Abbreviations and Acronyms

8-OHdG = 8-hydroxy-2′-deoxyguanosine
dp/dt = first derivative of left ventricular pressure
EGCG = epigallocatechin-3-gallate
HR = heart rate
IRI = ischemia–reperfusion injury
LV = left ventricle
MAPK = mitogen-activated protein kinase

to drink the respective EGCG solutions as their daily fluid intakes for 2 weeks. In the control group, rats drank regular water for 2 weeks. Fluid consumption was measured daily for each rat.

Measurement of Plasma EGCG Levels

A technique of liquid chromatography coupled with tandem mass spectrometry was used to measure EGCG in rat plasma as previously described.[12] Just before the Langendorff study, a blood sample was obtained from the inferior vena cava of each rat that had been given EGCG for 2 weeks. Samples were centrifuged at 3000 g for 10 minute. The resulting plasma (100 μL) was mixed with 20 μL of an internal standard (gallocatechin gallate, 0.6 μg/mL) and 20 μL buffer solution (2% ascorbic acid and 1-mol/L sodium acetate, pH 5.0). The mixture was then extracted with 100 μL of a methanol and acetic acid mixture (100/5 volume/volume) and centrifuged for 15 minutes at 11750*g*. After centrifugation, 100 μL of the clear supernatant were transferred to another centrifuge tube, dried under nitrogen, and reconstituted with 100 μL of mobile phase A. An aliquot of the solution was directly injected onto the setup for liquid chromatography coupled with tandem mass spectrometry for analysis, and the plasma EGCG concentration was calculated.

Heart Isolation and Perfusion

Isolated hearts were perfused with a Langendorff apparatus as previously described.[10] The rats were anesthetized by inhalation of diethyl ether and intraperitoneal injection of sodium pentobarbital (50 mg/kg). Anticoagulation was achieved with an intravenous injection of heparin (1000 IU/kg). Each heart was quickly excised, washed in ice-cold saline solution, and mounted on a nonrecirculating Langendorff apparatus. After cannulation of the aorta, the coronary circulation was quickly restarted at a constant pressure of 80 mmHg at 37°C. Krebs–Henseleit solution (sodium chloride 118 mmol/L, potassium chloride 4.7 mmol/L, magnesium sulfate 1.2 mmol/L, sodium hydrogen carbonate 25 mmol/L, monobasic potassium phosphate 1.2 mmol/L, calcium chloride 2.5 mmol/L, and glucose 11 mmol/L) bubbled with a mixture of 95% oxygen and 5% carbon dioxide was used for perfusion. For measurement of left ventricular (LV) function, a water-filled latex balloon was inserted through the left atrium into the LV after a pulmonary arteriotomy. The balloon pressure was adjusted to maintain LV end-diastolic pressure at 10 mm Hg. After a stabilization period of at least 20 minutes, the isolated heart was subjected to no-flow global ischemia at 37°C for 30 minutes, followed by 60 minutes of reperfusion. The LV pressure waveform was traced on a strip chart.

Evaluation of LV Function

To measure LV pressure, the latex balloon was connected by a fluid-filled polyethylene tube to a pressure transducer. After achievement of preischemic perfusion equilibrium, heart rate (HR), LV developed pressure, and positive and negative first derivatives of LV pressure (+dp/dt and −dp/dt, reflecting both systolic and diastolic function) were measured and recorded continuously with LV diastolic pressure stabilized at 10 mm Hg. Coronary flow was measured by collecting coronary effluent buffer. LV function was assessed by calculating the percentage recovery of each parameter (eg, % recovery of HR = HR after 60 minutes of reperfusion/baseline HR × 100%).

Immunohistochemical Assay for Evaluation of Oxidative Stress

The level of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a major product of oxidative DNA modifications,[13] is widely used as a marker of oxidative stress against DNA.[10,14] Cardiac tissues were fixed overnight in paraformaldehyde phosphate buffer solution immediately after reperfusion and then dehydrated sequentially with 50% and 70% ethanol for 24 hours. For immunohistochemical analysis, the avidin–biotin complex method was carried out as previously described.[14] Briefly, after deparaffinization of the specimens, appropriately diluted solutions of normal rabbit serum (Dako Japan Co, Ltd, Kyoto, Japan), mouse monoclonal antibody against 8-OHdG (Japan Institute for the Control of Aging, Fukuroi, Japan), biotin-labeled rabbit anti–mouse IgG serum (Dako), and avidin–biotin complex (Vector Laboratories, Inc, Burlingame, Calif) were sequentially applied. The substrate for alkaline phosphatase (black) was obtained from a vector.

Immunohistologic data (8-OHdG index) were quantified as follows[15]: 8-OHdG index = $\Sigma[(X - \text{threshold}) \times \text{area (pixels)}]$/total cell number, where X is the staining density indicated in gray scale with ImageJ (National Institutes of Health, http://rsbweb.nih.gov/ij/) and Photoshop (Adobe Systems Inc, San Jose, Calif) software.

