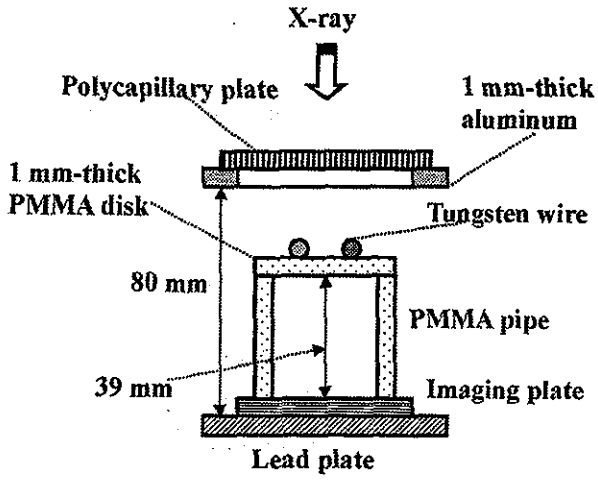

Fig. 12. Radiography for imaging tungsten wires using the polycapillary.

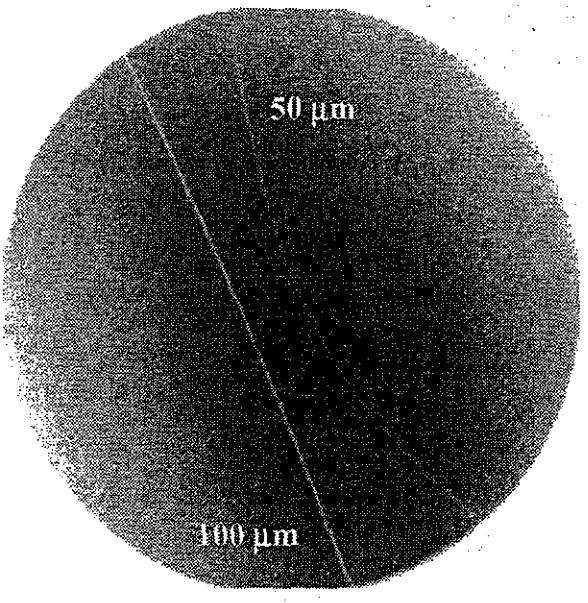

Fig. 13. Radiograms of tungsten wires on a PMMA spacer.

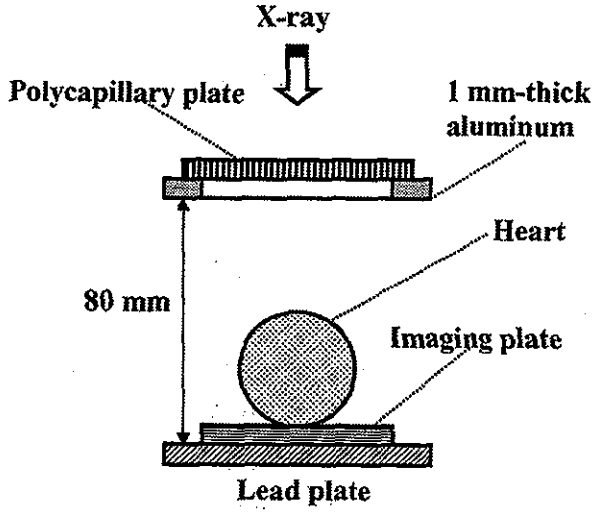

Fig. 14. Angiography using iodine-based microspheres of the heart extracted from a rabbit.

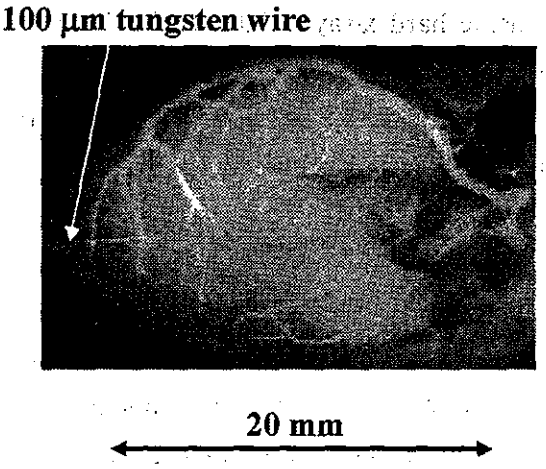

Fig. 15. Angiogram of the heart using the polycapillary.

5. Discussion

In this research, we carried out parallel radiography using a polycapillary plate in conjunction with monochromatic x-rays, and we obtained higher image resolutions as compared with those obtained without using the plate. Currently, the image resolution of the polycapillary is primarily determined by the inner capilliary diameter and the thickness, and it is improved with decreases in the diameter and increases in the thickness. In cases where the CR system is employed, although the resolution of the CR system is primarily determined by the minimum sampling pitch of 87.5 μm, we could observe 50 μm tungsten wires.

The photon energies of the characteristic x-rays are determined by the target element, and the capillary thickness should be increased according to increases in the photon energy because the transmission intensity through capillary glass increases. Subsequently, in order to increase the

parallelity for phase imaging, single crystals should be employed after passing the x-ray beam through the polycapillary.

Since it is possible to increase the irradiation field by increasing the distance between the x-ray source and the polycapillary, this system can be applied to image a wide variety of objects in various fields, including medical radiography.

Acknowledgments

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Optimal Windows of Statin Use for Immediate Infarct Limitation

5′-Nucleotidase as Another Downstream Molecule of Phosphatidylinositol 3-Kinase

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Background—Although statins are reported to have a cardioprotective effect, their immediate direct influence on ischemia-reperfusion injury and the underlying mechanisms remain obscure. We investigated these issues an in vivo canine model.

Methods and Results—Dogs were subjected to coronary occlusion (90 minutes) and reperfusion (6 hours) immediately after injection of pravastatin (0.2, 2, or 10 mg/kg), pitavastatin (0.01, 0.1, or 0.5 mg/kg), or cerivastatin (0.5, 5, or 50 μg/kg). Then myocardial phosphatidylinositol 3-kinase (PI3-K) and 5′-nucleotidase activities were measured, as well as infarct size. After 15 minutes of reperfusion, pravastatin caused dose-dependent activation of Akt and ecto-5′-nucleotidase in the ischemic zone, and the effect was significant at higher doses. Pitavastatin also significantly increased these activities, and its optimal dose was within the clinical range, whereas cerivastatin caused activation at the lowest dose tested. In all cases, both Akt and ecto-5′-nucleotidase showed activation in parallel, and this activation was completely abolished by wortmannin, a PI3-K inhibitor. The magnitude of the infarct-limiting effect paralleled the increase in Akt and ecto-5′-nucleotidase activity and was blunted by administration of wortmannin, α,β-methyleneadenosine-5′-diphosphate, or 8-sulfophenyltheophylline during reperfusion. Both collateral flow and the area at risk were comparable for all groups.

Conclusions—Activation of ecto-5′-nucleotidase after ischemia by PI3-K activation may be crucial for immediate infarct-size limitation by statins. There seems to be an optimal dose for each statin that is independent of its clinical cholesterol-lowering effect. (***Circulation.*** **2004;110:2143-2149.)**

Key Words: statins ■ myocardial infarction ■ adenosine ■ enzymes ■ phosphates

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) block the biosynthesis of cholesterol[1] and are widely used clinically to decrease serum cholesterol levels. Recent studies have focused on the pleiotropic effects of either hydrophilic[2-4] or hydrophobic[4,5] statins, which are independent of their cholesterol-lowering effect.[2,3,5] Protection against ischemia-reperfusion injury is one of them, which is particularly evident after 12 hours.[6,7] In addition, some studies showed that statins activate the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway within 1 hour,[8,9] as well as activating endothelial nitric oxide synthase (eNOS),[5,10] to cause immediate infarct limitation.[9]

On the other hand, other studies revealed that statins also acutely activate ecto-5′-nucleotidase,[11] which produces the endogenous cardioprotective substance adenosine,[12] especially in response to certain stresses.[13] Ecto-5′-nucleotidase can act only when localized on the cell membrane,[13] and the density of this enzyme on the membrane regulates its activity.[11,14] Endocytotic turnover of ecto-5′-nucleotidase (5′-nucleotidase localized on the cell surface) is inhibited by PI3-K activation,[14] which subsequently increases total 5′-nucleotidase activity within a period as short as 10 minutes.[14] Therefore, we hypothesized that an increase of ecto-5′-nucleotidase activity might be critical for early cardioprotec-

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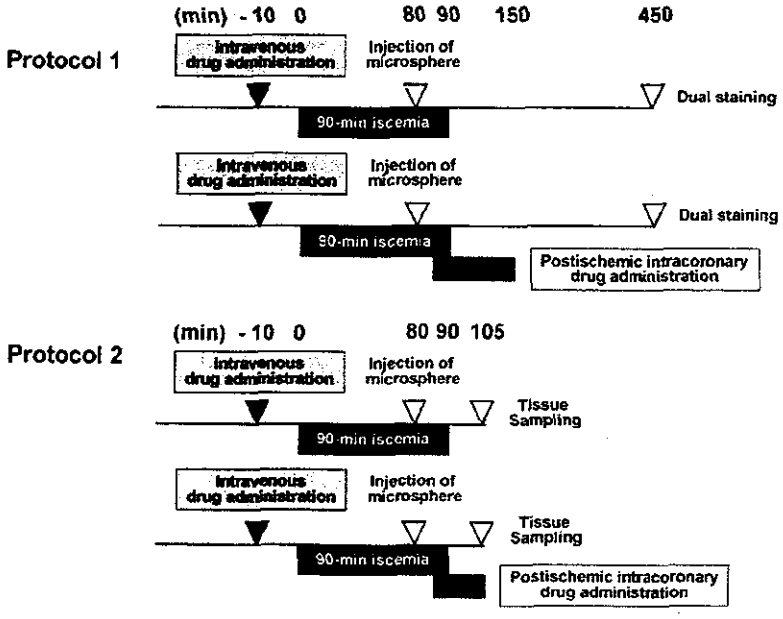

Figure 1. Experimental protocols to measure infarct size (protocol 1; Upper) and kinase activity (protocol 2; Lower).

tion mediated by statins and might be associated with rapid activation of PI3-K.

Here we used a dog model to determine whether 3 statins with different water solubilities (pravastatin, pitavastatin, and cerivastatin) could acutely limit infarct size, as well as whether adenosine and PI3-K were involved in the underlying mechanism.

