported that plasma glucose at admission is associated with increased mortality even after adjustment of hemoglobin A1c.[34] It is thus unlikely that the adverse outcome of nondiabetic patients with acute hyperglycemia is as a result of chronic hyperglycemia of undiagnosed diabetes mellitus.

This is a retrospective and observational study. However, it included all consecutive patients who were admitted to the participating institutions during the first year of the new millennium. Patients received contemporary management and >70% of the patients underwent PCI. Plasma glucose at admission was reported only in 76% of the patients enrolled in the JACSS. However, there was no significant difference in the incidence of diabetes mellitus (32% vs 28%, $P = .31$) and the inhospital mortality rate (9% vs 11%, $P = .34$) between patients with measurement of plasma glucose at admission and patients without. Because diabetes mellitus was defined as previous or current diagnosis of diabetes mellitus at the time of hospital admission, some of diabetic patients may not have been diagnosed as such. Oral glucose tolerance test was not usually performed during hospitalization, and it was not assessed how often nondiabetic patients with acute hyperglycemia at admission had newly diagnosed diabetes mellitus by the time of hospital discharge. We did not assess microvascular flow by using myocardial blush grade or TIMI frame count, which might have provided additional information.

In conclusion, acute hyperglycemia, but not diabetes mellitus, was associated with inhospital mortality after AMI in the PCI era. Angiographic no-reflow occurred more frequently during PCI in patients with acute hyperglycemia, suggesting that microvascular dysfunction might have contributed to adverse outcome of these patients.

## References

1. Oswald GA, Corcoran S, Yudkin JS. Prevalence and risk of hyperglycemia and undiagnosed diabetes in patients with acute myocardial infarction. Lancet 1984;1:1264-7.
2. Lakhdar A, Stromberg P, McAlpine SG. Prognostic importance of hyperglycemia induced by stress after acute myocardial infarction. BMJ 1984;288:288.
3. Bellodi G, Manicardi V, Malavasi V, et al. Hyperglycemia and prognosis of acute myocardial infarction in patients without diabetes mellitus. Am J Cardiol 1989;64:885-8.
4. O'Sullivan JJ, Conroy RM, Robinson K, et al. Inhospital prognosis of patients with fasting hyperglycemia after first myocardial infarction. Diabetes Care 1991;14:758-60.
5. Fava S, Aquilina O, Azzopardi J, et al. The prognostic value of blood glucose in diabetic patients with acute myocardial infarction. Diabet Med 1996;13:80-3.
6. Capes SE, Hunt D, Malmberg K, et al. Stress hyperglycemia and increased risk after myocardial infarction in patients without diabetes: a systematic overview. Lancet 2000;355:773-8.
7. Wahab NN, Cowden EA, Pearce NJ, et al. On behalf of the ICONS investigators. Is blood glucose an independent predictor of mortality in acute myocardial infarction in the thrombolytic era? J Am Coll Cardiol 2002;40:1748-54.
8. Keeley EC, Boura JA, Grines CL. Primary coronary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. Lancet 2003;361:13-20.

9. Zhu MM, Feit A, Chadow H, et al. Primary stent implantation compared with primary balloon angioplasty for acute myocardial infarction: a meta-analysis of randomized clinical trials. Am J Cardiol 2001;88:297-301.
10. Ito H, Maruyama A, Iwakura K, et al. Clinical implications of the 'no reflow' phenomenon. A predictor of complications and left ventricular remodeling in reperfused anterior wall myocardial infarction. Circulation 1996;93:223-8.
11. Morishima I, Sone T, Okumura K, et al. Angiographic no-reflow phenomenon as a predictor of adverse long-term outcome in patients treated with percutaneous transluminal coronary angioplasty for first acute myocardial infarction. J Am Coll Cardiol 2000;36:1202-9.
12. Iwakura K, Ito H, Ikushima M, et al. Association between hyperglycemia and the no-reflow phenomenon in patients with acute myocardial infarction. J Am Coll Cardiol 2003;41:1-7.
13. Kosuge M, Kimura K, Kojima S, et al. On behalf of Japanese Acute Coronary Syndrome Study (JACSS) investigators. Effects of pre-infarction angina pectoris on infarct size and inhospital mortality after coronary intervention for acute myocardial infarction. Am J Cardiol 2003;92:840-3.
14. The TIMI study group. The Thrombolysis in Myocardial Infarction (TIMI) trial. N Engl J Med 1985;312:932-6.
15. Ishihara M, Sato H, Tateishi H, et al. Attenuation of the no-reflow phenomenon after coronary angioplasty for acute myocardial infarction with intracoronary papaverine. Am Heart J 1996;132:959-63.
16. Sranders I, Diammant M, van Gelder RE, et al. Admission blood glucose level as risk indicator of death after myocardial infarction in patients with and without diabetes mellitus. Arch Intern Med 2004;164:982-8.
17. Wong VW, Ross DL, Park K, et al. Hyperglycemia: still an important predictor of adverse outcomes following AMI in the reperfusion era. Diabetes Res Clin Pract 2004;64:85-91.
18. Shiomi T, Tsutsui H, Ikeuchi M, et al. Streptozotocin-induced hyperglycemia exacerbates left ventricular remodeling and failure after experimental myocardial infarction. Circulation 2003;42:165-72.
19. Ishihara M, Inoue I, Kawagoe T, et al. Effect of acute hyperglycemia on the ischemic preconditioning effect of prodromal angina pectoris in patients with an anterior wall first acute myocardial infarction. Am J Cardiol 2003;92:288-91.
20. Kersten JR, Montgomery MW, Ghanssemi T, et al. Diabetes and hyperglycemia impair activation of mitochondrial KATP channels. Am J Physiol Heart Circ Physiol 2001;280:H1744-50.
21. Ishihara M, Inoue I, Kawagoe T, et al. Impact of acute hyperglycemia on left ventricular function after reperfusion therapy in patients with a first anterior wall acute myocardial infarction. Am Heart J 2003;146:674-8.
22. Scechter M, Merz NB, Paul-Labrador MJ, et al. Blood glucose and platelet-dependent thrombosis in patients with coronary artery disease. J Am Coll Cardiol 2000;35:300-7.
23. Marfella R, Esposito K, Giunta R, et al. Circulating adhesion molecules in humans: role of hyperglycemia and hyperinsulinemia. Circulation 2000;101:2247-51.
24. Title LM, Cummings PM, Giddens K, et al. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. J Am Coll Cardiol 2000;36:2185-91.
25. Kersten JR, Toller WG, Tessmer JP, et al. Hyperglycemia reduces coronary blood flow through a nitric oxide–mediated mechanism. Am J Physiol Heart Circ Physiol 2001; 281:H2097-104.
26. Mak KH, Moliterno DJ, Granger CB, et al. For the GUSTO-I investigators. Influence of diabetes mellitus on clinical outcomes in the thrombolytic era of acute myocardial infarction. J Am Coll Cardiol 1997;30:171.
27. Ishihara M, Sato H, Kawagoe T, et al. Impact of diabetes mellitus on long-term survival after acute myocardial infarction in patients with single vessel disease. Heart 2001;86:133-8.
28. Zairis MN, Handanis SM, Lyras AG, et al. Type 2 diabetes and intravenous thrombolysis outcome in the setting of ST elevation myocardial infarction. Diabetes Care 2004;27:971-6.
29. Angeja BG, de Lemos J, Murphy SA, et al. Impact of diabetes mellitus on epicardial and microvascular flow after fibrinolytic therapy. Am Heart J 2002;144:649-56.
30. Löndahl M, Katzman P, Nilsson A, et al. Cardiovascular prevention before admission reduces mortality following acute myocardial infarction in patients with diabetes. J Intern Med 2002;251:325-30.
31. Süselbeck T, Tuerkoglu A, Kralev S, et al. Diabetes mellitus is not a predictor of short- and long-term outcome in patients with acute coronary syndromes undergoing primary coronary intervention with concomitant glycoprotein IIb/IIIa inhibitors. Circulation [abstract] 2003;108:IV732-3.
32. Antoniucci D, Valenti R, Migliorini A, et al. Impact of insulin-requiring diabetes mellitus on effectiveness of reperfusion and outcome of patients undergoing primary percutaneous coronary intervention for acute myocardial infarction. Am J Cardiol 2004;93:1170-2.
33. Handour G, Ferrera R, Sebbag L, et al. Improved myocardial tolerance to ischemia in the diabetic rabbit. J Mol Cell Cardiol 1998;30:1869-75.
34. Hadjadji S, Coisne D, Mauco G, et al. Prognostic value of admission plasma glucose and HbA1c in acute myocardial infarction. Diabet Med 2004;21:305-10.