Western Blotting Analysis for Apoptosis-Related Proteins

Western blotting was performed to identify apoptosis in the myocardium at 60 minutes after reperfusion, as previously described.[16] Ventricular tissue samples obtained from ischemic, reperfused hearts (n = 5 per group) were homogenized on ice in a lysis buffer with protease inhibitor and 1-mmol/L orthovanadic acid and quantified for protein levels with a commercially available assay (BCA protein assay reagent kit; Thermo Fisher Scientific Inc, Rockford, Ill). Proteins (20 μg/sample) were separated with sodium dodecylsulfate polyacrylamide gels (20%) and electrotransferred onto nitrocellulose membranes (Immobilon P; Millipore, Billerica, Mass). After blocking with 5% nonfat milk in tris(hydroxymethyl)aminomethane-buffered saline solution containing 0.1% polysorbate 20, membranes were incubated overnight with the following first antibodies: (1) p38 kinase (sc-7972) at a 1:1000 dilution, (2) phosphorylated p38 (sc-7973) at a 1:1000 dilution, (3) caspase-3 (C9598) at a 1:1000 dilution, and (4) activated caspase-3 (C8487) at a 1:1000 dilution. After incubation with these primary antibodies, horseradish peroxidase–conjugated secondary antibodies were added (1:4000 dilution) for 1 hour at room temperature. Protein bands were enhanced with a chemiluminescence Western blotting determination kit (ECL-Plus; Amersham Pharmacia, Little Chalfont, UK). The band intensity was quantified with imaging software (ImageJ version 1.3).

Statistical Analysis

All data were expressed as mean ± SD. Statistical analyses were performed with statistical software (StatView for Windows version 5.0; SAS Institute Inc, Cary, NC). Data were analyzed by Student *t* test or 1-factor analysis of variance. When results were significant by analysis of variance, differences between individual groups were estimated with the Bonferroni–Dunn post hoc test.

RESULTS

Plasma EGCG Levels in Rats

In all groups, each rat received on average 30 mL/day of EGCG solution or tap water. As the dose of EGCG administered rose, plasma EGCG levels correspondingly increased

TABLE 1. Baseline parameters before ischemia

Variable	Control group (n = 12)	Epigallocatechin-3-gallate groups		
		0.1 mmol/L (n = 12)	1 mmol/L (n = 12)	10 mmol/L (n = 12)
Body weight (g)	422.8 ± 27.9	423.8 ± 19.2	420.1 ± 21.9	428.1 ± 19.1
Heart weight (g)	1.57 ± 0.08	1.61 ± 0.17	1.60 ± 0.14	1.62 ± 0.12
Heart weight/body weight (%)	0.37 ± 0.02	0.37 ± 0.03	0.38 ± 0.03	0.37 ± 0.03
Heart rate (beats/min)	315.4 ± 24.5	312.4 ± 20.4	326.0 ± 32.2	324.5 ± 38.4
LVDP (mm Hg)	96.6 ± 16.4	95.6 ± 11.6	98.5 ± 14.2	98.4 ± 14.3
Maximum +dp/dt (mm Hg/s)	2996.0 ± 538.2	2512.8 ± 301.7	3069.1 ± 727.3	2910.5 ± 654.3
Minimum −dp/dt (mm Hg/s)	2569.0 ± 514.8	2243.6 ± 267.7	2526.0 ± 450.0	2370.4 ± 425.6
Coronary flow (mL/min)	14.9 ± 3.8	16.8 ± 3.4	15.1 ± 3.3	17.1 ± 4.8

All values are mean ± SD. *LVDP*, Left ventricular developed pressure; *dp/dt*, first derivative of left ventricular pressure.

to a greater extent in rats given EGCG solution (0.1, 1, and 10 mmol/L) as drinking fluid for 14 days. Plasma EGCG was significantly higher in the 10-mmol/L group than in the control group (92.7 ± 29.8 vs 0 ng/mL, $P < .0001$), 0.1-mmol/L group (92.7 ± 29.8 vs 0 ng/mL, $P < .0001$), and 1-mmol/L group (92.7 ± 29.8 vs 6.2 ± 2.9 ng/mL, $P < .0001$). Plasma EGCG was undetectable in the 0.1-mmol/L group, just as in the control group.

Baseline Measurement Before Ischemia (Table 1)

There were no significant differences in body or heart weights before the Langendorff study among the 4 groups. Moreover, baseline LV function in the Langendorff study did not differ significantly among the groups.

Cardiac Function After Reperfusion

LV function measurements at 60 minutes after reperfusion are shown in Table 2. The percentage recoveries of LV developed pressure (Figure 1, *B*), maximum +dp/dt (Figure 1, *C*), and minimum −dp/dt (Figure 1, *D*) after 60 minutes of reperfusion were significantly higher in the 1-mmol/L and 10-mmol/L groups than in the control group. Among EGCG groups, the percentage recoveries of LV developed pressure (Figure 1, *B*), maximum +dp/dt (Figure 1, *C*), and minimum −dp/dt (Figure 1, *D*) were significantly higher in the 1-mmol/L group than in the 0.1-mmol/L and 10-mmol/L groups. There were no significant differences in HR among the 4 groups (Figure 1, *A*). Among all cardiac parameters, recovery after reperfusion in the 0.1-mmol/L group was almost equal to that in the control group. There were no significant differences in coronary flow at 60 minutes of reperfusion among the groups (Table 2).