Methods

All procedures were performed in conformity with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, 1996 revision) and were approved by the Osaka University Committee for Laboratory Animal Use. Pravastatin, pitavastatin, and cerivastatin were obtained from Sankyo, Kowa, and Takeda Pharmaceuticals, respectively. The other drugs were obtained from Sigma.

Instrumentation

Beagle dogs weighing 8 to 13 kg were anesthetized and connected to an extracorporeal bypass tube as described previously.[15,16] In all experiments, the average baseline values of mean aortic blood pressure (ABP), heart rate (HR), and arterial blood Po_2 were 102±2.2 mm Hg, 129±2.5 min^{-1}, and 109±4.1 mm Hg, respectively. Both ABP and HR were measured continuously during the study.

Experimental Protocols

Protocol 1: Measurement of Infarct Size and Myocardial Collateral Blood Flow

After hemodynamic stabilization, we infused pravastatin (0.2, 2, or 10 mg/kg), pitavastatin (0.01, 0.1, or 0.5 mg/kg), cerivastatin (0.5, 5, or 50 μg/kg) or saline intravenously for 10 minutes before 90 minutes of sustained ischemia, which was followed by 6 hours of reperfusion (n=9 to 13 each). Some groups also received intracoronary administration of a selective ecto-5′-nucleotidase inhibitor (α,β-methyleneadenosine-5′-diphosphate [AMP-CP: 80 $\mu g \cdot kg^{-1} \cdot min^{-1}$]; a nonselective adenosine receptor antagonist (8-sulfophenyltheophylline [8-SPT; 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$]); or a selective PI3-K inhibitor (wortmannin [1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$]) between 5 minutes before and 60 minutes after reperfusion. We measured infarct size and regional myocardial collateral blood flow during 90 minutes of ischemia as described previously.[15]

We have already confirmed in the same model that the doses of AMP-CP,[17] 8-SPT,[17,18] or wortmannin[19] used in this study were appropriate to block ecto-5′-nucleotidase, the adenosine receptors, or PI3-K, respectively. Figure 1 shows the details of this protocol, and the Table lists all of the groups studied.

Protocol 2: Myocardial Enzyme Assays

Another 54 dogs underwent a procedure identical to that of some groups from protocol 1 and were studied for enzyme assays (n=3 or 4 each). In this protocol, not only wortmannin (1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) but also LY294002 (60 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was used as another selective PI3-K inhibitor. After 15 minutes of reperfusion, a myocardial tissue sample was obtained from the ischemic border zone to ensure evaluation of viable ischemic myocardium and was used for the measurement of PI3-K and ecto-/endo-5′-nucleotidase activity. The myocardial tissue was rapidly frozen in LN_2 and stored at −80°C. Measurement of PI3-K and 5′-nucleotidase activity was done as reported previously[15,19] with minor modifications.

Criteria for Exclusion

To ensure that all of the animals included in analysis were healthy and were exposed to a similar extent of ischemia, the exclusion criteria reported previously[16] for hemodynamics, excessive collateral flow, and lethal arrhythmia were adopted.

Statistical Analysis

Results were expressed as mean±SEM, and the number of animals or experiments is shown as n. Statistical analysis was performed by ANOVA with a modified Bonferroni post hoc test, and significance was defined at $P<0.05$.

Results

Mortality and Exclusions in Protocol 1

Among 222 dogs used in protocols 1, 56 dogs met the exclusion criteria of ventricular fibrillation or excessive myocardial collateral blood flow (>15 mL · 100 $g^{-1} \cdot min^{-1}$). Therefore, 166 dogs completed these protocols satisfactorily and were included in the data analysis (Table).

Changes in Hemodynamic Parameters, Risk Area, and Collateral Blood Flow in Protocol 1

The changes in ABP and HR were comparable among all groups throughout the protocol (data not shown), and both the

TABLE 1. Mortality, Exclusion, Area at Risk, and Collateral Flow in Each Group in Protocol 1

Groups	Initial No.	Excluded: Lethal Arrhythmia: During Ischemia	Excluded: Lethal Arrhythmia: After Reperfusion	Excluded: Excessive Collateral Flow	Final No.	Area at Risk, %	Collateral Flow, mL/100 g per minute
Control	13	1	2	1	9	40.1±2.1	8.2±1.0
Prava							
0.2	9	0	1	0	8	38.8±2.0	8.4±1.2
2.0	10	0	0	2	8	39.1±2.2	8.9±1.1
10	10	0	0	2	8	39.6±2.1	8.9±1.4
Pitava							
0.01	9	1	1	0	7	38.7±2.2	8.1±1.3
0.1	11	0	1	2	8	39.3±2.0	9.2±1.5
0.5	10	1	0	2	7	39.9±1.9	8.8±1.5
Ceriva							
0.5	11	0	1	2	8	39.2±1.9	8.5±1.3
5.0	10	1	1	1	7	38.9±2.1	8.7±1.4
50	11	0	1	3	7	39.0±2.0	9.1±1.5
AMP-CP							
+Prava 10	9	0	2	0	7	40.4±2.3	8.6±1.3
+Pitava 0.1	9	0	1	1	7	39.8±2.0	8.4±1.5
+Ceriva 0.5	9	1	1	0	7	40.4±2.3	9.0±1.4
8SPT							
+Prava 10	10	0	1	1	8	38.7±2.2	8.3±1.3
+Pitava 0.1	11	1	2	0	8	39.9±2.1	8.2±1.6
+Ceriva 0.5	11	0	2	1	8	38.4±2.6	8.5±1.5
WTMN							
+Prava 10	10	0	2	1	7	38.6±2.3	9.5±1.5
+Pitava 0.1	10	0	2	0	8	38.9±2.1	9.2±1.6
+Ceriva 0.5	10	0	1	1	8	39.8±2.8	8.8±1.4
AMP-CP	9	0	2	0	7	38.8±2.5	8.5±1.6
8SPT	11	0	3	0	8	39.6±2.5	8.2±1.5
WTMN	9	1	2	0	6	40.5±2.3	8.6±1.6

Data expressed as mean±SEM. Prava indicates pravastatin (mg/kg); Pitava, pitavastatin (mg/kg); Ceriva, cerivastatin (μg/kg); 8SPT, 8-sulfophenyltheophilline; and WTMN, wortmannin.

area at risk and collateral blood flow were also comparable (Table).

Infarct Size

Figure 2 shows infarct size in the groups of protocol 1. Pravastatin (0.2, 2, and 10 mg/kg) dose-dependently reduced the infarct size (29.5±3.5%, 22.5±4.0%, and 18.8±3.4%, respectively) compared with that in the control group (39.8±3.6%), and the difference was significant at 2 mg/kg or more. Pitavastatin (0.01, 0.1, and 0.5 mg/kg) also reduced infarct size (32.9±3.9%, 23.6±3.8%, and 31.4±3.9%, respectively), although the optimal dose was 0.1 mg/kg (the only dose that produced a significant difference). Although cerivastatin (0.5, 5, and 50 μg/kg) caused infarct limitation (26.2±3.2%, 32.1±5.3%, and 37.1±4.4%, respectively), it was significant at the lowest dose only, and the effect was weaker at higher doses. Furthermore, cotreatment with AMP-CP, 8-SPT, or wortmannin between 5 minutes before and 60 minutes after reperfusion abrogated the infarct-limiting effect of pravastatin (39.9±4.0%, 42.6±4.0%, or 38.6±3.6%, respectively), pitavastatin (40.4±3.1%, 39.4±3.6%, or 39.1±3.1%, respectively), and cerivastatin (41.1±3.7%, 42.1±3.9%, or 40.4±4.0%, respectively), although these drugs per se did not affect infarct size (42.7±4.5%, 40.3±3.5%, or 42.7±4.5%, respectively).

5′-Nucleotidase Activity at Reperfusion

Figure 3 shows the activity of ecto-/endo-5′-nucleotidase in protocol 2. Sustained ischemia for 90 minutes and 15 minutes of subsequent reperfusion did not significantly change the activity of ecto-5′-nucleotidase (41.0±5.7 versus 33.2±1.2 nmol · mg protein^{-1} · min^{-1} at baseline). Preischemic treat-

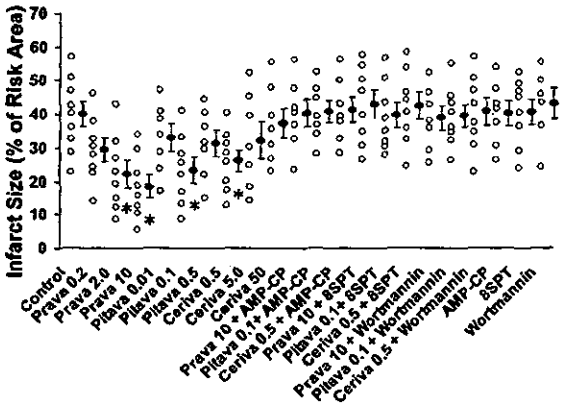

Figure 2. Infarct size in each group in protocol 1. Data are expressed as mean±SEM. *$P<0.05$ vs control. Open circles show infarct size in each individual. Prava indicates pravastatin; Pitava, pitavastatin; and Ceriva; cerivastatin. All other abbreviations are as defined in text.

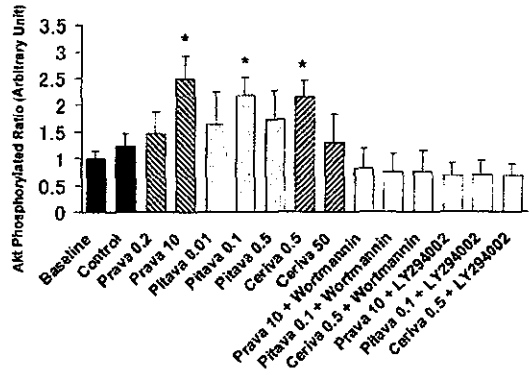

Figure 4. Myocardial PI3-K activity represented by phosphorylated ratio of Akt in each group in protocol 2. Data are expressed as mean±SEM. $n=4$ each, *$P<0.05$ vs control. Abbreviations are as defined in text and in legend to Figure 2.

ment with pravastatin caused a dose-dependent and acute increase of ecto-5′-nucleotidase activity in the ischemic zone, which became significant at the highest dose (72.6±6.0 nmol · mg protein^{-1} · min^{-1} at 10 mg/kg, $P<0.05$ versus control). Pitavastatin also caused significant activation at its optimal (medium) dose (66.7±6.1 nmol · mg protein^{-1} · min^{-1} at 0.1 mg/kg, $P<0.05$ versus control). Cerivastatin caused activation at the lowest dose (62.5±5.6 nmol · mg protein^{-1} · min^{-1} at 0.5 μg/kg, $P<0.05$ versus control). All of these increases were canceled by the selective PI3-K inhibitors wortmannin (39.5±6.8 nmol · mg protein^{-1} · min^{-1} for pravastatin, 37.0±7.1 nmol · mg protein^{-1} · min^{-1} for pitavastatin, and 38.4±6.5 nmol · mg protein^{-1} · min^{-1} for cerivastatin) or LY294002 (33.5±6.5 nmol · mg protein^{-1} · min^{-1} for pravastatin, 35.0±6.2 nmol · mg protein^{-1} · min^{-1} for pitavastatin, and 37.5±6.7 nmol · mg protein^{-1} · min^{-1} for cerivastatin). The activity of endo-5′-nucleotidase remained unchanged in all cases.