## Appendix A

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# Successful Excision of a Cystic Tumor of the Atrioventricular Nodal Region

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Cystic tumor of the atrioventricular nodal region is a rare cardiac primary tumor that can cause heart blockage and sudden death. Antemortem diagnosis and successful excision of the atrioventricular nodal region are extremely rare. A 45-year-old woman who presented with palpitations is reported. Electrocardiography revealed first-degree atrioventricular block. Echocardiography, computed tomography, and magnetic resonance imaging scans revealed a cystic mass attached to the interatrial septum. Complete surgical excision of the mass was achieved, although placement of a permanent pacemaker was required for complete heart blockage. Histopathological examination revealed the mass to be a cystic tumor of the atrioventricular nodal region. A 5-year follow-up has revealed no sign of recurrence. (*Circ J* 2005; **69:** 1293–1294)

**Key Words:** Atrioventricular node; Cystic tumor; Heart block; Pacemaker

Cystic tumor of the atrioventricular nodal region is a rare primary cardiac tumor. It can cause various degrees of heart blockage, and is the smallest tumor capable of causing sudden death[1–3] Although there have been approximately 70 reported cases of atrioventricular nodal region in published reports to date, most were diagnosed postmortem. Antemortem diagnosis and successful excision of this type of tumor are extremely rare. Here we report a case of cystic tumor of the atrioventricular nodal region in which the tumor was detected preoperatively and successfully excised.

## Case Report

A 45-year-old woman who presented with dyspnea on effort and palpitations visited her primary care physician. A resting electrcardiogram (ECG) revealed sinus rhythm and first-degree atrioventricular block. Sporadic ventricular paroxysmal contraction was found on Holter ECG. Because echocardiography showed an intracardiac tumor, she was referred to our institute for surgical evaluation. Transesophageal echocardiography and computed tomography (Fig 1A) revealed a 28–27 mm circular tumor with no stalk on the interatrial septum (IAS)[4] On magnetic resonance imaging (MRI), the tumor was of high intensity on $T_1$-weighted images and isointense with myocardium on $T_2$-weighted images (Fig 1B).

She underwent surgery to resect the tumor. Under a median sternotomy and standard cardiopulmonary bypass with warm blood cardioplegia, the right atrium was opened, to reveal a 30 mm round cyst attached to the IAS in the area of the triangle of Koch. The cyst was incised and yellow caseous material was found within it. Rapid cytodiagnosis was done and revealed neither malignant cells nor bacteria in the fluid. The IAS at the portion of the

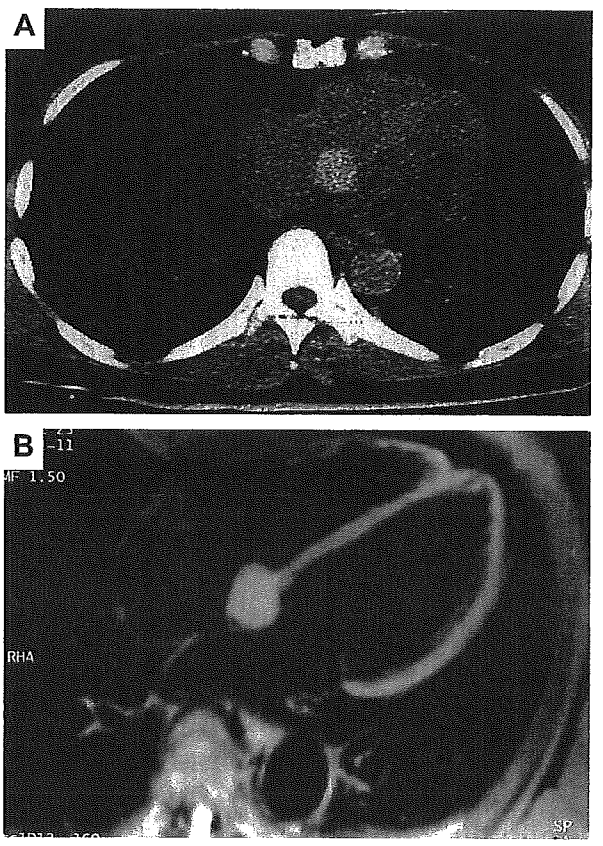


Fig 1. (A) Computed tomography scan and (B) magnetic resonance imaging showing intracardiac tumor with a broad connection to the interatrial septum.

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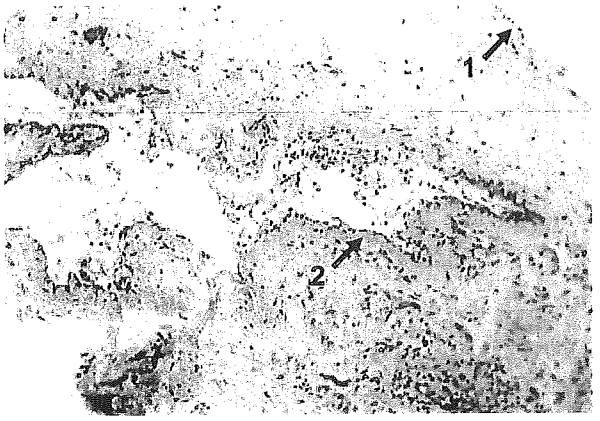


Fig 2. Photomicrograph (hematoxylin–eosin staining, ×400) of the cyst wall showing the large cyst lining of the squamous epithelium (arrow 1), and within the fibrous wall of the large cyst, a smaller cyst lined by similar squamous epithelium (arrow 2) is shown.

atrioventricular node became a defect after the wall of the cyst was completely resected. It was closed directly with 6-0 monofilament sutures. Complete atrioventricular block persisted after surgery and a permanent pacemaker was inserted 11 days later.

Histopathological examination revealed that the cyst wall was composed of fibrous connective tissue covered by a layer of squamous epithelium with partial cornification[4] Within the fibrous tissue were smaller cysts lined by a similar epithelium (Fig 2). Immunohistochemical staining showed that the cells of the cyst expressed carcinoembryonic antigen and epithelial membrane antigen, suggesting that they were of endodermal origin.

The patient's recovery was uneventful and her symptoms completely disappeared. A 5-year follow-up has revealed no evidence of recurrence of the tumor.

## Discussion

Cystic tumor of the atrioventricular nodal region was first described in 1911[5] It is a rare primary cardiac tumor located in the region of the atrioventricular node. According to previous reports[1–3,6] it can cause variable degrees of heart blockage, which gradually evolve to complete heart blockage. Moreover, ventricular tachycardia or fibrillation sometimes occurs in patients with this tumor[2,3,6] Tumor size varies from 0.5 mm to 30 mm, and there is no relationship between size and the occurrence of lethal arrhythmia. It is thus important to consider the possibility of cystic tumor of the atrioventricular nodal region in patients with electrocardiographic evidence of heart block limited to the atrioventricular node (ie, with a narrow QRS), particularly in women[2]

This tumor is thought to be of embryologic origin and not a true neoplasm because of its benign histological appearance and invariable location in the atrioventricular area. The atrioventricular nodal region is an area of embryologic fusion, suggesting that either mesothelium or nearby foregut inclusion could be incorporated in it. The immunohistochemical findings in this case, which corresponded with those of other recently reported cases[1,6–9] suggested that the tumor was an endodermal and not mesothelial remnant.

The cause of lethal arrhythmia in patients with cystic tumor of the atrioventricular nodal region is uncertain. Complete heart blockage or associated dysfunction of the sinus node might cause excessive distention of the ventricle and lead to ventricular fibrillation. However, this hypothesis does not explain why lethal arrhythmia often occurs just after the beginning of electronic pacing[10]

The first case of successful resection of this tumor was reported in 1992 by Balasundaram et al[7] In their case, the atrioventricular node tumor was only 0.5 mm in diameter and was an unexpected finding associated with an ostium secundum atrial septal defect. Paniagua et al reported the first case in which this type of tumor was detected preoperatively by echocardiography and MRI[8] Kaminishi et al recently reported the successful prevention of heart blockage by leaving the cyst wall attached to the base of the IAS[9] There was no sign of residual mass or recurrence 12 months after surgery. It is controversial whether the cyst should be resected completely from the base of the IAS. However, because one complication of this tumor is sudden death as a result of ventricular tachycardia or ventricular fibrillation[2,3,6] we believe that complete resection is essential, even if subsequent pacemaker implant is required.