Oxidative Stress on DNA

Representative myocardial images of 8-OHdG immunohistochemical staining are shown in Figure 2, *A*. In the hearts of the 1-mmol/L and 10-mmol/L group rats, there were fewer darkly stained nuclei than in the control and 0.1-mmol/L groups. As shown in Figure 2, *B*, the 8-OHdG index calculated from staining was significantly lower in the 1-mmol/L group than in the control group (98.2 ± 39.4 vs 244.5 ± 105.0 × 10^2, $P < .01$) and 0.1-mmol/L (98.2 ± 39.4 vs 221.9 ± 79.1 × 10^2, $P < .05$) groups. This index was also significantly lower in the 10-mmol/L group than in the control group (125.9 ± 83.2 vs 244.5 ± 105.0 × 10^2, $P < .05$).

Expression of Apoptosis-Related Proteins

Western blotting analyses for p38 (Figure 3, *A*) and caspase-3 (Figure 3, *B*) showed lower expressions of phosphorylated p38 and active caspase-3 in the 1-mmol/L group than in the control group ($P < .05$). Phosphorylation of p38 and caspase-3 cleavage were also significantly lower in the 10-mmol/L group than in the control group ($P < .05$).

DISCUSSION

We found oral pretreatment with EGCG to attenuate myocardial IRI and to preserve LV function after reperfusion in a Langendorff-perfused rat heart model. The antioxidative and antiapoptotic properties of EGCG may be involved in this cardioprotective effect. In addition, oral intake of

TABLE 2. Left ventricular function measurements at 60 minutes after reperfusion

Variable	Control group (n = 12)	Epigallocatechin-3-gallate groups		
		0.1 mmol/L (n = 12)	1 mmol/L (n = 12)	10 mmol/L (n = 12)
Heart rate (beats/min)	311.0 ± 46.1	301.6 ± 26.1	309.9 ± 22.3	298.4 ± 28.3
LVDP (mm Hg)	61.0 ± 17.4	59.3 ± 7.9	83.9 ± 12.8*†‡	72.3 ± 10.2§‖
Maximum +dp/dt (mm Hg/s)	1920.7 ± 591.0	1637.7 ± 174.8	2648.6 ± 599.5*‡	2262.0 ± 595.4‖
Minimum −dp/dt (mm Hg/s)	1589.2 ± 378.9	1403.1 ± 167.1	2033.1 ± 291.7*†‡	1745.0 ± 345.9‖
Coronary flow (mL/min)	10.5 ± 2.7	12.2 ± 1.9	13.0 ± 2.4	12.7 ± 3.3

All values are mean ± SD. *LVDP*, Left ventricular developed pressure; *dp/dt*, first derivative of left ventricular pressure. *$P < .01$ versus control. †$P < .01$ versus 0.1 mmol/L. ‡$P < .05$ versus 10 mmol/L. §$P < .05$ versus control. ‖$P < .05$ versus 0.1 mmol/L.