PI3-K Activity at Reperfusion

Figure 4 shows the activity of PI3-K in protocol 2. Sustained ischemia for 90 minutes and subsequent reperfusion for 15 minutes did not change PI3-K activity significantly (123±23% versus 100±14% at baseline). Preischemic treatment with pravastatin caused dose-dependent and acute activation of ecto-5′-nucleotidase in the ischemic zone, which was significant at the highest dose (249±44% at 10 mg/kg, $P<0.05$ versus control). Pitavastatin also caused significant activation at its medium dose (218±34% at 0.1 mg/kg, $P<0.05$ versus control), whereas cerivastatin caused activation at the lowest dose (214±31% at 0.5 μg/kg, $P<0.05$ versus control). We confirmed that all of these increases were also blocked by wortmannin (81±38% for pravastatin,

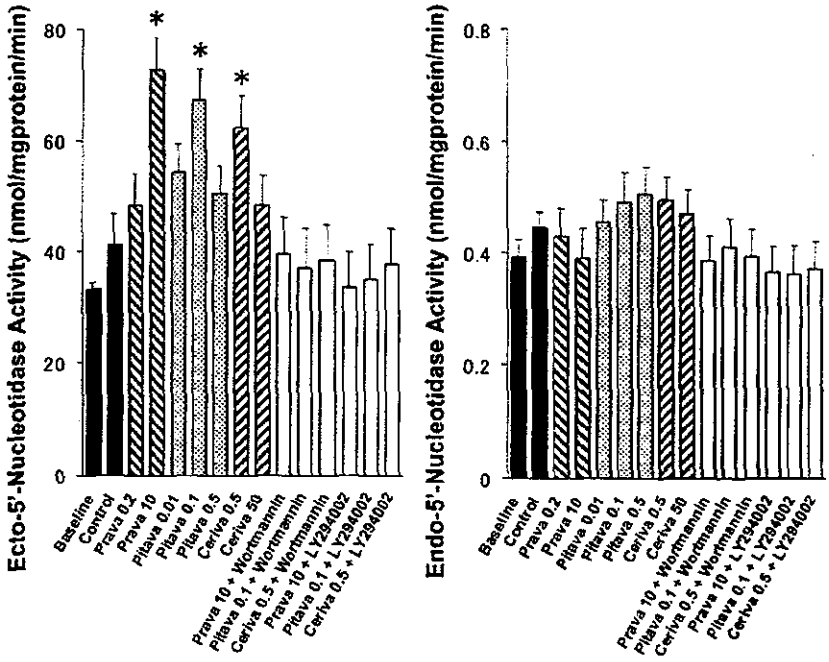

Figure 3. Myocardial ecto-/endo-5′-nucleotidase activity in each group in protocol 2. Data are expressed as mean±SEM. $n=4$ each, *$P<0.05$ vs control. Abbreviations are as defined in text and in legend to Figure 2.

77±32% for pitavastatin, and 76±39% for cerivastatin) or LY294002 (69±23% for pravastatin, 70±27% for pitavastatin, and 68±21% for cerivastatin).

Discussion

The present study demonstrates that several statins provide immediate infarct limitation of different magnitudes and at different optimal doses. Our results also suggest that activation of ecto-5′-nucleotidase through the activation of PI3-K after ischemia was involved in this cardioprotective mechanism of statins.

Cholesterol-Lowering Effects and Immediate Infarct Limitation of Statins

In this study, we set the doses of statins in line with their clinical cholesterol-lowering properties. In Japan, the standard clinical doses to obtain a 20% to 30% reduction of total plasma cholesterol levels were 10 mg/d for pravastatin, 2 mg/d for pitavastatin, and 0.15 mg/d for cerivastatin. Our preliminary trials in the same dog model revealed that a single intravenous injection of 0.2 mg/kg pravastatin, 0.1 mg/kg pitavastatin, or 5 μg/kg cerivastatin approximated the clinical cholesterol-lowering dose based on the maximal plasma concentration of each statin (data not shown). Because (1) the maximal infarct limitation was achieved by a higher dose of pravastatin than the clinical dose, whereas the dose was similar to the clinical dose for pitavastatin and lower for cerivastatin, and (2) these statins showed early cardioprotection within 2 hours of administration in this model, it is strongly suggested that the magnitude of immediate infarct limitation by each statin is not correlated with its cholesterol-lowering effect.

Existence of Optimal Cardioprotective Doses for Each Statin

In the present report, we have directly shown that pitavastatin has the optimal dose to reduce infarct size. Obviously, there is also an optimal dose for cerivastatin under the lowest dose we tried, because infarct size with far lower doses of cerivastatin near zero will converge with those of control levels. In the case of pravastatin, our additional experiment, within the limitation with regard to the total amount of the drug we could obtain, showed that 100 mg/kg pravastatin administered in the same manner as in protocol 1 exerted similar (but a slightly weaker) magnitude of reducing infarct size (20.9±4.5%, n=5) compared with that achieved with 10 mg/kg of this agent. Although we could not show direct evidence in this case, it would at least not deny the possibility for the existence of an optimal dose of pravastatin. Furthermore, other reports also showed the existence of an optimal dose of atorvastatin for infarct limitation[9] or of simvastatin for PI3-K activation.[8] Taken together, the existence of optimal doses should be ubiquitous among all (or at least all hydrophobic) statins.

Although direct exhibition of the reason for this phenomenon remains unclear in this study, there might be some reasons to regulate the respective optimal windows for each statin, eg, differences in the ability to attenuate inflammatory response[20] or in the potency of direct absorption into cellular membrane to modulate intracellular signaling systems. In addition, our present finding that infarct limitation completely paralleled the activation of PI3-K leads us to hypothesize that the lesser effects by the higher doses of statins should be regulated upstream of PI3-K. One possibility is that all hydrophobic statins can dose-dependently activate apoptosis-related signals,[21] which might also explain the wide range of higher cardioprotective doses for pravastatin specifically. Finally, additional studies will need to be performed to obtain direct evidence.

Cardioprotective Mechanisms

Our observations that (1) activation of PI3-K and ecto-5′-nucleotidase was coincident with a substantial limitation of infarct size, (2) either wortmannin or AMP-CP abolished cardioprotection by all 3 statins, (3) different PI3-K inhibitors at reperfusion actually inhibited PI3-K activity (Figure 4) and subsequently reduced ecto-5′-nucleotidase activity (Figure 3), and (4) our preliminary documentation that PI3-K inhibition by either wortmannin of LY294002 before ischemia did not abolish the infarct limitation by statins in the present study (n=4 or 5, data not shown), together suggest that infarct limitation in this model was linked to the activation of PI3-K during reperfusion, not before ischemia, followed by ecto-5′-nucleotidase activation.

In this study, we did not determine the exact mechanism of how PI3-K activates ecto-5′-nucleotidase. Although we have previously reported that phosphorylation of ecto-5′-nucleotidase might be crucial,[22] other mechanisms may also be involved, such as endocytotic turnover.[17] In addition, although we did not evaluate real-time regional myocardial production of adenosine in each group, treatment with a potent adenosine receptor antagonist (8-SPT) during reperfusion also blunted infarct limitation by statins along with the inhibition of ecto-5′-nucleotidase, further suggesting that cardioprotection against ischemia-reperfusion injury via ecto-5′-nucleotidase activation might be mediated by an increase of adenosine, the main product of ecto-5′-nucleotidase.[11,13,22] However, other implicated mechanism of enhanced activation of the adenosine receptor (eg, increased receptor sensitivity) should be determined by future studies.

Possible Link Between Cardioprotection by Adenosine and NO

Previous studies support our present findings that statins rapidly activate the PI3-K/Akt pathway,[8,9] and we obtained another preliminary finding that the cotreatment with N^{ω}-nitro-L-arginine methyl ester ($10\ \mu g \cdot kg^{-1} \cdot min^{-1}$) in the same manner as in protocol 1, which we confirmed did not affect baseline infarct size in the present model,[23] blunted the infarct limitation by pravastatin (36.8±4.1%, n=7), pitavastatin (39.9±3.9%, n=6), and cerivastatin (42.6±4.6%, n=5). Therefore, there is a possibility that ecto-5′-nucleotidase and NO act in series to cause statin-induced cardioprotection.

Although elucidation of a direct effect should be the focus of future studies, there are at least 2 lines of evidence to support the explanation that adenosine and NO synergistically caused infarct limitation in this study. First, NO directly exerts cardioprotection[24]: NO inhibits cell-to-cell adhesion,

such as that between platelets[25] or between neutrophils and endothelial cells,[26,27] by reducing expression of P-selectin,[27] E-selectin, and intercellular adhesion molecule-1,[28] which leads to attenuation of the inflammatory response[22,24,25] or protects against ischemia-reperfusion injury.[25–28] In addition, NO is reported to inhibit caspase-3 activity and to block apoptosis of cardiac myocytes.[29] On the other hand, adenosine also rescues injured myocardium through activating adenosine receptors.[13,30–32] Either administration of adenosine or enhancement of endogenous adenosine release during reperfusion after sustained ischemia limits infarct size.[13,17] We and others have shown that (1) adenosine receptor (A_1 and A_2) activation improves contractile dysfunction after reperfusion,[14] (2) inhibition of norepinephrine release from the presynaptic vesicles and attenuation of calcium influx occur through the A_1 receptor and the coupled inhibitory G protein,[33,34] (3) inhibition of platelet aggregation and leukocyte activation occurs through the A_2 receptor and the coupled stimulatory G protein,[34–36] and (4) activation of extracellular signal–regulated kinase, one of the reperfusion injury survival kinase pathways,[37] takes place during reperfusion through the A_3 receptor.[38] Therefore, either adenosine or NO similarly and potentially protects injured myocardium through multiple pathways.