## References

1. Bruke AP, Anderson PG, Virmani R, James TN, Herrera GA, Ceballos R. Tumor of the atrioventricular nodal region. *Arch Pathol Lab Med* 1990; **114:** 1057–1062.
2. Nishida K, Kamijima G, Nagayama T. Mesothelioma of the atrioventricular node. *Br Heart J* 1985; **53:** 468–470.
3. Travers H. Congenital polycystic tumor of the atrioventricular node: Possible familial occurrence and critical review of reported cases with special emphasis on histogenesis. *Hum Pathol* 1982; **13:** 25–35.
4. Nojima Y, Ishibashi-Ueda H, Yamagishi M. Cystic tumor of the atrioventricular node. *Heart* 2003; **89:** 122.
5. Armstrong H, Monckeberg JG. Herzblock, bedingt druch primaren Herztumor, bei einem 5 jahrigen Kinde. *Dtsch Arch Klin Med* 1911; **102:** 144–166.
6. Monma N, Sotodate R, Tashiro A, Segawa I. Origin of so-called mesothelioma of the atrioventricular node. *Arch Pathol Lab Med* 1991; **115:** 1026–1029.
7. Balasundaram S, Halees SA, Duran C. Mesothelioma of the atrioventricular node: First successful follow-up after excision. *Eur Heart J* 1992; **13:** 718–719.
8. Paniagua JR, Sadaba JR, Davidson LA, Munsch CM. Cystic tumor of the atrioventricular nodal region: Report of a case successfully treated with surgery. *Heart* 2000; **83:** E6.
9. Kaminishi Y, Watanabe Y, Nakata H, Shimokama T, Jikuya T. Cystic tumor of the atrioventricular nodal region. *Jpn J Thorac Cardiovasc Surg* 2002; **50:** 37–39.
10. James TN, Galakhov I. Fatal electrical instability of the heart associated with benign congenital polycystic tumor of the atrioventricular node. *Circulation* 1977; **56:** 667–678.

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# Elevation of Plasma Matrix Metalloproteinase-9 in the Culprit Coronary Artery in Patients With Acute Myocardial Infarction

## —— Clinical Evidence From Distal Protection ——

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**Background** Although the elevation of circulating plasma matrix metalloproteinase (MMP)-9 levels in patients with acute myocardial infarction (AMI) has been documented, the origin of MMP-9 remains unclear.
**Methods and Results** Plasma MMP-9 levels in both the peripheral circulation and coronary arteries were measured in patients with AMI (n=23) and with stable angina pectoris (SAP, n=10) during percutaneous coronary intervention (PCI) with a distal protection device. Blood samples were collected from the femoral artery (FA) and the coronary artery before (Initial) and after (Second) dilation of the culprit lesion. Coronary sinus blood samples were obtained immediately after PCI (n=7). Coronary artery plaque fragments were aspirated in patients with AMI (n=20) and compared with those from patients with SAP who underwent directional atherectomy (n=10). MMP-9 levels in Initial and Second were significantly higher in patients with AMI than in patients with SAP ($p<0.01$). In AMI patients MMP-9 levels were significantly higher in Initial than in the FA ($p<0.05$), and were further increased in Second ($p<0.0001$), whereas those in the coronary sinus were similar to the FA. Immunohistochemistry revealed augmented MMP-9 expression in the coronary artery plaque fragments from AMI patients.
**Conclusions** MMP-9 is mainly released into the coronary circulation from the coronary artery plaque in patients with AMI. (*Circ J* 2005; **69:** 1180–1185)

**Key Words:** Acute myocardial infarction; Coronary intervention; Matrix metalloproteinase

Matrix metalloproteinase (MMP), an extracellular matrix degrading enzyme, plays a crucial role in the breakdown of the fibrous cap of plaque and subsequent rupture in the pathogenesis of acute coronary syndrome (ACS).[1,2] The MMP family has been identified in the shoulder regions of human atherosclerotic plaque,[3] and is more frequently expressed in the coronary plaque of patients with ACS than with stable angina pectoris (SAP).[4] MMP-9 (92-kDa gelatinase) is associated with atherosclerotic arterial remodeling[5] and is actively synthesized in vulnerable plaque.[6] MMP-9 affects plaque stability in association with various inflammatory cytokines.[7,8] Elevated plasma levels of MMP-9 in the peripheral blood have been shown in patients with ACS,[9] and are associated with severe coronary stenosis[10] and cardiovascular mortality.[11] In addition, the plasma MMP-9 level is elevated in the coronary circulation of patients with ACS,[12,13] indicating that the production of MMP-9 may be enhanced in ACS.

MMP release, however, may also occur in the interstitium of ischemic myocardium in the early period after a myocardial infarction (MI),[14] as well as from coronary plaque. The serum MMP concentration is associated with left ventricular remodeling after MI,[15] and in particular, an elevated plasma MMP-9 level is associated with infarct size.[16,17] Hence, whether the main source of increased plasma MMP-9 is the culprit coronary plaque or the infarcted myocardium still remains unclear.

The aim of this study was to determine the major source of plasma MMP-9 in patients with early phase acute MI (AMI) by measuring the plasma levels of MMP-9 in the coronary artery as well as a peripheral artery of patients with AMI and in those with SAP during percutaneous coronary intervention (PCI) using a distal protection device. We also investigated in situ MMP-9 expression in coronary artery plaque aspirated from patients with AMI and compared it with samples obtained by directional coronary atherectomy (DCA) in patients with SAP.

## Methods

*Patients*

We studied 23 consecutive patients with AMI within 24 h from the onset of chest pain (6 women; age range 44–87 years, 64±12 years) and 10 consecutive patients with SAP as the control (5 women; age range 50–71 years, 66±10 years, $p=0.61$ vs AMI). The diagnosis of AMI was determined on the basis of chest pain lasting longer than 30 min with ST-segment elevation >2 mm in at least 2

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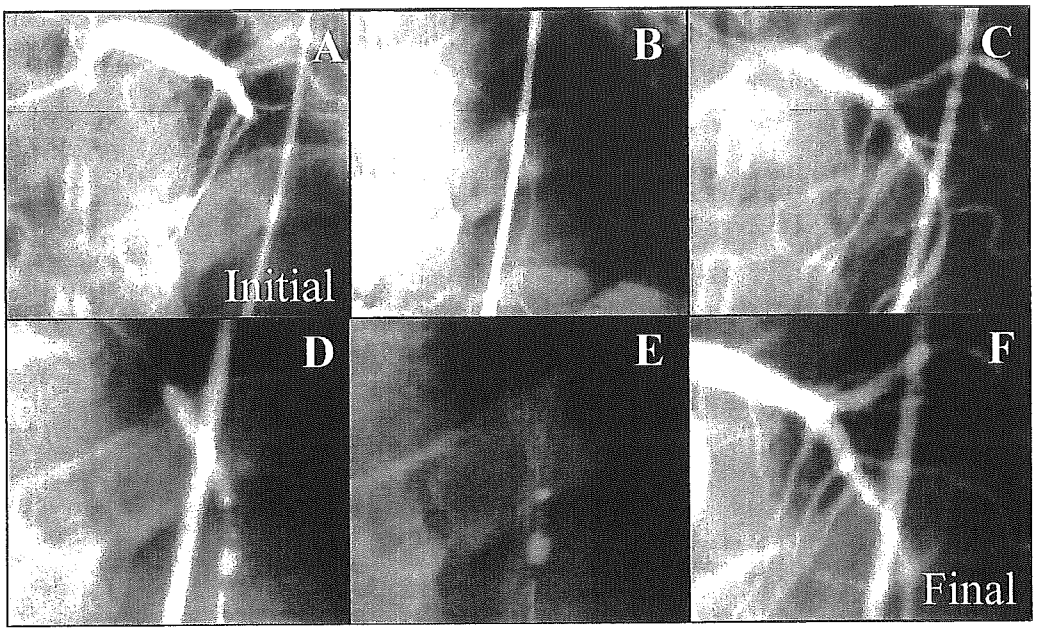


Fig 1. Serial coronary arteriograms showing the intervention procedure. (A) Complete occlusion of the left anterior descending coronary artery. (B) Coronary guidewire crossing the culprit lesion, followed by thrombus aspiration with an aspiration catheter (Initical blood sampling). (C) Coronary arteriogram immediately after the initial aspiration. Thrombolysis In Myocardial Infarction 3 flow grade was obtained in 21 of 23 cases (91%). (D) PercuSurge GuardWire™ also crossing the lesion, parallell with the coronary guidewire. Direct stenting and/or balloon angioplasty was performed under distal protection. (E) Second aspiration (Second blood sampling) to collect material released from the disrupted plaques. (F) Final coronary arteriogram after deflation of the distal occlusion balloon shows restored coronary flow.

contiguous leads of the electrocardiogram, and with more than a 3-fold increase in serum creatine kinase level. Patients with SAP were those who complained of chest pain on exertion with evidence of myocardial ischemia in whom critical coronary stenosis was confirmed by coronary arteriography. All subjects underwent PCI under distal protection with the PercuSurge GuradWire System (Medtronic AVE). Patients with AMI were transferred to the catheterization laboratory and PCI was performed within 1 h of admission. Patients unsuitable for the distal protection procedure because of triple vessel disease, cardiogenic shock or any other serious conditions were excluded. Patients with renal failure, liver function abnormality, and those with a systemic inflammatory disease were also excluded. Oral antiplatelet therapy (200 mg/day aspirin and 200 mg/day ticlopidine) was begun after PCI unless contraindicated. The Ethics Committee of the Kansai Rosai Hospital approved the study protocol and all patients gave written informed consent before cardiac catheterization.