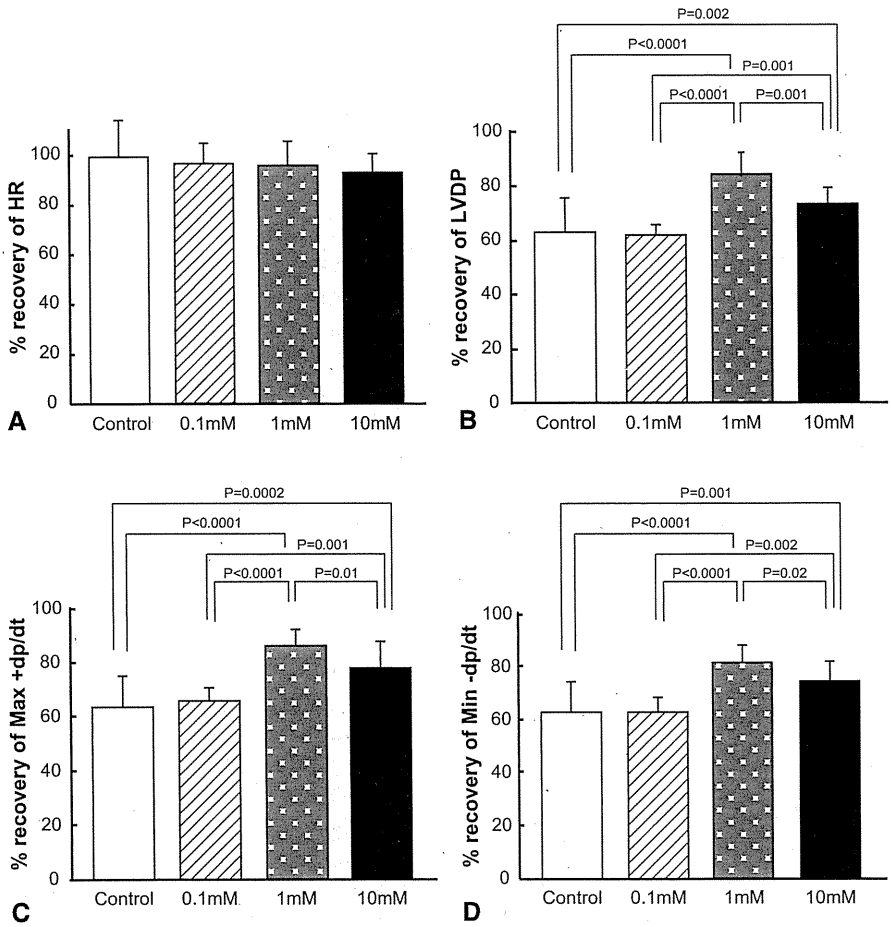

FIGURE 1. Recovery of left ventricular function at 60 minutes of reperfusion as percentage before ischemia. A, Heart rate *(HR)* did not differ significantly among groups. B–D, Recoveries of left ventricular developed pressure (*LVDP*, B), maximum positive first derivative of left ventricular pressure (*Max+dp/dt*, C), and minimum negative first derivative of left ventricular pressure (*Min−dp/dt*, D) in 1-mmol/L and 10-mmol/L groups were higher than in control and 0.1-mmol/L groups. Recoveries of left ventricular developed pressure and maximum positive and minimum negative first derivatives of left ventricular pressure were higher in 1-mmol/L group than in 10-mmol/L group. All values shown as mean ± SD, n = 12 per group.

a high dose of EGCG did not dramatically improve cardiac function after ischemia–reperfusion.

EGCG and Antioxidative Capacity

This novel pretreatment with EGCG, putting the whole body into an antioxidation state before IRI, may serve as a quite reasonable preconditioning method. One of the major theories, supported by most experimental evidence, suggests that reactive oxygen species generation is responsible for postischemic contractile dysfunction.[17] Furthermore, clinical studies have shown that reactive oxygen species generation starts before cardiac surgery and that the availabilities of protective antioxidants depend on preoperative plasma antioxidant status.[18] In this experiment, oral pretreatment with EGCG for 2 weeks before the Langendorff study had no effects on the animals' baseline cardiac parameters. Under IRI conditions, however, LV function was better maintained in the group pretreated with 1 mmol/L EGCG. The potent antioxidative capacity of green tea polyphenols could contribute to LV function recovery after reperfusion, as shown by the lower 8-OHdG indices in the 1-mmol/L and 10-mmol/L groups.

EGCG and Antiapoptotic Effects

Another mechanism possibly underlying the cardioprotective effects of EGCG is its antiapoptotic effect, exerted through oral pretreatment, which may be associated with the preservation of LV function. Oxidative stress is now considered to be a major contributor, serving as a trigger, to myocardial apoptosis. Oxidative stress–induced apoptosis and its prevention by antioxidants have therefore also been analyzed.[19] With respect to catechins, several in vitro investigations have shown EGCG to modulate multiple signal transduction pathways of apoptosis, such as mitogen-activated protein kinases (MAPKs). Furthermore, several studies have found caspase-3 cleavage, a downstream

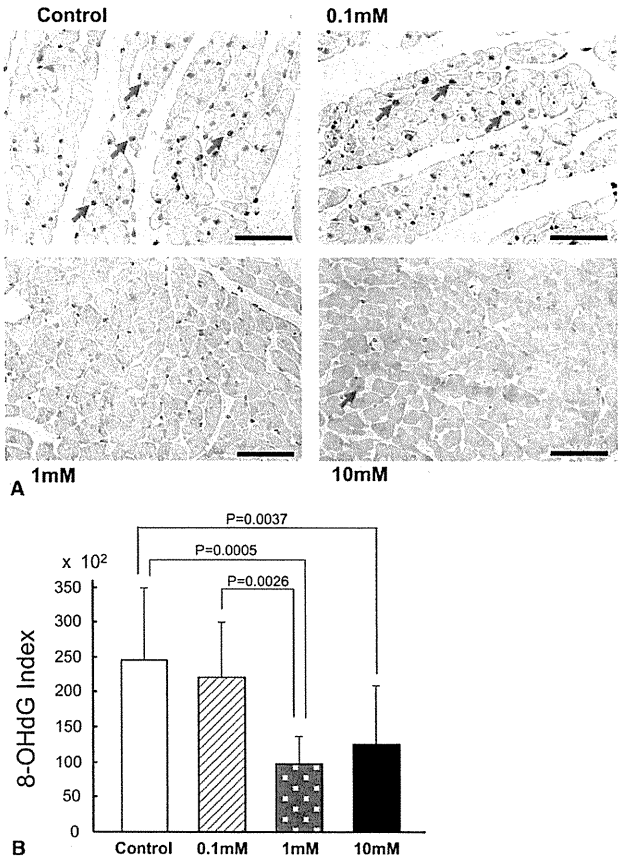

FIGURE 2. Oxidative stress on DNA in cardiomyocytes. A, Representative immunohistochemical analysis images for 8-hydroxy-2′-deoxyguanosine *(8-OHdG)* were obtained with specific monoclonal antibody. Stained nuclei are indicated by *arrows*. *Bar* represents 50 μm. B, Quantification of oxidative stress on DNA, as indicated by 8-hydroxy-2′-deoxyguanosine index. All values shown as mean ± SD.

substrate in caspases known to play an important role in regulating apoptosis, to be blocked by EGCG. In particular, EGCG has been shown to reduce the levels of p38 MAPK phosphorylation,[20] and several studies have confirmed inhibition of p38 MAPK phosphorylation to decrease apoptosis and improve cardiac function after myocardial IRI.[21] Consistent with these findings, our Western blotting analysis demonstrated EGCG to protect against myocardial apoptosis by blocking p38 MAPK phosphorylation and caspase-3 cleavage, indicating that oral pretreatment with EGCG preserved LV function possibly by inhibiting myocardial apoptosis.