Second, recent articles have shown that either adenosine[38–40] or NO[41] can reactivate PI3-K downstream. However, increasing the production of both agents is known to negatively regulate further increases of production of these molecules,[42,43] suggesting the requirement of both pathways to confer sufficient cardioprotection in the physiological system. Taking all of these together, it is likely that adenosine and NO synergistically confer the statin-derived immediate cardioprotection shown in this study.

In conclusion, our findings suggest the cellular mechanism by which statins attenuate myocardial injury, which may indicate the possibility of acute protective therapies for ischemia and associated myocardial stresses.

Acknowledgments

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Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis

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Departments of [1]Regenerative Medicine and Tissue Engineering, [3]Cardiac Physiology, and [6]Biochemistry, National Cardiovascular Center Research Institute, Osaka 565-8565; Departments of [2]Internal Medicine and [7]Cardiovascular Surgery, National Cardiovascular Center, Osaka; [4]Tissue Engineering Research Center, National Institute of Advanced Industrial Science and Technology, Hyogo; and [5]Cardiovascular Division, Kansai Rosai Hospital, Hyogo 660-8511, Japan

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Nagaya, Noritoshi, Takafumi Fujii, Takashi Iwase, Hajime Ohgushi, Takefumi Itoh, Masaaki Uematsu, Masakazu Yamagishi, Hidezo Mori, Kenji Kangawa, and Soichiro Kitamura. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. *Am J Physiol Heart Circ Physiol* 287: H2670–H2676, 2004. First published July 29, 2004; doi:10.1152/ajpheart.01071.2003.—Mesenchymal stem cells (MSCs) are pluripotent cells that differentiate into a variety of cells, including cardiomyocytes and endothelial cells. However, little information is available regarding the therapeutic potency of systemically delivered MSCs for myocardial infarction. Accordingly, we investigated whether intravenously transplanted MSCs induce angiogenesis and myogenesis and improve cardiac function in rats with acute myocardial infarction. MSCs were isolated from bone marrow aspirates of isogenic adult rats and expanded ex vivo. At 3 h after coronary ligation, 5×10^6 MSCs (MSC group, $n = 12$) or vehicle (control group, $n = 12$) was intravenously administered to Lewis rats. Transplanted MSCs were preferentially attracted to the infarcted, but not the noninfarcted, myocardium. The engrafted MSCs were positive for cardiac markers: desmin, cardiac troponin T, and connexin43. On the other hand, some of the transplanted MSCs were positive for von Willebrand factor and formed vascular structures. Capillary density was markedly increased after MSC transplantation. Cardiac infarct size was significantly smaller in the MSC than in the control group (24 ± 2 vs. 33 ± 2%, $P < 0.05$). MSC transplantation decreased left ventricular end-diastolic pressure and increased left ventricular maximum dP/d*t* (both $P < 0.05$ vs. control). These results suggest that intravenous administration of MSCs improves cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis in the ischemic myocardium.

left ventricular end-diastolic pressure; cell transplantation; differentiation; homing

INTERRUPTION OF MYOCARDIAL blood flow leads to cardiomyocyte death (20). Although myocyte mitosis and the presence of cardiac precursor cells in adult hearts have recently been reported (6, 17), death of large numbers of cardiomyocytes results in the development of heart failure (16). Thus it would be desirable to induce angiogenesis and myogenesis for the treatment of ischemic heart disease.

Mesenchymal stem cells (MSCs) are pluripotent adult stem cells residing within the bone marrow microenvironment (11, 18). In contrast to their hematopoietic counterparts, MSCs have an adherent nature and are expandable in culture. MSCs can differentiate into not only osteoblasts, chondrocytes, neurons, and skeletal muscle cells but also vascular endothelial cells (19) and cardiomyocytes (23, 24). In vitro, MSCs have the potential to induce a neovascular response in murine Matrigel angiogenesis assay (2). In vivo, local MSC implantation induces therapeutic angiogenesis in a rat model of hindlimb ischemia (1). On the other hand, MSCs directly injected into the infarcted heart have been shown to induce myocardial regeneration and improve cardiac function (21). Stem or progenitor cells have been shown to circulate in peripheral blood and home to ischemic tissues (4). These results raise the possibility that intravenously administered MSCs participate in repair of the ischemic myocardium primarily by angiogenesis, which prevents apoptosis of native cardiomyocytes, and by direct regeneration of lost cardiomyocytes. However, little information is available regarding the therapeutic potential of systemically delivered MSCs for myocardial infarction.

Thus the purpose of this study was to investigate whether *1*) intravenously administered MSCs are able to engraft in the ischemic myocardium, *2*) transplanted MSCs induce angiogenesis and myogenesis after myocardial infarction, and *3*) transplantation of MSCs decreases infarct size and improves cardiac function.

METHODS

Animals. Male Lewis rats ($n = 70$) weighing 220–250 g were used in this study. These isogenic rats ($n = 8$) served as donors and recipients of MSCs to simulate autologous implantation. The Animal Care Committee of the National Cardiovascular Center approved the experimental protocol.

Model of myocardial infarction and cell transplantation. Fifty-one rats underwent ligation of the left coronary artery to produce myocardial infarction, as described previously (15). Briefly, after rats were anesthetized by injection of pentobarbital sodium (30 mg/kg body wt ip), they were artificially ventilated using a volume-regulated respirator. The heart was exposed via a left thoracotomy, and the left coronary artery was ligated 2–3 mm from its origin between the pulmonary artery conus and the left atrium using a 6-0 Prolene suture.

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At 3 h after coronary ligation, 40 rats survived (78% survival rate): 30 were randomized to receive an intravenous injection of MSCs (MSC group, $n = 14$) or PBS (control group, $n = 16$), and 10 received fluorescence-labeled MSCs for examination of MSC differentiation ($n = 5$) and incorporation ($n = 5$). Eleven rats underwent a sham operation consisting of thoracotomy and cardiac exposure but without coronary artery ligation. At 3 h after coronary ligation, we administered 5×10^6 MSCs/100 μl in PBS or PBS alone through a catheter inserted into the left jugular vein in ~30 s. The subsequent mortality for 4 wk was 25% in the control group and 14% in the MSC group. This protocol resulted in the creation of three groups: normal rats given PBS (sham group, $n = 11$), myocardial infarction rats given PBS (control group, $n = 12$), and myocardial infarction rats given MSCs (MSC group, $n = 12$).

Expansion of bone marrow MSCs. MSC expansion was performed according to previously described methods (18). Briefly, we killed the male Lewis rats and harvested the bone marrow by flushing the cavity of the femurs and tibias with PBS. Bone marrow cells were introduced into 100-mm dishes and cultured in α-MEM supplemented with 10% FBS and antibiotics. A small number of cells developed visible symmetrical colonies by day 5–7. Nonadherent hematopoietic cells were removed, and the medium was replaced. The adherent, spindle-shaped MSC population expanded to $>5 \times 10^7$ cells by approximately four to five passages after the cells were first cultured.

Flow cytometry. Adherent cells were analyzed by fluorescence-activated cell sorting (FACS SCAN flow cytometer, Becton Dickinson). Cells were incubated for 30 min at 4°C with the FITC-conjugated mouse monoclonal antibodies against rat CD34 (clone ICO-115, Santa Cruz Biotechnology) and CD45 and CD90 (clones OX-1 and OX-7, respectively, Becton Dickinson). 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 Biotechnology) were used. Isotype-identical antibodies served as controls.

Echocardiographic studies. Echocardiographic studies were performed by an investigator blinded to treatment allocation 4 wk after coronary ligation. Two-dimensional targeted M-mode traces were obtained at the level of the papillary muscles using an echocardiographic system equipped with a 7.5-MHz phased-array transducer (SONOS 5500, Hewlett-Packard, Andover, MA). Anterior and posterior end-diastolic wall thickness and left ventricular (LV) end-diastolic and end-systolic dimensions were measured by the American Society for Echocardiology leading-edge method from at least three consecutive cardiac cycles. LV fractional shortening was calculated as follows: $(LVD_d - LVD_s)/LVD_d \times 100$, where LVD_d is LV diastolic dimension and LVD_s is LV systolic dimension. LV volume and ejection fraction were calculated on the basis of the Teichholtz formula.

Hemodynamic studies. Hemodynamic studies were performed 4 wk after coronary ligation. A 1.5-Fr micromanometer-tipped catheter (Millar Instruments) was inserted in the right carotid artery for measurement of mean arterial pressure. Then the catheter was advanced into the LV for measurement of LV pressure. Hemodynamic variables were measured using 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. Infarction size was determined as a percentage of the entire LV area, as reported previously (8). Briefly, incisions were made in the LV, so that the tissue could be pressed flat. The circumference of the entire flat LV and the visualized infarcted area, as judged from the epicardial and endocardial sides, was outlined on a clear plastic sheet. The difference in weight between the two marked areas on the sheet was used to determine infarction size and was expressed as a percentage of LV surface area.

Histological examination. To detect fibrosis in cardiac muscle, the LV myocardium ($n = 5$ each group) was fixed in 10% formalin, cut transversely, embedded in paraffin, and stained with Masson's trichrome. To detect capillary endothelial cells in the peri-infarct area, samples of the harvested muscle ($n = 5$ each) were embedded in OCT compound (Miles Scientific), snap frozen in liquid nitrogen, and cut into transverse sections. Tissue sections were stained for alkaline phosphatase with an indoxyltetrazolium method. The number of capillary vessels was counted in the peri-infarct area using a light microscope at ×200 magnification. The numbers in five high-power fields were averaged and expressed as the number of capillary vessels. These morphometric studies were performed by two examiners who were blinded to treatment.

An additional five rats were used to examine whether transplanted MSCs differentiated into cardiomyocytes or vascular endothelial cells. Suspended MSCs were labeled with fluorescent dyes with a PKH-26 red fluorescent cell linker kit (Sigma Chemical, St. Louis, MO) before implantation, as reported previously (13). Fluorescence-labeled MSCs were intravenously administered 3 h after coronary ligation. This subgroup of rats was killed 4 wk after coronary ligation. After the LV was excised and dissected free, muscle samples were embedded in OCT compound, snap frozen in liquid nitrogen, and cut into sections. Immunofluorescent staining for cardiac and endothelial cell markers was performed using monoclonal mouse antidesmin (Dako), anti-cardiac troponin T (Novo), anticonnexin43 (Sigma Chemical), and polyclonal rabbit anti-von Willebrand factor (Dako). FITC-conjugated IgG antibody (BD Pharmingen and Molecular Probes) was used as a secondary antibody.