*Distal Protection Device*

A balloon-type temporary occlusion and aspiration system for the coronary artery (PercuSurge GuardWire™ System) was used for distal protection during PCI and blood sampling from the coronary artery. Detailed specifications of the system are described elsewhere[18,19] In brief, it consists of a guidewire with a distal occlusion balloon (GuardWire Plus), an aspiration catheter (Export), MicroSeal Adapter and EZ Flator inflating system. Guard Wire Plus comprises a 0.014-inch nitinol hypotube with an inflatable internal lumen, which is connected to the occlusion balloon (3–6 mm in diameter) at the end. Export Aspiration catheter is a 0.071-inch monorail-type aspiration catheter incorporating a 0.040-inch lumen that requires an 8Fr guide catheter. GuardWire Plus is connected to the MicroSeal Adapter and EZ Flator, which adjust the size of the distal occlusion balloon under low pressures less than 1 atm. This distal protection and aspiration system enabled us to take selective blood samples from the PCI site in the coronary artery.

*PCI With Distal Protection* Prior to PCI each patient was given 10,000 units and additional heparin was administrated during the procedure to maintain the activated clotting time >250 s. First, the culprit lesion was crossed with a 0.014-inch angioplasty guidewire and then the Export Aspiration catheter was advanced to the culprit lesion for extensive aspiration while advancing and retracting the catheter (initial aspiration: Initial). The SAP patients underwent the same procedure, although none had a total occlusive lesion. Next, the PercuSurge GuardWire was advanced beyond the lesion, parallel with the guidewire and balloon angioplasty or direct stenting was performed while the distal occlusion balloon inflated the lumen 0.5–1.0 mm larger than the diameter of the reference vessel. After satisfactory dilation had been achieved, the Export Aspiration catheter was again inserted just proximal to the distal occlusion balloon, and aspiration was repeated (second aspiration: Second). Finally, the distal balloon was deflated and antegrade coronary flow was restored. The procedure was completed by ascertaining Thrombolysis In Myocardial Infarction (TIMI) 3 coronary blood flow without any interventional complications. Additional angioplasty was performed if necessary. Patients with AMI and those with SAP underwent the similar procedure. A representative series of coronary arteriograms during the procedure are shown in Fig 1.

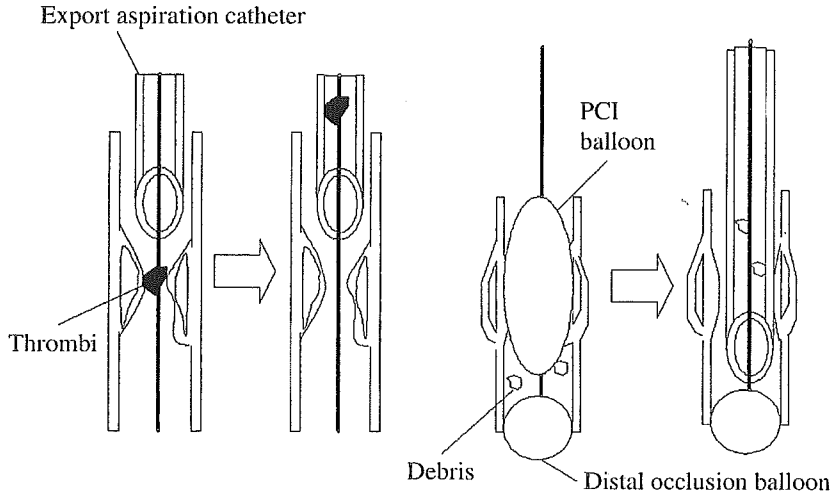


Fig 2. Schematic illustrations of the Initial (Left) and Second (Right) blood samplings. Coronary blood was initially aspirated with an Export aspiration catheter in all patients, regardless of the presence or absence of occlusive thrombi (Left). Immediately after balloon dilatation of the culprit lesion under distal protection, coronary blood, including plaque debris, was aspirated again (Right). PCI, percutaneous coronary intervention.

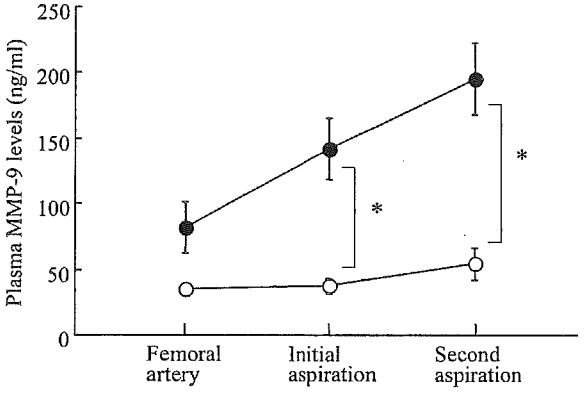


Fig 3. Comparison of plasma matrix metalloproteinase (MMP)-9 levels in patients with acute myocardial infarction (AMI) and those with stable angina pectoris (SAP) during percutaneous coronary intervention. MMP-9 levels in Initial and Second are significantly elevated in patients with AMI compared with those in patients with SAP. Data are expressed as mean ± SE. (Solid circles) Patients with AMI; (Open circles) patients with SAP. *p<0.01.

*MMP-9 Measurement*

Blood samples were obtained from the femoral artery (FA) at the beginning of PCI and during the first and second aspirations from the coronary artery (Fig 2). Blood from the coronary artery was directly collected into the collection bottle without filtration. In 7 AMI patients, a thermodilution catheter was inserted via the internal jugular vein after the PCI, and samples from the coronary sinus (CS) were collected simultaneously using a 5F Multipurpose catheter (Goodman). The blood was immediately mixed with sodium EDTA, centrifuged at 3,000 rpm for 5 min, separated and stored at −80°C. Plasma levels of MMP-9 were measured by one-step sandwich enzyme immunoassay using 2 monoclonal antibodies (Daiichi Fine Chemical).[20]

*Immunohistochemistry*

Subgroups of AMI and SAP patients were selected for immunohistochemistry. The AMI group comprised 20 patients (3 women; age range 44–82 years) from whom coronary plaque was successfully obtained during the emergency PCI. These patients underwent aggressive intracoronary aspiration using an aspiration catheter (Resque™ Thrombectomy System, Boston Scientific), and thrombi that included fragments of the coronary plaque were obtained. The SAP group comprised 10 patients (4 women; age range 55–65 years) from whom coronary plaque samples were while they underwent elective DCA.

*MMP-9 Staining* Specimens were placed in tissue fixative (Histochoice, Hedwin, Baltimore, MD, USA). After overnight fixation, they were embedded in paraffin and sectioned at 4• m intervals. Tissue sections were deparaffinized with xylene followed by immersion in a graded alcohol series. They were washed 3 times for 5 min each in phosphate-buffered saline (PBS) and blocked with bovine serum albumin for 60 min. Specimens were then incubated with primary antibodies against MMP-9 (Fuji Chemical, Tokyo, Japan) overnight at 4°C. After being washed in PBS, they were incubated with biotinylated rabbit anti-mouse IgG for 60 min at room temperature. Specimens were then washed with PBS, stained with horseradish peroxidase-conjugated streptavidin, and finally incubated with substrate solution for 1–15 min. The tissue sections were also stained with hematoxylin-eosin. MMP expression was semiquantitatively graded as 0 if there was no staining in any visual field, 1 if <25% cells were stained, 2 if <50% cells were stained and 3 if >50% cells and extracellular matrix were positive for staining. Grading of immunohistochemistry staining was performed by pathologists unaware of the patients' backgrounds.

*Statistical Analysis*

Data are expressed as mean ± SEM, unless otherwise indicated. One-way factorial analysis of variance (ANOVA) followed by Sheffe's post hoc test was used for intergroup comparisons. Two-factor ANOVA or unpaired t-test was applied to evaluate the difference between the groups. Mann-Whitney's U test was used to compare the staining grade. A probability value of less than 0.05 was considered statistically significant.