Dose Effects of EGCG

In this study, oral pretreatment with a high dose of EGCG did not yield the most potent cardioprotective effects. The cardiac function recovery rates after reperfusion were actually lower in the 10-mmol/L group than in the 1-mmol/L group, despite the blood EGCG concentration remaining higher in the 10-mmol/L group. The plasma EGCG levels of rats given 1-mmol/L EGCG for 2 weeks may have exceeded the saturation point for cardioprotective effects. This result may also, however, be related to some additional adverse effects of EGCG. One possible adverse effect is that EGCG at high concentrations produces an excess of nitric oxide through activation of endothelial nitric oxide synthase. If nitric oxide exceeds a certain level, it reacts with superoxide anion under postischemic reperfusion conditions and yields more toxic radicals, such as hydroxyl radical and peroxynitrite anion, as demonstrated in several studies.[22] The toxic activity of these reaction products may exceed the scavenger activity of EGCG, possibly leading to the suppression of heart function. These results underscore the importance of establishing the optimal oral EGCG dose to maintain recovery of cardiac function and minimize surgical complications, in anticipation of clinical application of EGCG.

Optimal Delivery of EGCG

We advocate that EGCG should be administered orally as a preoperative measure. Recently, with respect to the safety of EGCG, excessively high concentrations have been shown to be cytotoxic and to trigger genotoxic events in mammalian cells.[23] Direct administration of EGCG to the myocardium, such as by intravenous or intracoronary injection, reportedly produces harmful side effects, particularly at high doses. Oral EGCG, however, even when given as a very high bolus dose, is safe in human beings, as shown by clinical trial.[24] In this study, we selected the 2-week oral protocol for administration of EGCG on the basis of a report on the bioavailability of green tea polyphenols in rodents during long-term green tea consumption in drinking fluid.[25] Plasma concentrations of EGCG were shown to gradually increase in the first 2 weeks and then reach a plateau in rats given EGCG in drinking fluid.[25] In considering clinical applications, we will need to establish a period of oral administration of EGCG before heart surgery, referring to several clinical studies to determine the safety and pharmacokinetics of green tea polyphenols with long-term oral administration of EGCG.[24]

Study Limitations

This study has several limitations. First, the heart model used in our experiment may not fully reflect in vivo conditions because of the lack of blood components such as neutrophils, platelets, and blood-derived cytokines. Several studies have demonstrated that neutrophils play an important role in the pathogenesis of IRI. An in vivo study taking into account the influence of blood is therefore in progress. Second, the type of ischemia used in this experimental model was global no-flow ischemia without cardioplegia, which is unlike the type of ischemia used in human heart surgery. This type of ischemia was adopted for this model to facilitate

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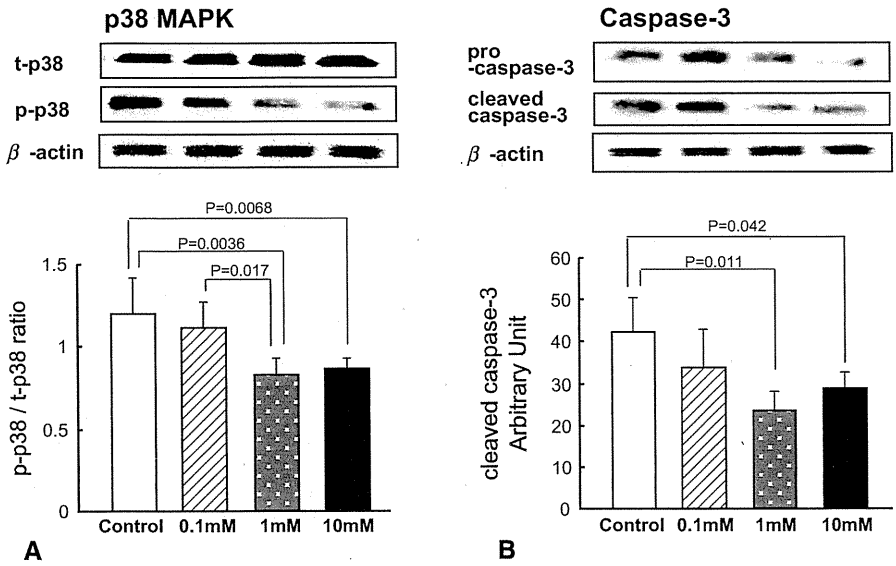

FIGURE 3. Western blotting analysis for apoptosis-related proteins. A, p38 mitogen-activated protein kinase *(MAPK)*, as phosphorylated p38 *(p-p38)* and nonphosphorylated p38 *(t-p38)*. B, Caspase-3. All values shown as mean ± SD.