At 24 h after intravenous administration of PKH-26-labeled MSCs, cardiac muscle was embedded in OCT compound and snap frozen in liquid nitrogen. Then the cardiac muscle from base to apex was

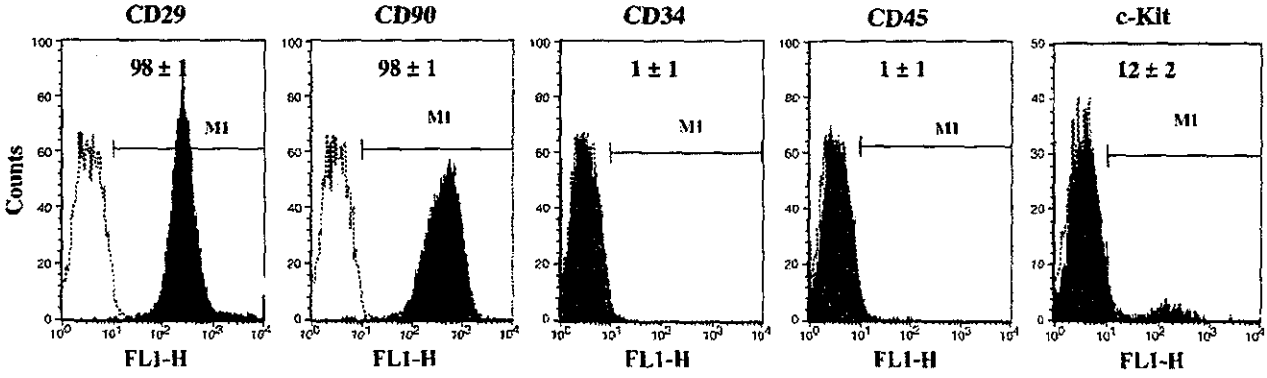

Fig. 1. Flow cytometric analysis of adherent, spindle-shaped mesenchymal stem cell (MSC) population expanded to 4–5 passages. Most of the cells expressed CD29 and CD90 but were negative for CD34 and CD45. Some cells were positive for c-Kit. MI, myocardial infarction.

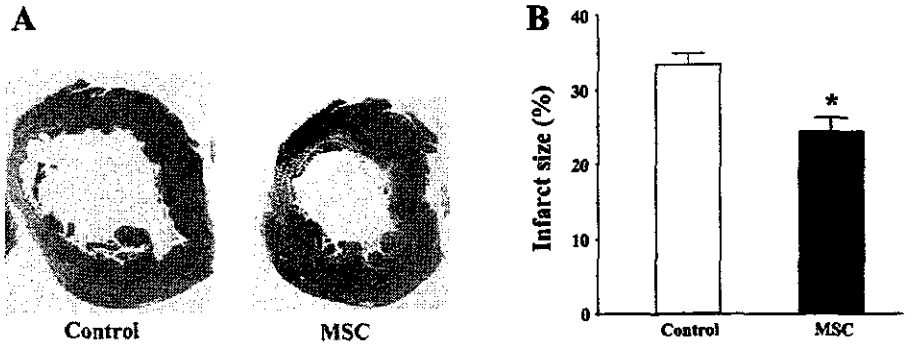

Fig. 2. Effect of MSC transplantation on myocardial infarct size 4 wk after myocardial infarction. *A*: representative Masson's trichrome-stained myocardial sections from control and MSC groups. *B*: quantitative analysis demonstrating that MSC transplantation significantly decreased infarct size. Values are means ± SE. $*P < 0.05$ vs. control.

transversely cut into 5-μm slices for calculation of the numbers of transplanted MSCs in the heart ($n = 5$).

Statistical analysis. Numerical values were expressed as means ± SE unless otherwise indicated. Comparisons of parameters among the three groups were made using one-way analysis of variance (ANOVA) followed by Scheffé's multiple comparison test. Comparisons of parameters between two groups were made by unpaired Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Characterization of cultured MSCs. Most of cultured adherent cells expressed CD29 and CD90 (Fig. 1). In contrast, a majority of adherent cells were negative for CD34 and CD45. A small fraction of the adherent cells expressed c-Kit. Thus we confirmed that the major population of adherent cells was MSCs.

Reduction of myocardial infarct size after MSC transplantation. Moderate-to-large infarcts were observed in Masson's trichrome-stained myocardial sections 4 wk after coronary ligation (control group; Fig. 2*A*). However, MSC transplantation markedly decreased the infarct size after myocardial infarction (MSC group). Quantitative analysis also demonstrated that cardiac infarct size was significantly smaller in the MSC than in the control group: 24 ± 2 vs. 33 ± 2% ($n = 12$ each, $P < 0.05$; Fig. 2*B*).

Hemodynamic effects of MSC transplantation. At 4 wk after coronary ligation, hemodynamic studies were performed in the sham ($n = 11$), control ($n = 12$), and MSC ($n = 12$) groups. LV end-diastolic pressure showed a marked elevation in the control group (18 ± 1 mmHg); the elevation was significantly attenuated in the MSC group (13 ± 1 mmHg, $P < 0.05$; Fig. 3*A*). LV maximum dP/d*t* was significantly higher in the MSC than in the control group (Fig. 3*B*). LV minimum dP/d*t* tended to be lower in the MSC than in the control group (Fig. 3*C*). Although mean arterial pressure was significantly lower in the control than in the sham group, no decrease was observed in the MSC group (Table 1). Heart rate did not significantly differ among the three groups.

LV diastolic dimension was significantly smaller in the MSC than in the control group (Table 2). Fractional shortening was significantly greater in the MSC than in the control group (Fig. 3*D*). LV ejection fraction was also higher in the MSC than in

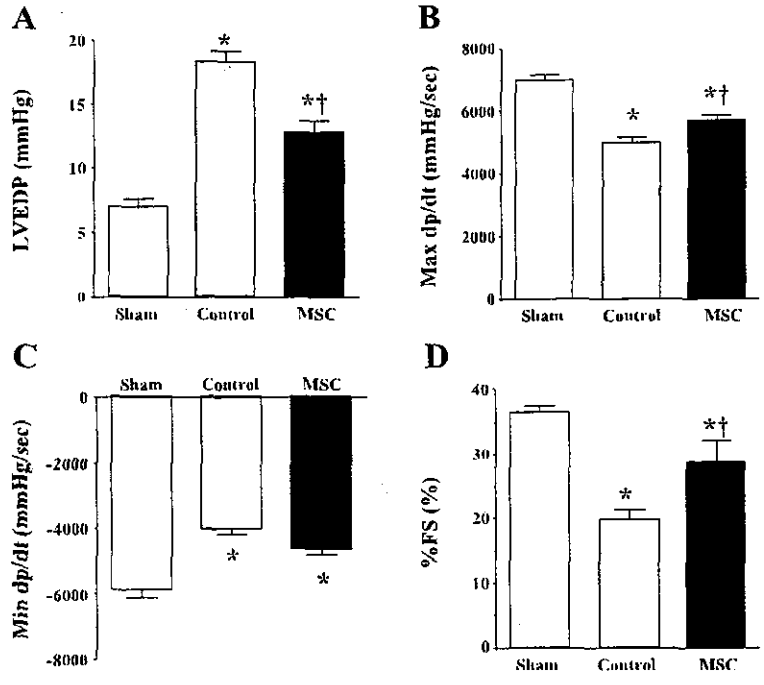

Fig. 3. Effects of MSC transplantation on hemodynamic parameters. LVEDP, LV end-diastolic pressure (*A*); max dP/d*t*, LV maximum dP/d*t* (*B*); Min dP/d*t*, LV minimum dP/d*t* (*C*); %FS, LV fractional shortening (*D*). Values are means ± SE. $*P < 0.05$ vs. sham. $\dagger P < 0.05$ vs. control.

Table 1. *Characterization of animals*

	Sham (n = 11)	Control (n = 12)	MSC (n = 12)
Body wt, g	331±4	301±7*	321±7†
LV wt/body wt, g/kg	1.83±0.11	2.22±0.10*	2.17±0.09*
RV wt/body wt, g/kg	0.55±0.02	0.83±0.04*	0.71±0.03*†
Heart rate, beats/min	404±15	428±17	418±15
Mean arterial pressure, mmHg	128±2	113±4*	119±3

Values are means ± SE. Sham, sham-operated rats given vehicle; control, myocardial infarction rats given vehicle; MSC, myocardial infarction rats given mesenchymal stem cells; LV, left ventricle; RV, right ventricle. *P < 0.05 vs. sham. †P < 0.05 vs. control.

Table 2. *Echocardiographic data*

	Sham	Control	MSC
LVD_d, mm	6.3±0.1	8.6±0.2*	7.5±0.3*†
LVD_s, mm	4.0±0.1	6.9±0.3*	5.5±0.5*†
%FS, %	37±1	20±2*	29±3*†
LVEF, %	65±1	39±3*	53±5*†
AWT diastole, mm	1.6±0.1	1.1±0.1*	1.4±0.1†
PWT diastole, mm	1.6±0.1	1.7±0.1	1.7±0.1

Values are means ± SE. LVD_d, LV diastolic dimension; LVD_s, LV systolic dimension; %FS, LV fractional shortening; LVEF, LV ejection fraction; AWT, anterior wall thickness; PWT, posterior wall thickness. *P < 0.05 vs. sham. †P < 0.05 vs. control.

the control group (Table 2). Diastolic anterior wall thickness was significantly attenuated in the MSC group compared with the control group.

Myogenesis and angiogenesis induced by MSCs. Red fluorescence-labeled MSCs were intravenously administered 3 h after coronary ligation (n = 5). Semiquantitative analysis demonstrated that ~3% of the transplanted MSCs were incorporated into the heart 24 h after transplantation. At 4 wk after transplantation (n = 5), MSCs were incorporated predominantly into the border zone of infarcts (Fig. 4), whereas few MSCs were detected in the noninfarcted myocardium. Immunofluorescence analyses demonstrated that the engrafted MSCs were positive for desmin (Fig. 4), cardiac troponin T (Fig. 5*A*), and connexin43 (Fig. 5*B*). These results suggest the ability of MSCs to engraft in the ischemic myocardium and differentiate into cardiomyocytes. On the other hand, some of the transplanted MSCs were positive for von Willebrand factor and formed vascular structures (Fig. 6). Alkaline phosphatase staining of the ischemic myocardium showed marked augmentation of neovascularization in the MSC group (Fig. 7*A*). Quantitative analysis demonstrated that capillary density was significantly higher in the MSC than in the control group (n = 5 each; Fig. 7*B*).