## Results

*Plasma MMP-9 Measurement*

*AMI vs SAP* Plasma MMP-9 levels were elevated at any sampling point in patients with AMI, whereas they fell within the normal range of 38±13 ng/ml[20] in patients with

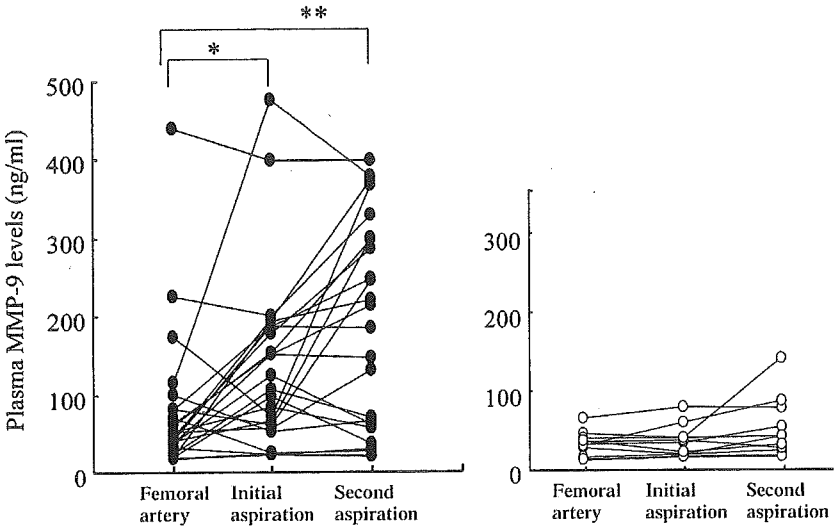


Fig 4. Individual values of plasma matrix metalloproteinase (MMP)-9. In patients with acute myocardial infarction (AMI), MMP-9 levels in Initial were significantly higher than those in the femoral artery, and further increased in Second (Left panel). In patients with stable angina pectoris (SAP), MMP-9 levels remained within the normal range throughout the percutaneous coronary intervention procedure (Right panel). (Solid circles) Patients with AMI; (Open circles) patients with SAP. *p<0.05, **p<0.0001.

SAP (Fig 3). MMP-9 levels in the coronary artery, both Initial and Second, were significantly higher in patients with AMI than in those with SAP (Initial, 141.6±23.3 ng/ml vs 37.2±6.3 ng/ml, p<0.01; Second, 194.9±27.3 ng/ml vs 54.2±12.0 ng/ml, p<0.01) although there was no statistical difference regarding the MMP levels in the systemic circulation, in FA, between patients with AMI and those with SAP.

*Variation of MMP-9 Level Among the Sampling Points*

MMP-9 levels in Initial were significantly higher than those in the FA (141.6±23.3 ng/ml vs 81.9±19.2 ng/ml, p<0.05) in patients with AMI, and they further increased in Second (194.9±27.3 ng/ml, p<0.0001 vs FA) (Fig 4). MMP-9 levels in the FA were also elevated in some patients with AMI and they remained high throughout the PCI procedure in those patients. In contrast, MMP-9 levels remained within the normal range in patients with SAP throughout the PCI procedure (Fig 4). MMP-9 levels in the CS were similar to those in the FA despite the elevation in the coronary artery, particularly in Second (Fig 5).

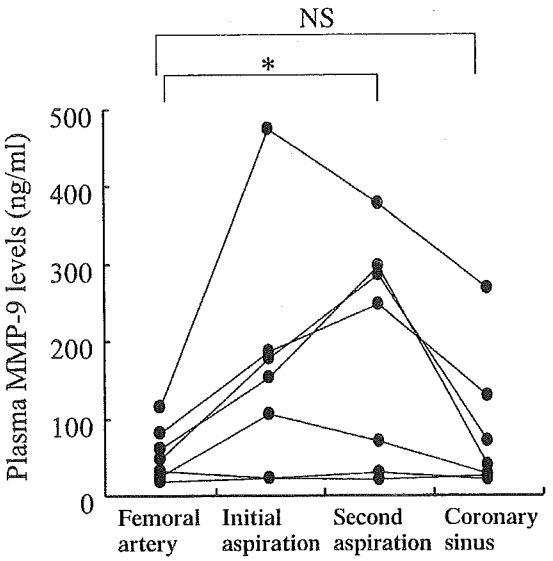


Fig 5. Comparison of matrix metalloproteinase (MMP)-9 levels in the femoral artery, coronary artery and coronary sinus in 7 patients with acute myocardial infarction. MMP-9 levels are elevated in the coronary artery, but not in the coronary sinus, compared with the femoral artery. *p<0.05.

*Immunohistochemistry*

Hematoxylin-eosin staining of the plaque samples aspirated from patients with AMI revealed both thrombi and atheromatous tissue, in which many macrophages strongly positive for MMP-9 were present (Fig 6). In contrast, specimens from patients with SAP had MMP-9 negative to moderately positive cells (Fig 6). The cell-associated staining grade for MMP-9 was 0 in 2 samples (10%), 2 in 3 samples (15%), and 3 in 15 samples (75%) in patients with AMI. Compared with 0 in 8 samples (80%), 1 in 1 sample (10%), 2 in 1 sample (10%), and no samples showing grade 3 from patients with SAP. This semiquantitative grading demonstrated that MMP-9 expression was significantly augmented in the specimens from patients with AMI (p<0.0001).

## Discussion

The present study demonstrates for the first time that the plasma levels of MMP-9 are significantly higher in the coronary artery than in the systemic circulation (FA and CS) in patients with AMI. Mechanical disruption of the culprit plaque by PCI induces a further elevation of the MMP-9 level in the coronary artery. These elevations were not observed in patients with SAP. In addition, immunohistochemistry revealed augmented MMP-9 expression in the coronary plaque from patients with AMI. Given these findings, the increase in the plasma MMP-9 level in patients with AMI is mainly attributable to the rupture of the culprit plaque in the coronary artery, rather than to the production from necrotic myocardium downstream.

Earlier immunohistochemistry studies on human coronary artery specimens obtained by directional atherectomy revealed localization of MMP-9 in the coronary artery plaque, associated with ischemic heart disease. Larger numbers of MMP-9-positive macrophages were contained within atherectomy specimens from patients with unstable angina pectoris than in those with SAP.[4] MMP-9 was shown to be actively synthesized by macrophages and

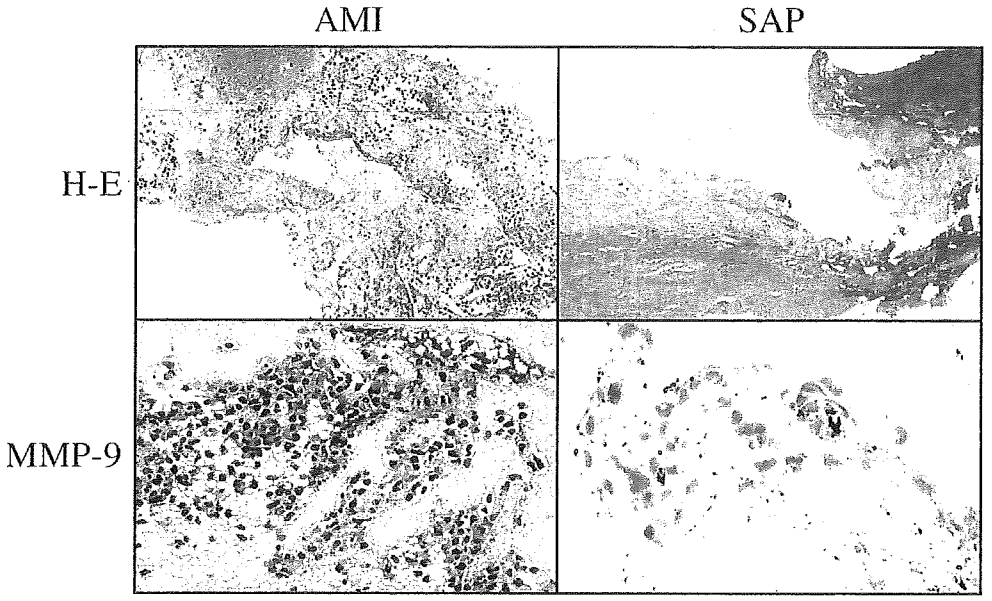


Fig 6. Histology of the aspirated specimen from a case of acute myocardial infarction (AMI) (Left) and the specimen obtained by directional atherectomy in a patient with stable angina pectoris (SAP) (Right). (Upper panels) Hematoxylin-eosin (HE) staining; (Lower panels) immunohistochemistical staining for matrix metalloproteinase (MMP)-9. Left panels demonstrate both thrombotic and atheromatous plaque segments. A number of macrophages are strongly positive for MMP-9 (Lower left). In contrast, MMP-9 was negative to moderately positive in the SAP specimen (Lower right).