the evaluation of the cardioprotective effect of EGCG alone, eliminating other cardioprotective factors as far as possible. In the future, further investigations are needed to clarify the cardioprotective effects of oral pretreatment with EGCG in a larger animal model of ischemia with cardioplegia and cardiopulmonary bypass, in view of its clinical application to human heart surgery. These issues will be addressed in future green tea polyphenol clinical trials. Third, in this study, we did not incorporate preischemic measurement of oxidative stress or apoptosis. Although the baseline cardiac function did not differ among the groups, we cannot rule out the possibility that preischemic oxidative stress and apoptosis were suppressed in the EGCG groups. This point needs further investigation. Fourth, the involvement of p38 MAPK expression in apoptosis is still controversial. We cannot definitely conclude that the cardioprotective effect of EGCG exhibited in this study is attributable to p38-mediated apoptosis suppression. We selected p38 for evaluation in this study because in several previous reports EGCG was shown to suppress apoptosis through inhibition of p38 activation under conditions of myocardial IRI.[20]

CONCLUSIONS

Oral pretreatment with EGCG, a green tea polyphenol, attenuated myocardial IRI and enhanced cardiac function recovery after ischemia followed by reperfusion in an isolated rat heart model. The mechanisms of oxidative stress suppression and myocardial apoptosis suppression may be involved in the cardioprotective effects of EGCG. In addition, oral intake of a high dose of EGCG did not dramatically improve cardiac function after ischemia–reperfusion. EGCG pretreatment by oral intake could be a novel and simple preconditioning cardioprotective method for preventing perioperative cardiac dysfunction in cardiac surgical patients.

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ORIGINAL ARTICLE
Regenerative Medicine

Gene and Protein Expression Analysis of Mesenchymal Stem Cells Derived From Rat Adipose Tissue and Bone Marrow

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Background: Mesenchymal stem cells (MSC) are multipotent and reside in bone marrow (BM), adipose tissue and many other tissues. However, the molecular foundations underlying the differences in proliferation, differentiation potential and paracrine effects between adipose tissue-derived MSC (ASC) and BM-derived MSC (BM-MSC) are not well-known. Therefore, we investigated differences in the gene and secretory protein expressions of the 2 types of MSC.

Methods and Results: ASC and BM-MSC were obtained from subcutaneous adipose tissue and BM of adult Lewis rats. ASC proliferated as rapidly as BM-MSC, and had expanded 200-fold in approximately 2 weeks. On microarray analysis of 31,099 genes, 571 (1.8%) were more highly (>3-fold) expressed in ASC, and a number of these genes were associated with mitosis and immune response. On the other hand, 571 genes (1.8%) were more highly expressed in BM-MSC, and some of these genes were associated with organ development and morphogenesis. In secretory protein analysis, ASC secreted significantly larger amounts of growth factor and inflammatory cytokines, such as vascular endothelial growth factor, hepatocyte growth factor and interleukin 6, whereas BM-MSC secreted significantly larger amounts of stromal-derived factor-1α.

Conclusions: There are significant differences between ASC and BM-MSC in the cytokine secretome, which may provide clues to the molecule mechanisms associated with tissue regeneration and alternative cell sources.
(*Circ J* 2011; **75:** 2260–2268)

Key Words: Cell therapy; Mesenchymal stem cells; Microarray; Secretory protein

Mesenchymal stem cells (MSC) are multipotent cells that reside within various tissues, including bone marrow (BM), adipose tissue and many other tissues,[1,2] and can differentiate into a variety of cell types of mesodermal lineage.[1,3] MSC can be expanded in vitro over the short term, and they are thought to be an attractive tool for cell therapy. It has been demonstrated in animal and human studies of cardiovascular disease that transplanted BM-MSC induce neovascularization and differentiate into functional cells.[4–8] In addition, recent studies suggest that MSC exert tissue regeneration, secreting various kinds of angiogenic and cytoprotective factors.[6,9,10]

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Subcutaneous adipose tissue can be harvested more safely and noninvasively than BM, and ASC have emerged as a possible alternative cell source to BM-MSC.[9,11] We and others have demonstrated that ASC transplantation induces neovascularization in animal models of myocardial infarction and hindlimb ischemia.[12,13] ASC are similar to BM-MSC in terms of morphology and surface marker expression.[14] However, few data exist regarding their differences in biological activity, such as proliferative activity, differentiation potential and productive ability. Using microarray and enzyme-linked immunosorbent

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assay (ELISA), we have performed a comprehensive analysis to evaluate both the differences between ASC and BM-MSC, and their usage as an effective transplanted cell source from the point of view of the gene and protein expression profile of the 2 MSC sources.

Methods

Isolation and Culture of ASC and BM-MSC

All protocols were performed in accordance with the guidelines of the Animal Care Committee of the National Cardiovascular Center Research Institute and Kanazawa University. MSC isolation and culture were performed according to previously described methods.[15] In brief, we harvested BM from male Lewis rats (Japan SLC, Hamamatsu, Japan) weighing 200–250 g by flushing their femoral cavities with phosphate-buffered saline. Subcutaneous adipose tissue was harvested from the inguinal region and minced with scissors, then digested with 0.1% type I collagenase (300 U/ml; Worthington Biochemical, Lakewood, NJ, USA) for 1 h at 37°C in a water-bath shaker. After filtration with 100-μm filter mesh (Cell Strainer; Becton Dickinson, Bedford, MA, USA) and centrifugation at 1,240 g for 5 min, MSC were cultured in complete culture medium: α-minimal essential medium (α-MEM; Invitrogen, Carlsbad, CA, USA), 10% fetal bovine serum (FBS, Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). A small number of cells developed visible symmetric colonies by days 5–7. Nonadherent hematopoietic cells were removed, and the medium was replaced. The adherent, spindle-shaped MSC population expanded to >5×10^7 cells within 3–5 passages after the cells were first plated.