DISCUSSION

In the present study, we demonstrated that intravenously administered MSCs were capable of engraftment in the ischemic myocardium and that the engrafted MSCs differentiated into cardiomyocytes and vascular endothelial cells, resulting in myogenesis and angiogenesis. We also demonstrated that MSC transplantation decreased myocardial infarct size and improved cardiac function after acute myocardial infarction in rats.

Earlier studies showed that MSCs directly injected into the myocardium or those injected into coronary arteries improve cardiac function after myocardial infarction. However, little information is available regarding the therapeutic potential of systemically delivered MSCs for myocardial infarction. This study demonstrated that intravenous administration of MSCs

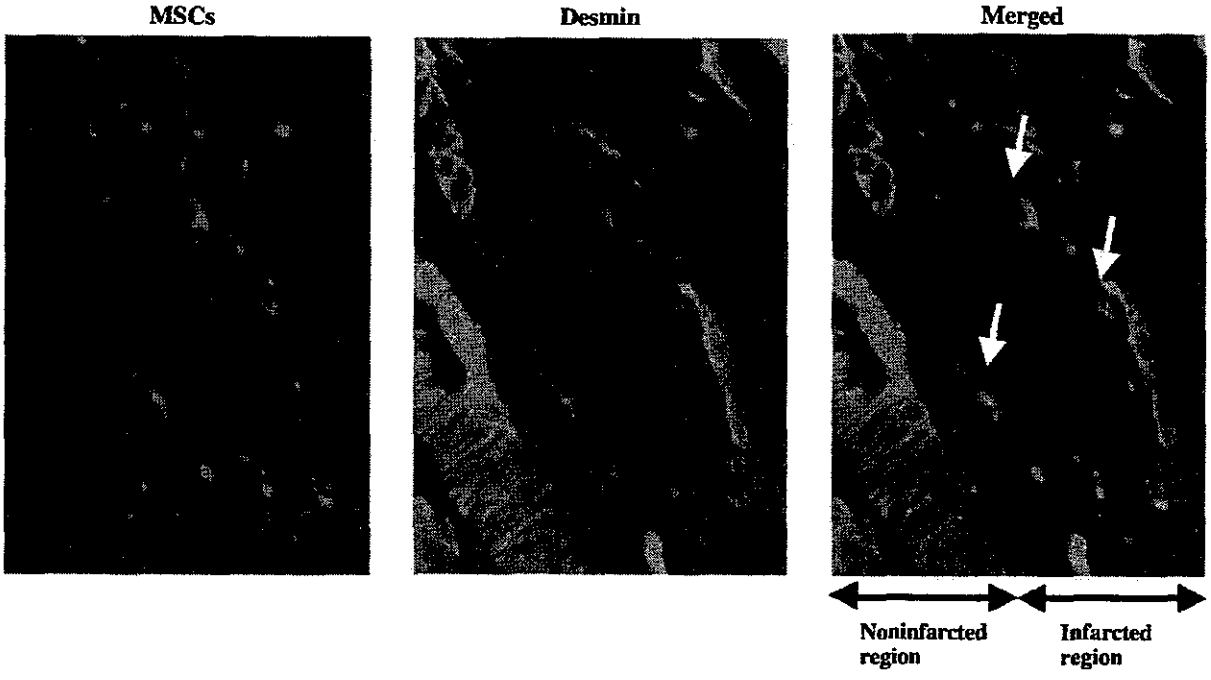

Fig. 4. Distribution of intravenously administered MSCs in myocardium after acute myocardial infarction. Red fluorescence-labeled MSCs were incorporated into ischemic boundary zone of the heart. These cells were positive for desmin (arrows), a cardiac marker. Magnification ×400.

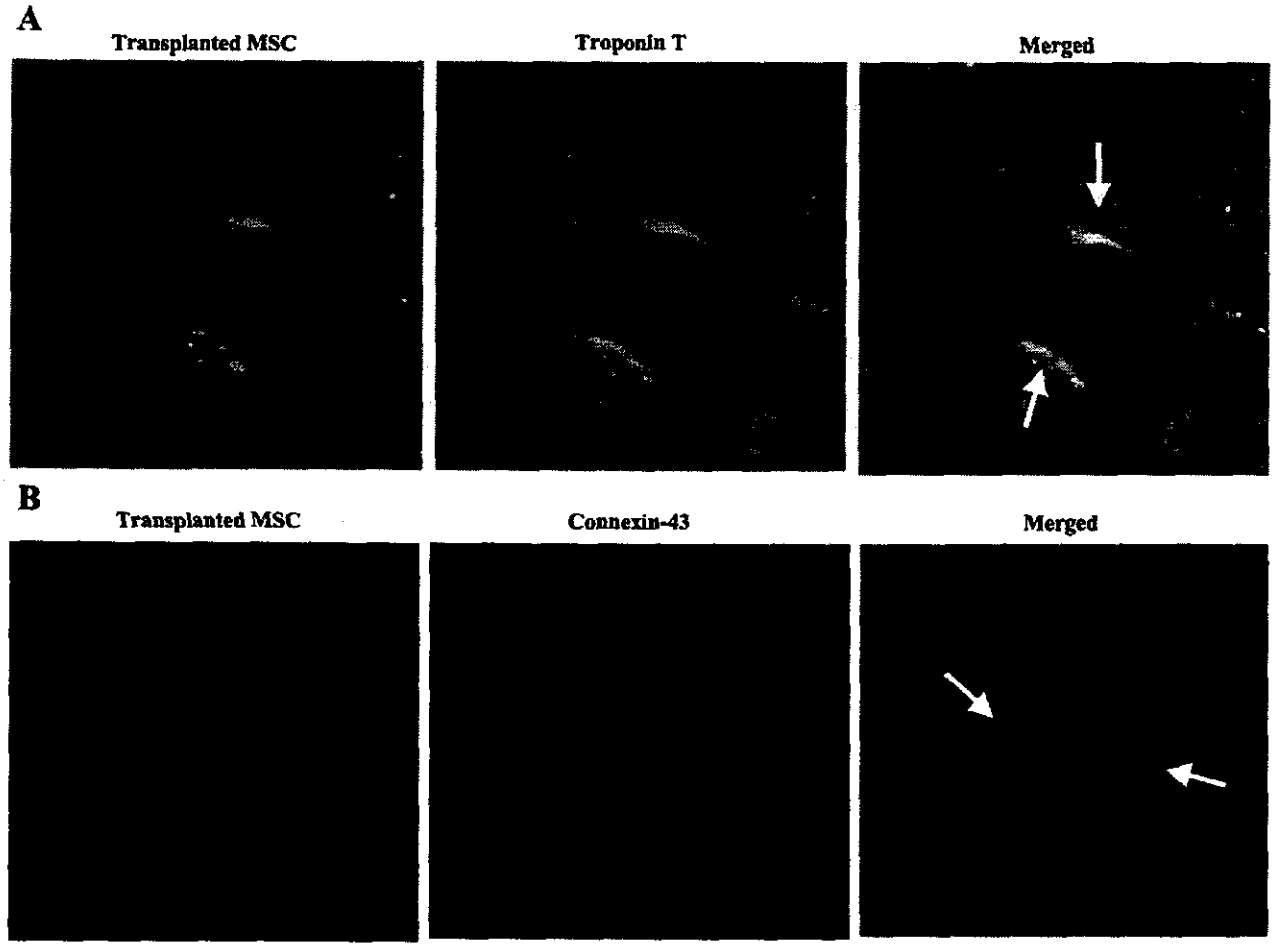

Fig. 5. Differentiation of transplanted MSCs in ischemic myocardium. Engrafted MSCs were positive (arrows) for cardiac troponin T (*A*) and connexin43 (*B*). Magnification ×400.

improves cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis in the ischemic myocardium.

Earlier studies showed that endothelial progenitor cells are mobilized from bone marrow into the peripheral blood in response to tissue ischemia and home to and incorporate into sites of neovascularization (21). Similar to epithelial progenitor cells, in the present study, transplanted MSCs were preferentially attracted to and retained in the border zone of infarcts. This is consistent with recent findings in the ischemic heart (5)

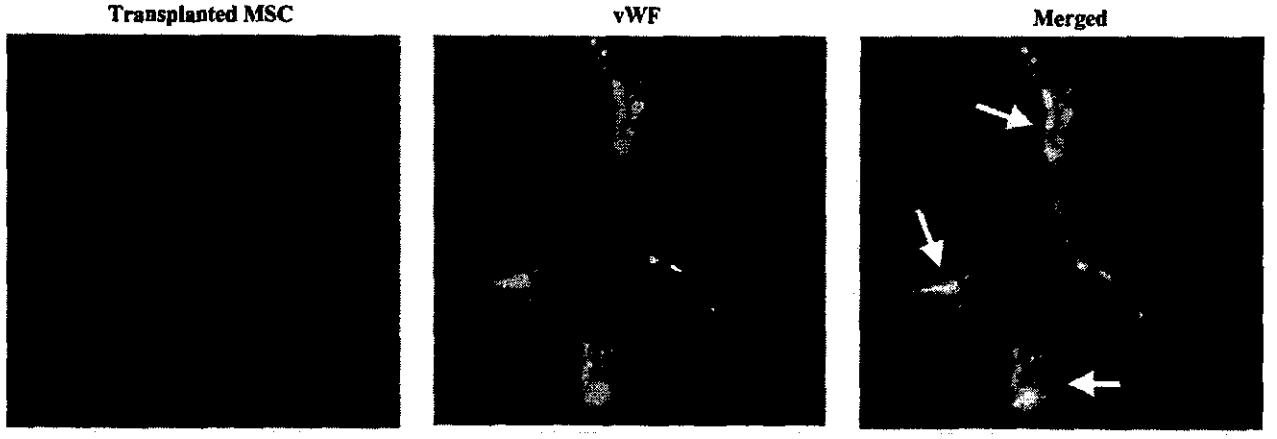

Fig. 6. Transplanted MSCs were positive for von Willebrand factor (vWF) and formed vascular structures. Magnification ×400.