smooth muscle cells in the atherosclerotic lesions of the coronary artery in patients with unstable angina pectoris[6] These reports uniformly indicated augmented in vivo MMP-9 localization in the atheromatous plaque and its potential role in plaque vulnerability. Thus, atherosclerotic plaque may be the major source of MMP-9 released into the circulation. However, MMP-9 release and activation were also demonstrated in the ischemic myocardial interstitium in the early post-MI period in animals[14,21] Thus, the necrotic myocardium could also be the predominant source of MMP-9. Previous blood sampling from the CS[12] was unable to exclude the production of MMP by necrotic myocardial tissue. In the present study, however, selective blood sampling using the PercuSurge distal protection device together with CS sampling demonstrated in vivo the elevation of plasma MMP-9 levels in the coronary artery in patients during the acute phase of an AMI. Recently, Funayama et al also reported elevated MMP-9 levels in the human coronary artery[13] but they did not refer to the influence of PCI nor did they simultaneously measure the MMP-9 levels in the CS blood.

Local blood concentration or dilution may affect the measured values, and elevation of circulating plasma MMP-9 levels alone does not directly prove in situ expression in coronary artery plaque. Hence, we immunohistochemically demonstrated augmented expression of MMP-9 and associated inflammatory cells in the coronary artery plaque obtained from patients with AMI. Immunohistochemistry, as well as the blood samples, indicated that culprit plaque and associated inflammatory cells contained large amounts of MMP-9, which would be released into the coronary circulation by mechanical disruption.

Interestingly, patients with AMI who demonstrated TIMI 0 flow in the first coronary arteriogram showed relatively higher levels of MMP-9 in the initial aspiration samples than those who demonstrated TIMI 1–3 flow (165.7±30.6 ng/ml vs 86.8±21.0 ng/ml, p=0.12). It is interesting to speculate that MMP-9 content may vary depending on the local thrombus burden. Of note, MMP-9 may induce thrombus formation through degradation of the tissue factor pathway inhibitor, as reported for porcine coronary arteries[22] Further increases in the MMP-9 level in the second aspiration samples taken in our study indicate that mechanical disruption of the culprit plaque in AMI causes MMP-9 release into the coronary circulation, which may also further aggravate thrombus formation during coronary intervention.

Recently, plasma MMP-9 has been identified as a novel indicator of cardiovascular mortality in patients with coronary artery disease[11] Together with the data from our study, this suggests that plasma MMP-9 may not only be a risk marker, but also an indicator of atherosclerotic plaque vulnerability in patients with ischemic heart disease. Nonetheless, our data failed to show a difference between the AMI and SAP groups in MMP-9 levels in the FA, suggesting that care should be taken to interpret systemic biomarkers for coronary artery disease.

*Study Limitations*

The study population was relatively small, although the elevation of the MMP-9 level reached statistical significance. A larger population including a variety of re-flow conditions would be of interest. Because all blood samples were obtained only during a PCI procedure performed in the acute phase, these results can not refer to the subsequent clinical course of AMI patients. In the immunohistochemistry study, aspirated specimens were compared with DCA samples. It would be desirable to use the same DCA method to obtain tissue specimens from patients with AMI, but as this is seldom performed in the clinical setting of AMI, we used fragmented coronary artery plaque obtained by the aspiration technique.

## Conclusion

Analysis of blood samples from a peripheral artery, the coronary artery and the CS during PCI, together with immunohistochemical staining of plaque, indicate that MMP-9 is mainly released into the coronary circulation, not from the myocardium, but from the culprit coronary artery plaque in patients with AMI.

### *Acknowledgments*

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## References

1. Libby P. Molecular bases of the acute coronary syndromes. *Circulation* 1995; **91:** 2844–2850.
2. Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, et al. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques: Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* 1995; **92:** 1565–1569.
3. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 1994; **94:** 2493–2503.
4. Kaartinen M, van der Wal AC, van der Loos CM, Piek JJ, Koch KT, Becker AE, et al. Mast cell infiltration in acute coronary syndromes: Implications for plaque rupture. *J Am Coll Cardiol* 1998; **32:** 606–612.
5. Pasterkamp G, Schoneveld AH, Hijnen DJ, de Kleijn DP, Teepen H, van der Wal AC, et al. Atherosclerotic arterial remodeling and the localization of macrophages and matrix metalloproteases 1, 2 and 9 in the human coronary artery. *Atherosclerosis* 2000; **150:** 245–253.
6. Brown DL, Hibbs MS, Kearney M, Loushin C, Isner JM. Identification of 92-kD gelatinase in human coronary atherosclerotic lesions: Association of active enzyme synthesis with unstable angina. *Circulation* 1995; **91:** 2125–2131.
7. Saren P, Welgus HG, Kovanen PT. TNF-alpha and IL-1beta selectively induce expression of 92-kDa gelatinase by human macrophages. *J Immunol* 1996; **157:** 4159–4165.
8. Kim SH, Kang YJ, Kim WJ, Woo DK, Lee Y, Kim DI, et al. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. *Circ J* 2004; **68:** 396–399.
9. Kai H, Ikeda H, Yasukawa H, Kai M, Seki Y, Kuwahara F, et al. Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. *J Am Coll Cardiol* 1998; **32:** 368–372.
10. Kalela A, Koivu TA, Sisto T, Kanervisto J, Hoyhtya M, Sillanaukee P. Serum matrix metalloproteinase-9 concentration in angiographically assessed coronary artery disease. *Scand J Clin Lab Invest* 2002; **62:** 337–342.
11. Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 2003; **107:** 1579–1585.
12. Inokubo Y, Hanada H, Ishizaka H, Fukushi T, Kamada T, Okumura K. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. *Am Heart J* 2001; **141:** 211–217.
13. Funayama H, Ishikawa SE, Kubo N, Katayama T, Yasu T, Saito M, et al. Increases in interleukin-6 and matrix metalloproteinase-9 in the infarct-related coronary artery of acute myocardial infarction. *Circ J* 2004; **68:** 451–454.
14. Etoh T, Joffs C, Deschamps AM, Davis J, Dowdy K, Hendrick J, et al. Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. *Am J Physiol Heart Circ Physiol* 2001; **281:** 987–994.
15. Soejima H, Ogawa H, Sakamoto T, Miyamoto S, Kajiwara I, Kojima S, et al. Increased serum matrix metalloproteinase-1 concentration predicts advanced left ventricular remodeling in patients with acute myocardial infarction. *Circ J* 2003; **67:** 301–304.
16. Kaden JJ, Dempfle CE, Sueselbeck T, Brueckmann M, Poerner TC, Haghi D, et al. Time-dependent changes in the plasma concentration of matrix metalloproteinase 9 after acute myocardial infarction. *Cardiology* 2003; **99:** 140–144.
17. Sundström J, Evans JC, Benjamin EJ, Levy D, Larson MG, Sawyer DB, et al. Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: The Framingham Heart Study. *Circulation* 2004; **109:** 2850–2856.
18. Carlino M, De Gregorio J, Di Mario C, Anzuini A, Airoldi F, Albiero R, et al. Prevention of distal embolization during saphenous vein graft lesion angioplasty: Experience with a new temporary occlusion and aspiration system. *Circulation* 1999; **99:** 3221–3223.
19. Baim DS, Wahr D, George B, Leon MB, Greenberg J, Cutlip DE, et al. Randomized trial of a distal embolic protection device during percutaneous intervention of saphenous vein aorto-coronary bypass grafts. *Circulation* 2002; **105:** 1285–1290.
20. Fujimoto N, Hosokawa N, Iwata K, Shinya T, Okada Y, Hayakawa T. A one-step sandwich enzyme immunoassay for inactive precursor and complexed forms of human matrix metalloproteinase 9 (92 kDa gelatinase/type IV collagenase, gelatinase B) using monoclonal antibodies. *Clin Chim Acta* 1994; **231:** 79–88.
21. Cleutjens JP, Kandala JC, Guarda E, Guntaka RV, Weber KT. Regulation of collagen degradation in the rat myocardium after infarction. *J Mol Cell Cardiol* 1995; **27:** 1281–1292.
22. Morishige K, Shimokawa H, Matsumoto Y, Eto Y, Uwatoku T, Abe K, et al. Overexpression of matrix metalloproteinase-9 promotes intravascular thrombus formation in porcine coronary arteries in vivo. *Cardiovasc Res* 2003; **57:** 572–585.