Cell Proliferation

We compared the proliferative activity of ASC and BM-MSC in cell culture, as reported previously.[16] In brief, cells (3×10^5 cells/dish) at passage 1 were cultured in a 10-cm dish with complete culture medium, and harvested at 70–90% confluency at each passage. Cell number was counted with a hemocytometer (n=5).

Differentiation of ASC and BM-MSC Into Adipocytes and Osteoblasts

MSC (1×10^5 cells/well) were seeded onto 12-well plates, and differentiation into adipocytes and osteocytes was induced when MSC were 70–80% confluent. MSC were cultured in α-MEM with MSC osteogenesis supplements (Dainippon Sumitomo Pharma, Osaka, Japan) according to the manufacturer's instructions. After 14–17 days of differentiation, cells were fixed and stained with Alizarin Red S (Sigma-Aldrich, St Louis, MO, USA). To induce differentiation into adipocytes, MSC were cultured with adipocyte differentiation medium: 0.5 mmol/L 3-isobutyl-1-methylxanthine (Wako Pure Chemical Industries, Osaka, Japan), 1 μmol/L dexamethasone (Wako Pure Chemical Industries), 50 μmol/L indomethacin (Wako Pure Chemical Industries), and 10 μg/ml insulin (Sigma-Aldrich) in Dulbecco's modified Eagle medium (DMEM, Invitrogen) containing 10% FBS. After 21 days of differentiation, adipocytes were stained with Oil Red O (Sigma-Aldrich). In order to measure lipid accumulation, isopropyl alcohol was added to the stained culture plate, the extracted dye was immediately collected, and the absorbance was measured spectrophotometrically at 490 nm (Bio-Rad, Hercules, CA, USA).

Microarray Analysis of ASC and BM-MSC

To compare the gene expression of ASC and BM-MSC, microarray analysis was performed according to previously reported methods.[17] Total RNA was extracted from cells using an RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was quantified by spectrometry, and its quality was confirmed by gel electrophoresis. Double-stranded cDNA was synthesized from 10 μg of total RNA, and in-vitro transcription was performed to produce biotin-labeled cRNA using GeneChip One-Cycle Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. After fragmentation, 10 μg of cRNA was hybridized with a GeneChip Rat Genome 230 2.0 Array (Affymetrix) containing 31,099 genes. The GeneChips were then scanned in a GeneChip Scanner 3000 (Affymetrix). Normalization, filtering and Gene Ontology analysis of the data were performed with GeneSpring GX 7.3.1 software (Agilent Technologies, Palo Alto, CA, USA). The raw data from each array were normalized as follows: each CEL file was preprocessed with RMA, and each measurement for each gene was divided by the 80th percentile of all measurements. Genes showing at least a 3-fold change were then selected.

Quantitative Real-Time Reverse-Transcription–Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from cultured BM-MSC and ASC as described, and 5 μg of total RNA was reverse-transcribed into cDNA using a QuantiTect reverse-transcription kit (Qiagen) according to the manufacturer's instructions. PCR amplification was performed in 50 μl containing 1 μl of cDNA and 25 μl of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, amplified from the same samples, served as an internal control. After an initial denaturation at 95°C for 10 min, a 2-step cycle procedure was used (denaturation at 95°C for 15s, annealing and extension at 60°C for 1 min) for 40 cycles in a 7700 sequence detector (Applied Biosystems). Gene expression levels were normalized according to that of GAPDH.

ELISA

To investigate differences in protein secretion between ASC and BM-MSC, we measured the levels of various bioactive proteins, including proliferative and anti-apoptotic factors such as hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and adrenomedullin (AM); chemokines such as stem cell-derived factor-1α (SDF-1α); inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6); and adipokines such as leptin and plasminogen activator inhibitor-1 (PAI-1). Protein levels were measured in conditioned medium 24 h after medium replacement. MSC (1×10^6 cells/dish) were plated in 10-cm dishes and cultured in complete culture medium. After 24 h, conditioned medium (n=6) was collected and centrifuged at 2,000 g for 10 min, and the supernatant was filtered through a 0.22-μm filtration unit (Millipore, Bedford, MA, USA). Angiogenic and growth factors were measured by ELISA according to each of the manufacturer's instructions (VEGF, TNF-α: R&D Systems, Minneapolis, MN, USA; HGF: Institute of Immunology, Tokyo, Japan; AM: Phoenix Pharmaceuticals, Burlingame, CA, USA; IL-6: Pierce, Rockford, IL, USA; adiponectin: AdipoGen, Seoul, Korea; PAI-1, Oxford Biomedica Reseach, Oxford, CT, USA).