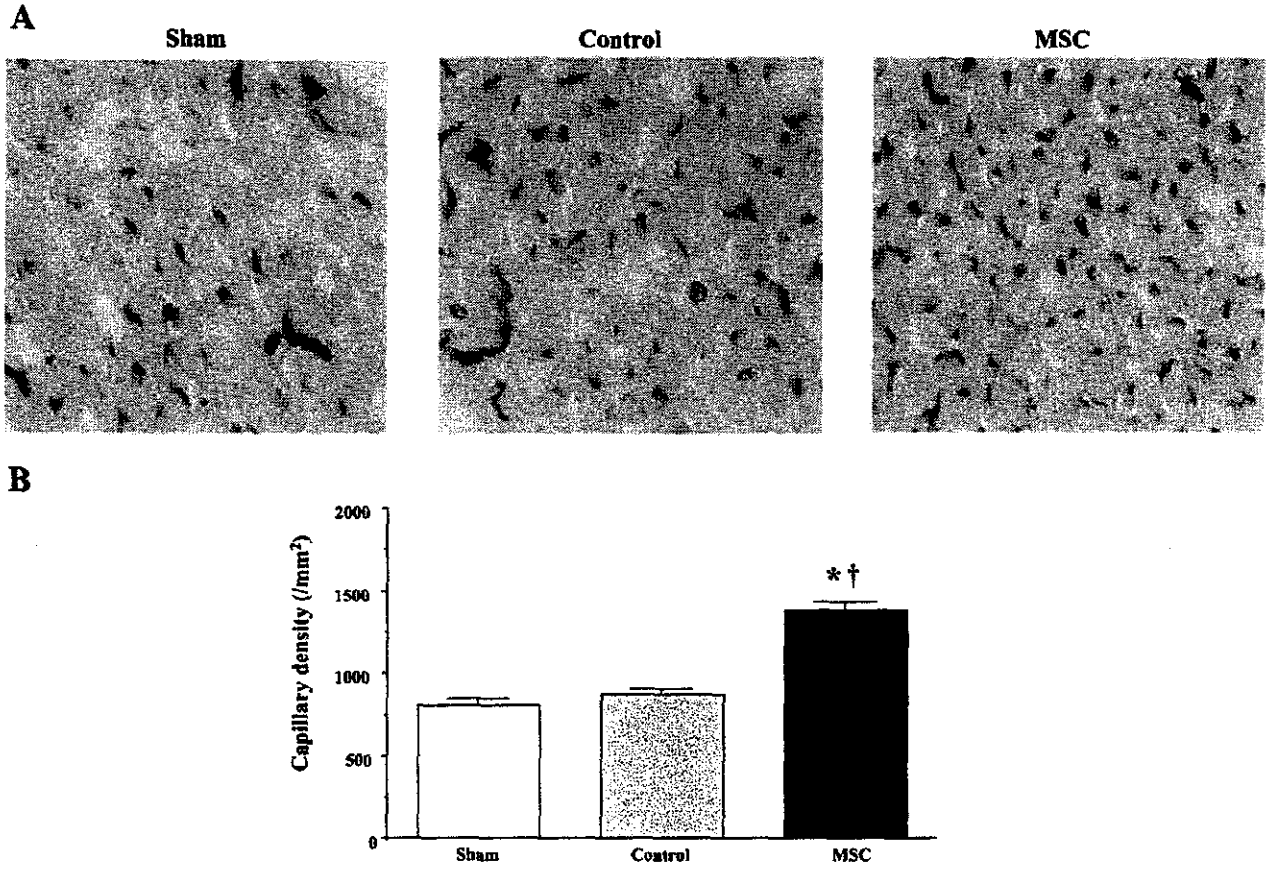

Fig. 7. *A*: representative samples of alkaline phosphatase staining in peri-infarct area. Magnification ×200. *B*: quantitative analysis of capillary density in peri-infarct area. Values are means ± SE. *$P < 0.05$ vs. sham. †$P < 0.05$ vs. control.

or brain (7). Although the underlying mechanisms remain unclear, ischemic tissue may express specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to ischemic sites.

In the present study, some of the engrafted MSCs were stained by cardiac proteins such as desmin and cardiac troponin T. Transplanted MSCs also expressed connexin43, a gap junction protein, at contact points with native cardiomyocytes. These results suggest that MSCs differentiated into cardiomyocytes in the ischemic myocardium and formed connections with native cardiomyocytes. In contrast to skeletal myoblasts, which have been used as a tool for myocardial repair, MSCs may have the capacity for electromechanical coupling. Earlier studies demonstrated the importance of the microenvironment for cardiomyogenic differentiation. Possible factors might include direct cell-cell contact (9), electrical and mechanical stimulation (10), and unknown growth factors. On the other hand, recent studies showed that stem cells may fuse with existing native cells (22, 25). Although the mechanisms by which MSCs develop into cardiomyocyte-like cells remain unclear, it is possible that the direct attachment with host cardiomyocytes in the ischemic myocardium contributes to the cardiogenic differentiation of transplanted MSCs. Further studies are necessary to investigate whether engrafted MSCs are actually becoming contractile.

In the present study, some of the transplanted MSCs were positive for an endothelial cell marker and participated in vessel formation. MSC transplantation significantly increased the capillary density in ischemic myocardium. The recently reported phenotypic plasticity of MSCs to transform into endothelial-like cells provides a rationale for their potential role in neovascularization. Hypoxia has been shown to induce MSC migration and capillary-like structure formation by upregulation of membrane type 1 matrix metalloproteinase (3). MSC implantation has been shown to induce therapeutic angiogenesis in a rat model of chronic hindlimb ischemia (1). These findings support the theory that intravenously administered MSCs are able to differentiate into vascular endothelial cells in the ischemic myocardium. Interestingly, MSCs enhance angiogenesis partly by increasing endogenous levels of vascular endothelial growth factor and vascular endothelial growth factor type 2 receptor (7). Together, these findings suggest that MSCs may contribute to neovascularization in the ischemic myocardium not only through their ability to generate capillary-like structures and but also through growth factor-mediated paracrine regulation.

The present study showed that MSC transplantation significantly reduced infarct size and attenuated wall thinning after acute myocardial infarction. Cardiomyocyte apoptosis during ischemia is one of the major contributors to the development of myocardial infarcts (16, 20). It is possible that newly formed vessels after MSC transplantation improve tissue perfusion around the ischemic boundary zone, resulting in functional recovery after acute myocardial infarction. We also demonstrated that transplanted

MSCs differentiated into cardiomyocytes in the ischemic myocardium. These results suggest that the decrease in infarct size and the increase in wall thickness may be attributable not only to MSC-induced neovascularization but also to myocardial regeneration. In the present study, MSC transplantation improved cardiac function after acute myocardial infarction, as indicated by a significant decrease in LV end-diastolic pressure, a tendency for an increase in maximum LV dP/dt, and a decrease in minimum LV dP/dt. Thus MSC-induced angiogenesis and myogenesis and the resultant reduced infarct size may have contributed to the hemodynamic improvement after acute myocardial infarction.

The low percentage of MSC migration to the heart is in agreement with some previous studies (5, 14). The present study also showed that only a small percentage of transplanted MSCs were incorporated into the heart. This may be explained by MSC apoptosis (12), tracking in the lung (5), and a dilution of the fluorescent dyes as the cells reproduce. Nevertheless, when MSCs were intravenously administered in an acute phase of myocardial infarction, MSCs induced angiogenesis and myogenesis and modestly, but significantly, improved cardiac function. Thus systemic delivery of MSCs may be beneficial for the treatment of myocardial infarction.

A limitation of this study is that the cell population may be mixed, rather than limited to MSCs, although cell surface markers of cultured cells were consistent with those of previously reported MSCs (12, 18).

In conclusion, intravenously administered MSCs were preferentially attracted to the infarcted myocardium and differentiated into vascular endothelial cells and cardiomyocytes. MSC transplantation decreased the infarct size and improved cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis. Thus MSC transplantation may be a new therapeutic strategy for the treatment of myocardial infarction.

GRANTS

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Demonstration of enhanced *K*-edge angiography using a cerium target x-ray generator

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The cerium target x-ray generator is useful in order to perform enhanced *K*-edge angiography using a cone beam because *K*-series characteristic x rays from the cerium target are absorbed effectively by iodine-based contrast mediums. The x-ray generator consists of a main controller, a unit with a Cockcroft-Walton circuit and a fixed anode x-ray tube, and a personal computer. The tube is a glass-enclosed diode with a cerium target and a 0.5-mm-thick beryllium window. The maximum tube voltage and current were 65 kV and 0.4 mA, respectively, and the focal-spot sizes were 1.0 $\times$1.3 mm. Cerium $K\alpha$ lines were left using a barium sulfate filter, and the x-ray intensity was 0.48 μC/kg at 1.0 m from the source with a tube voltage of 60 kV, a current of 0.40 mA, and an exposure time of 1.0 s. Angiography was performed with a computed radiography system using iodine-based microspheres. In coronary angiography of nonliving animals, we observed fine blood vessels of approximately 100 μm with high contrasts. © *2004 American Association of Physicists in Medicine.* [DOI: 10.1118/1.1803433]

Key words: x-ray source, x-ray tube, x-ray spectra, attenuation coefficient, angiography

I. INTRODUCTION

Synchrotrons generate monochromatic parallel x-ray beams using single crystals. These beams with photon energies of approximately 35 keV have been employed to perform enhanced *K*-edge angiography,[1–4] since the beams are absorbed effectively by iodine-based contrast mediums. However, it is difficult to increase the irradiation field, due to the parallel beam, and to obtain sufficient machine times for various research projects, including medical applications.

Currently, flash x-ray generators utilize cold-cathode radiation tubes and produce extremely short x-ray pulses of less than 1 μs. So far, several different flash x-ray generators have been developed,[5] and the generators with photon energies of lower than 150 keV[6–11] can be employed to perform biomedical radiography. In order to produce monochromatic x rays, plasma flash x-ray generators are useful, since quite intense and clean characteristic x rays have been produced from weakly ionized linear plasmas of nickel, copper,[12] and molybdenum,[13] while bremsstrahlung rays are hardly detected at all. Using these generators, the characteristic x-ray intensity substantially increased with corresponding increases in the charging voltage.

Since *K*-series characteristic x rays from cerium target are absorbed effectively by iodine-based contrast mediums, a cerium-target x-ray tube is very useful in order to perform high contrast angiography. On the other hand, cerium is a rare earth element and has a high reactivity, and it is difficult to design the target. However, we are very interested in pro-

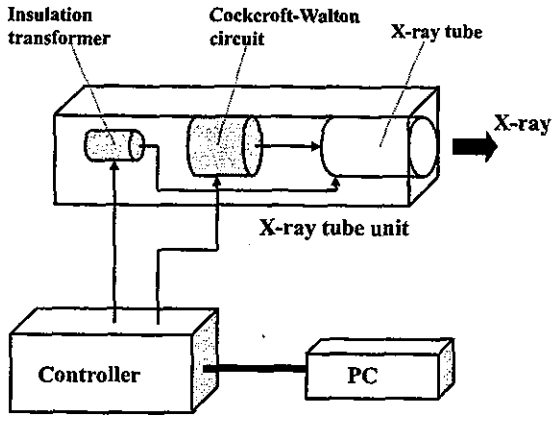

FIG. 1. Block diagram of the compact x-ray generator with a cerium-target radiation tube, which is used specially for *K*-edge angiography using iodine-based contrast mediums.

ducing cerium characteristic x rays to perform cone beam angiography because the irradiation field can be increased easily.