# Transplantation of Mesenchymal Stem Cells Improves Cardiac Function in a Rat Model of Dilated Cardiomyopathy

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***Background***—Pluripotent mesenchymal stem cells (MSCs) differentiate into a variety of cells, including cardiomyocytes and vascular endothelial cells. However, little information is available about the therapeutic potency of MSC transplantation in cases of dilated cardiomyopathy (DCM), an important cause of heart failure.

***Methods and Results***—We investigated whether transplanted MSCs induce myogenesis and angiogenesis and improve cardiac function in a rat model of DCM. MSCs were isolated from bone marrow aspirates of isogenic adult rats and expanded ex vivo. Cultured MSCs secreted large amounts of the angiogenic, antiapoptotic, and mitogenic factors vascular endothelial growth factor, hepatocyte growth factor, adrenomedullin, and insulin-like growth factor-1. Five weeks after immunization, MSCs or vehicle was injected into the myocardium. Some engrafted MSCs were positive for the cardiac markers desmin, cardiac troponin T, and connexin-43, whereas others formed vascular structures and were positive for von Willebrand factor or smooth muscle actin. Compared with vehicle injection, MSC transplantation significantly increased capillary density and decreased the collagen volume fraction in the myocardium, resulting in decreased left ventricular end-diastolic pressure (11±1 versus 16±1 mm Hg, $P<0.05$) and increased left ventricular maximum *dP/dt* (6767±323 versus 5138±280 mm Hg/s, $P<0.05$).

***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 might be mediated not only by their differentiation into cardiomyocytes and vascular cells but also by their ability to supply large amounts of angiogenic, antiapoptotic, and mitogenic factors. **(*Circulation*. 2005;112:1128-1135.)**

**Key Words:** myocytes ■ angiogenesis ■ heart failure ■ growth substances ■ transplantation

Despite advances in medical and surgical procedures, congestive heart failure remains a leading cause of cardiovascular morbidity and mortality.[1] Idiopathic dilated cardiomyopathy (DCM), a primary myocardial disease of unknown etiology characterized by a loss of cardiomyocytes and an increase in fibroblasts, is an important cause of heart failure.[2] Although myocyte mitosis and the presence of cardiac precursor cells in adult hearts have recently been reported,[3] the death of large numbers of cardiomyocytes results in the development of heart failure. Thus, restoring lost myocardium would be desirable for the treatment of DCM.

Mesenchymal stem cells (MSCs) are pluripotent, adult stem cells residing within the bone marrow microenvironment.[4] In contrast to their hematopoietic counterparts, MSCs are adherent and can be expanded in culture. MSCs can differentiate not only into osteoblasts, chondrocytes, neurons, and skeletal muscle cells but also into vascular endothelial cells[5] and cardiomyocytes.[6,7] In vitro, MSCs can be induced to differentiate into beating cardiomyocytes by 5-azacytidine treatment.[8] In vivo, MSCs directly injected into an infarcted heart have been shown to induce myocardial regeneration and improve cardiac function.[9] In addition, MSC implantation induces therapeutic angiogenesis in a rat model of hindlimb ischemia through vascular endothelial growth factor (VEGF) production by MSCs.[10,11] Myocardial blood flow abnormalities, even in the presence of angiographically normal coronary arteries, have been documented in patients with DCM.[12]

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These findings raise the possibility that transplanted MSCs have beneficial effects on myocardial structure and function via myogenesis and angiogenesis. However, little information is available about the therapeutic potential of MSCs for DCM.

A unique model of myocarditis in the rat has been created by immunization with porcine cardiac myosin,[13] which results in severe heart failure characterized by increased cardiac fibrosis and left ventricular (LV) dilation.[14] Thus, the late phase of this model can serve as a model of DCM.

The purpose of this study was to investigate the following topics: (1) whether transplantation of MSCs induces myogenesis and angiogenesis, decreases collagen deposition in the myocardium, and thereby improves cardiac function in a rat model of DCM and (2) whether the beneficial effects of MSCs are mediated by their differentiation into cardiomyocytes and vascular cells and/or by their supplying angiogenic, antiapoptotic, and mitogenic factors.

## Methods

### Expansion of Bone Marrow MSCs

MSC expansion was performed according to previously described methods.[4] In brief, we humanely killed male Lewis rats and harvested bone marrow by flushing their femoral and tibial cavities with phosphate-buffered saline (PBS). Bone marrow cells were cultured in α-minimal essential medium supplemented with 10% fetal bovine serum and antibiotics. A small number of cells developed visible symmetric colonies by days 5 to 7. Nonadherent hematopoietic cells were removed, and the medium was replaced. The adherent, spindle-shaped MSC population expanded to $>5\times10^7$ cells within ≈4 to 5 passages after the cells were first plated.

### Flow Cytometry

Cultured MSCs were analyzed by fluorescence-activated cell sorting (FACS) (FACScan flow cytometer, Becton Dickinson). Cells were incubated with fluorescein isothiocyanate (FITC)–conjugated mouse monoclonal antibodies against rat CD31 (clone TLD-3A12, Becton Dickinson), CD34 (clone ICO-115, Santa Cruz), CD45 (clone OX-1, Becton Dickinson), CD90 (clone OX-7, Becton Dickinson), vimentin (clone V9, Dako), and smooth muscle actin (SMA; clone 1A4, Dako). FITC-conjugated hamster anti-rat CD29 monoclonal antibody (clone Ha2/5, Becton Dickinson) and rabbit anti-rat c-Kit polyclonal antibody (clone C-19, Santa Cruz) were used. Isotype-identical antibodies served as controls.

### Model of DCM

Male Lewis rats weighing 220 to 250 g (Japan SLC Inc, Hamamatsu, Japan) were used in this study. These isogenic rats served as donors and recipients of MSCs to simulate autologous implantation. DCM was produced by inducing experimental myocarditis, as described previously.[13,14] In brief, 1 mg (0.1 mL) of porcine heart myosin (Sigma) was mixed with an equal volume of Freund's complete adjuvant (Sigma) and injected into a footpad on days 1 and 7. Five weeks after immunization, these rats served as a model of heart failure due to DCM.

### MSC Transplantation

In a preliminary experiment, we performed dose-response studies to obtain the maximal effects of cell transplantation. Because the effect of $10^6$ MSCs was modest, we used $5\times10^6$ MSCs for transplantation. Five weeks after immunization, we injected a total of $5\times10^6$ MSCs/100 μL PBS, or PBS alone, into the myocardium at 10 points. In brief, the LV was divided into 3 levels (basal, middle, and apical). The basal and middle levels were each subdivided into 4 segments, and the apical level was subdivided into 2 segments. Injection into each segment was performed with a 27-gauge needle. Sham rats received intramyocardial injections of 100 μL PBS. This protocol resulted in the creation of 3 groups: DCM rats given MSCs (MSC-treated DCM group, n=10); DCM rats given PBS (untreated DCM group, n=10); and sham rats given PBS (sham group, n=10). The Animal Care Committee of the National Cardiovascular Center approved this experimental protocol.

### Echocardiographic Studies

Echocardiographic studies were performed by an investigator, blinded to treatment allocation, at 5 weeks after immunization (before treatment) and 4 weeks after cell transplantation (after treatment). Two-dimensional, targeted M-mode tracings were obtained at the level of the papillary muscles with an echocardiographic system equipped with a 7.5-MHz transducer (HP Sonos 5500, Hewlett-Packard).[15] LV dimensions were measured according to the American Society for Echocardiology leading-edge method from at least 3 consecutive cardiac cycles. Fractional shortening was calculated as (LVDd−LVDs)/LVDd×100, where LVDd=LV diastolic dimension and LVDs=LV systolic dimension.

### Hemodynamic Studies

Hemodynamic studies were performed 4 weeks after cell transplantation. A 1.5F micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery for measurement of mean arterial pressure.[16] Next, the catheter was advanced into the LV for measurement of LV pressure. Hemodynamic variables were measured with a pressure transducer (model P23 ID, Gould) connected to a polygraph. After completion of these measurements, the left and right ventricles were excised and weighed.

### Histological Examination

To detect fibrosis in cardiac muscle, the LV myocardium (n=5 from each group) was fixed in 10% formalin, cut transversely, embedded in paraffin, and stained with Masson's trichrome. Transverse sections were randomly obtained from the 3 levels (basal, middle, and apical), and 20 randomly selected fields per section (n=60 per animal) were analyzed. After each field was scanned and computerized with a digital image analyzer (WinRoof, Mitani Co), collagen volume fraction was calculated as the sum of all areas containing connective tissue divided by the total area of the image.[15]

To detect capillaries in the myocardium, samples of harvested muscle (n=5 each) were embedded in OCT compound (Miles Scientific), snap-frozen in $LN_2$, cut into transverse sections, and stained for alkaline phosphatase by an indoxyltetrazolium method. Transverse sections were randomly obtained from the 3 levels (basal, middle, and apical), and 5 randomly selected fields per section (n=15 per animal) were analyzed. The number of capillaries was counted by light microscopy at a magnification of ×200. The number of capillaries in each field was averaged and expressed as the number of capillary vessels. These morphometric studies were performed by 2 examiners who were blinded to treatment assignment.

### Assessment of Cell Differentiation

Suspended MSCs were labeled with fluorescent dyes with use of a PKH26 red fluorescent cell linker kit (Sigma), as reported previously.[17] Fluorescence-labeled MSCs were injected into the myocardium 5 weeks after immunization. Rats (n=5) were humanely killed 4 weeks after cell transplantation. LV samples were embedded in OCT compound, snap-frozen in $LN_2$, and cut into sections. Immunofluorescence staining was performed with monoclonal mouse anti-cardiac troponin T (Novo), anti-desmin (Dako), anti-connexin-43 (Sigma), polyclonal rabbit anti–von Willebrand factor (Dako), and monoclonal mouse SMA (Dako). FITC-conjugated IgG antibody (BD Pharmingen) was used as a secondary antibody. To perform quantitative analysis of the magnitude of MSC differentiation into cardiomyocytes, heart cells from each rat (n=5) were isolated by incubation in balanced salt solution containing 0.06% collagenase type II (Worthington Biochemical Co), as reported previously.[18] PKH26/troponin T double-positive cells were detected by FACS.

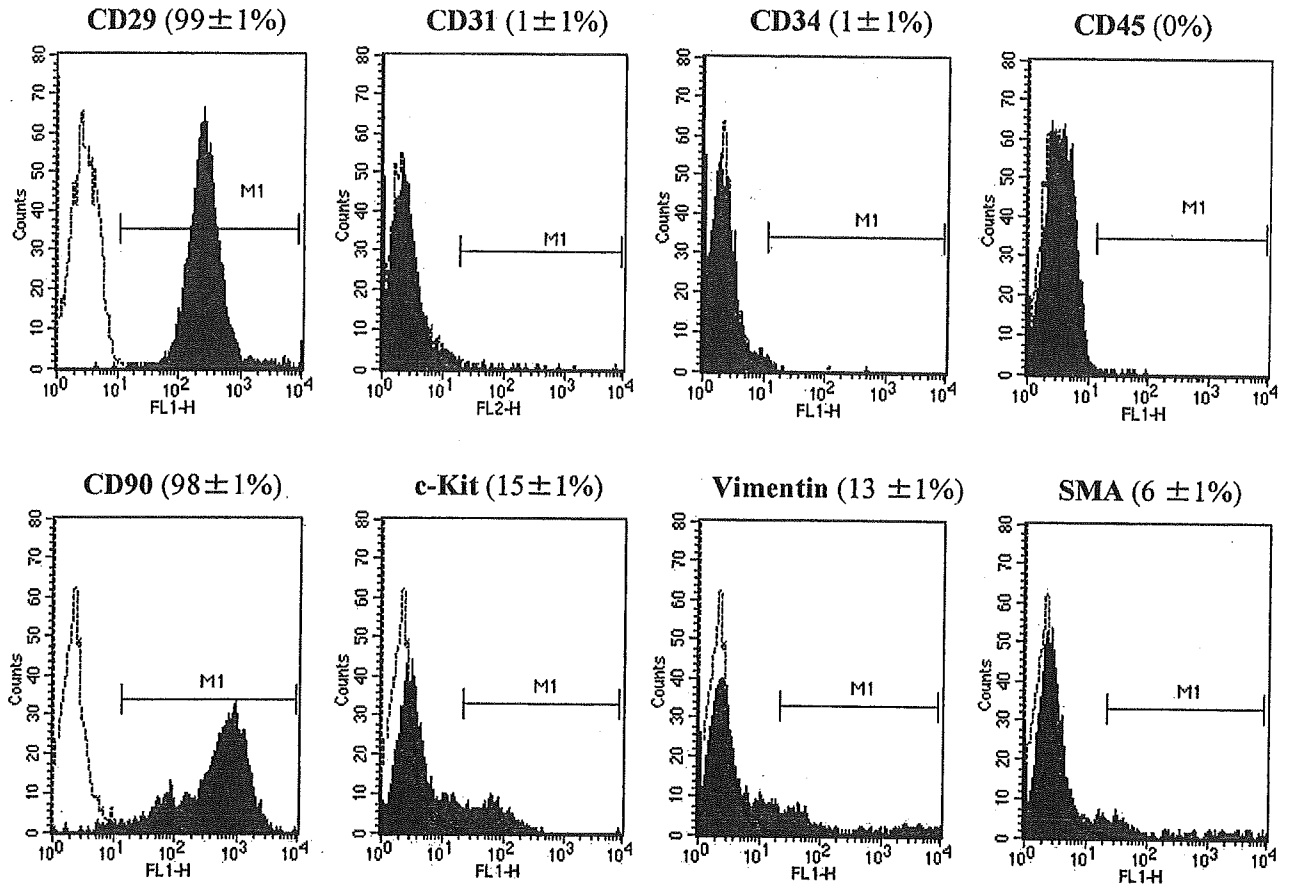


**Figure 1.** Flow-cytometric analysis of the adherent, spindle-shaped MSC population expanded to 4 to 5 passages. Most of the MSCs expressed CD29 and CD90, whereas they were negative for CD31, CD34, CD45, and SMA. Some of the cells were positive for c-Kit and vimentin.

### Western Blot Analysis of Matrix Metalloproteinases

To identify the protein expression of matrix metalloproteinases (MMPs)-2 and -9, Western blotting was performed with rabbit polyclonal antibody raised against MMP-2 (Laboratory vision Co) and MMP-9 (Chemicon Co). The LV obtained from individual rats was used for comparison among the 3 groups (n=5 each). These samples were homogenized on ice in 0.1% Tween 20 homogenization buffer with a protease inhibitor. Then, 40 $\mu$g of protein was transferred into sample buffer, loaded on a 7.5% sodium dodecyl sulfate–polyacrylamide gel, and blotted onto a polyvinylidene fluoride membrane (Millipore Co). After being blocked for 120 minutes, the membrane was incubated with primary antibody at a dilution of 1:200. The membrane was incubated with peroxidase labeled with secondary antibody at a dilution of 1:1000. Positive protein bands were visualized with an ECL kit (Amersham) and measured by densitometry. Western blot analysis with a mouse polyclonal antibody raised against $\beta$-actin (Santa Cruz) was used as a protein loading control.

### Assay for Angiogenic, Antiapoptotic, and Mitogenic Factors

To investigate whether MSCs produce angiogenic and growth factors, we measured VEGF, hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), and adrenomedullin (AM) levels in conditioned medium 24 hours after medium replacement. VEGF, HGF, and IGF-1 were measured by enzyme immunoassay (VEGF immunoassay, R&D Systems Inc; rat HGF enzyme immunoassay, Institute of Immunology Co, Ltd; and active rat IGF-1 enzyme immunoassay, Diagnostic Systems Laboratories, Inc). AM level was measured with a radioimmunoassay kit (Shionogi Co), as reported previously.[19] The amounts of these products produced by MSCs were compared with those produced by bone marrow–derived mononuclear cells (MNCs) because MNCs have commonly been used for regenerative therapy.[19–21] There was no significant difference in cell viability between MSCs and MNCs 24 hours after seeding (88±5% versus 85±4% by trypan blue solution). In vivo, circulating levels of VEGF, HGF, IGF-1, and AM were measured before and 24 hours after administration of MSCs or vehicle (n=6 from each group).

### Statistical Analysis

Numerical values are expressed as mean±SEM unless otherwise indicated. Comparisons of parameters between 2 groups were made with unpaired Student $t$ test. Comparisons of parameters among 3 groups were made with a 1-way ANOVA, followed by the Scheffe multiple-comparison test. Comparisons of changes in parameters among the 3 groups were made by a 2-way ANOVA for repeated measures, followed by the Scheffe multiple-comparison test. A value of $P<0.05$ was considered significant.

## Results

### Characterization of Cultured MSCs

Most cultured MSCs expressed CD29 and CD90 (Figure 1). In contrast, the majority of MSCs were negative for CD31, CD34, CD45, and SMA. Some of the MSCs expressed c-Kit and vimentin.