In the present research, we developed a compact x-ray generator with a cerium target tube, and used it to perform a preliminary study on enhanced *K*-edge angiography achieved with cerium $K\alpha$ rays.

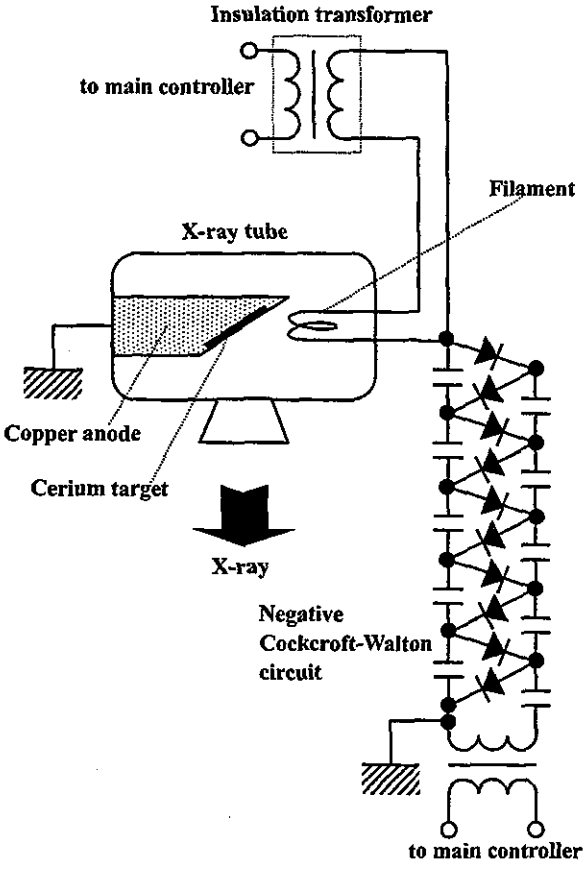

FIG. 2. Main circuit of the x-ray generator.

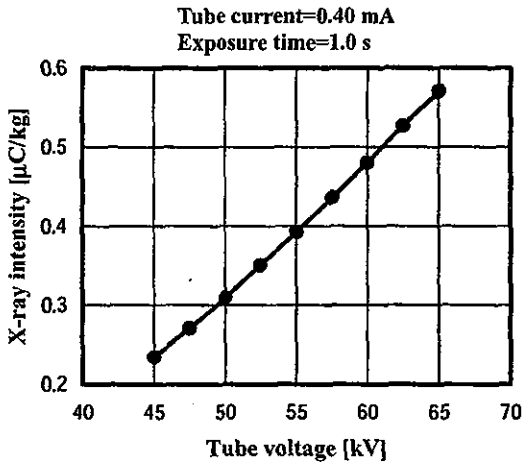

FIG. 3. X-ray intensity measured at 1.0 m from the x-ray source according to changes in the tube voltage.

II. GENERATOR

Figure 1 shows the block diagram of the x-ray generator, which consists of a main controller, an x-ray tube unit with a Cockcroft-Walton circuit, and a cerium-target tube, and a personal computer. The tube voltage, the current, and the exposure time can be controlled by both the controller and the computer. The main circuit for producing x rays is illustrated in Fig. 2, and employed the Cockcroft-Walton circuit in order to decrease the dimensions of the tube unit. In the circuit, the condensers are always in series, and are charged serially. In the x-ray tube, the negative high voltage is applied to the cathode electrode, and the anode (target) is connected to the tube unit case (ground potential) to cool the anode and the target effectively. The filament heating current is supplied by an ac power supply in the controller in conjunction with an insulation transformer which is used for isolation from the high voltage from the Cockcroft-Walton circuit. In this experiment, the tube voltage applied was from 45 to 65 kV, and the tube current was regulated to within 0.40 mA (maximum current) by the filament temperature. The exposure time is controlled in order to obtain optimum x-ray intensity. Monochromatic $K\alpha$ lines were left using a 5-mm-thick barium sulfate filter in which barium sulfate powder was mixed with polymethyl methacrylate (PMMA) resin, since both the bremsstrahlung and the $K\beta$ rays were absorbed effectively by the filter. In designing the filter, the surface density of the barium sulfate powder is important, since the x rays are absorbed effectively by the powder as compared with the PMMA resin. In this case, the density was 7.6 mg/cm^2.

III. CHARACTERISTICS

A. X-ray intensity

X-ray intensity was measured by a Victoreen 660 ionization chamber at 1.0 m from the x-ray source using the filter with an exposure time of 1.0 s (Fig. 3). At a constant tube current of 0.40 mA, the x-ray intensity increased when the tube voltage was increased. In this measurement, the inten-

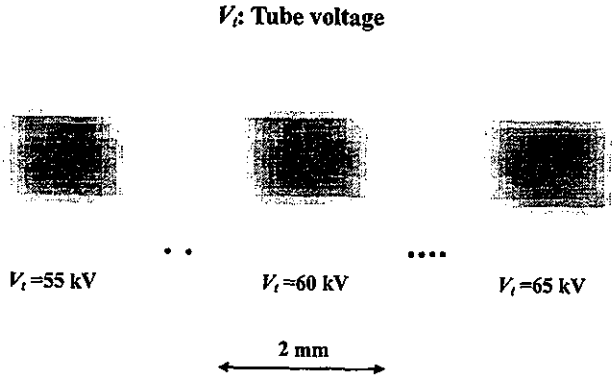

FIG. 4. Effective focal spots with changes in the tube voltage.

sity with a tube voltage of 60 kV and a current of 0.40 mA was 0.48 μC/kg at 1.0 m from the source with errors of less than 0.2%.

B. Focal spot

In order to measure images of the x-ray source after the barium sulfate filtration, we employed a pinhole camera with a hole diameter of 50 μm (magnification ratio of 1:1) in conjunction with a computed radiography (CR) system[14,15] with a sampling pitch of 87.5 μm. When the tube voltage was increased, spot dimensions seldom varied and had values of 1.0×1.3 mm (Fig. 4).

C. X-ray spectra

In order to measure x-ray spectra, we employed a cadmium tellurium detector (CDTE2020X, Hamamatsu Photonics Inc.) (Fig. 5). Compared with a germanium detector, this detector has a lower energy resolution of 1.7 keV. When the tube voltage was increased, the characteristic x-ray intensities of $K\alpha$ lines increased, and both the maximum photon energy and the intensities of bremsstrahlung x rays increased. The barium sulfate filter significantly attenuate the spectra above the barium K-edge energy of 37.399 keV. The areas under the spectral curves correlate closely to the total x-ray intensities shown in Fig. 3.

IV. ANGIOGRAPHY

Figure 6 shows the mass attenuation coefficients of iodine at the selected energies; the coefficient curve is discontinuous at the iodine K edge. The average photon energy of the cerium $K\alpha$ lines is shown just above the iodine K edge. Cerium is a rare earth element and has a high reactivity; however, the average photon energy of $K\alpha$ lines is 34.566 keV, and iodine contrast mediums with a K-absorption edge of 33.155 keV absorb the lines easily. Therefore, blood vessels were observed with high contrasts. Subsequently, in angiography testing, we usually employ nonliving animal phantoms using microspheres.

The angiography was performed by the CR system (Konica Regius 150) using the filter, and the distance (between the x-ray source and the imaging plate) was 1.5 m.

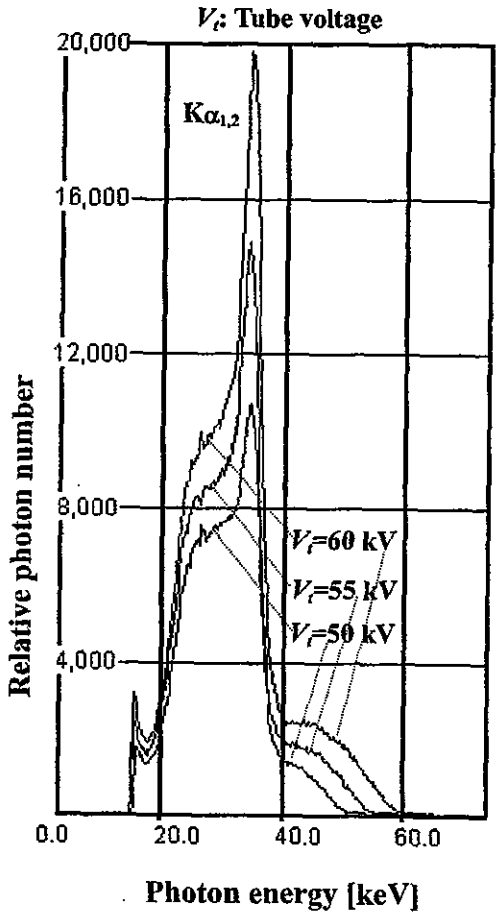

FIG. 5. X-ray spectra measured by a cadmium tellurium detector with changes in the tube voltage.

First, rough measurements of image resolution were made using wires. Figure 7 shows radiograms of tungsten wires in a rod made of PMMA with a tube voltage of 60 kV. Although the image contrast decreased somewhat with decreases in the wire diameter, due to blurring of the image caused by the sampling pitch of 87.5 μm, a 50-μm-diameter wire could be observed.

Angiograms of rabbit hearts are shown in Fig. 8. These two images were obtained using iodine and cerium micro-

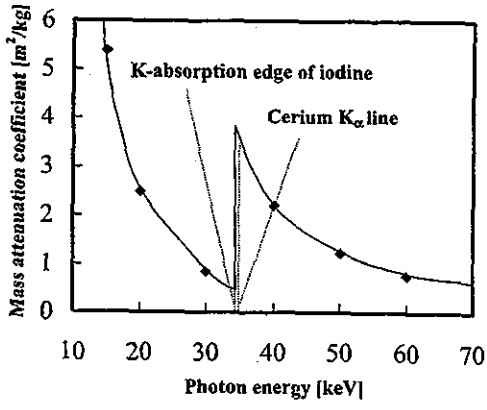

FIG. 6. Mass attenuation coefficients of iodine, and the average photon energy of the cerium $K\alpha$ lines is shown just above the iodine K edge.