Statistical Analysis

Data are expressed as mean±standard error of the mean. Comparisons of parameters among groups were made by 1-way ANOVA, followed by Newman-Keuls' test. Differences were

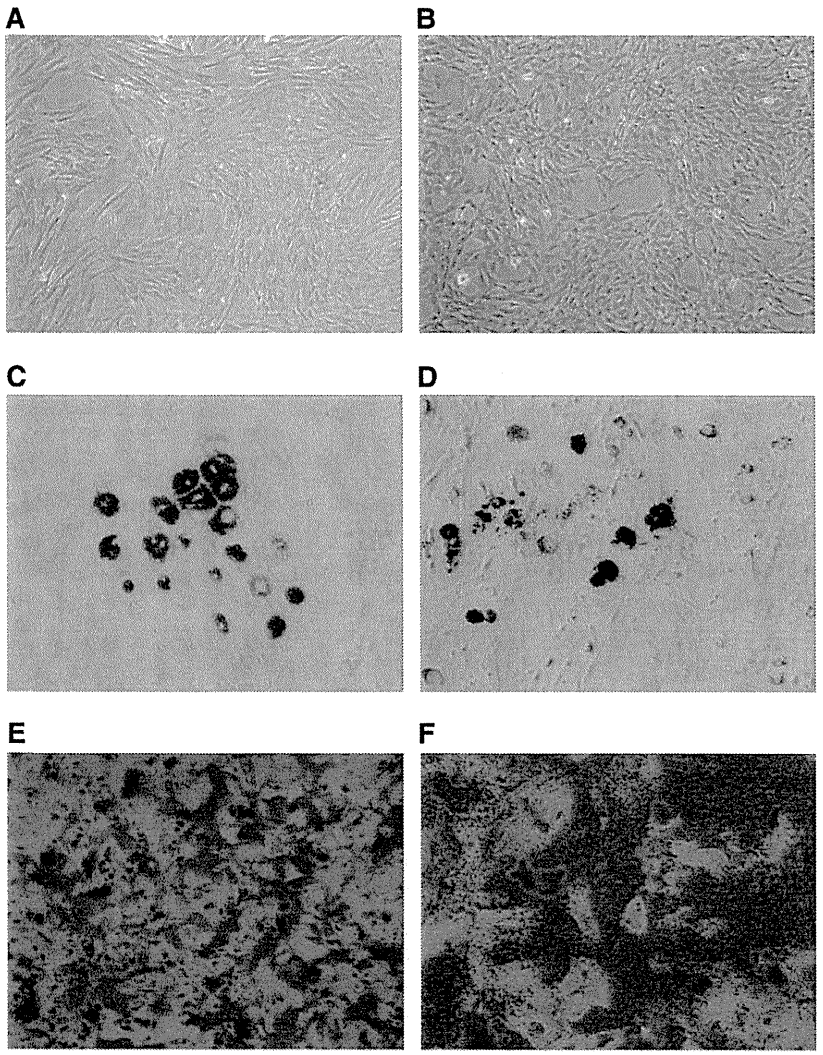

Figure 1. Morphology and differentiation of adipose tissue-derived mesenchymal stem cells (ASC) and bone marrow-derived mesenchymal stem cells (BM-MSC). Cultured ASC (**A**) and BM-MSC (**B**) at passage 2 (×100). Their typical morphology is a similar spindle shape, but ASC are slightly longer than BM-MSC. ASC (**C**) and BM-MSC (**D**) differentiated into adipocytes (×100; Oil Red O). ASC (**E**) and BM-MSC (**F**) differentiated into osteoblasts (×100; Alizarin Red S).

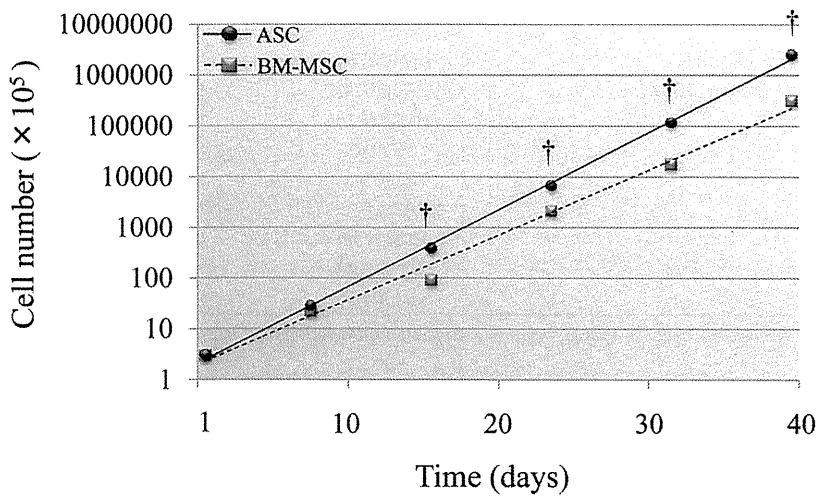

Figure 2. Proliferation of adipose tissue-derived mesenchymal stem cells (ASC) and bone marrow-derived mesenchymal stem cells (BM-MSC) shown as a growth chart. Vertical axis, cell number; horizontal axis, day after first passage. Day 1 is the first day at passage 1. Values are mean±SEM. *P<0.05, †P<0.01 vs. BM-MSC.

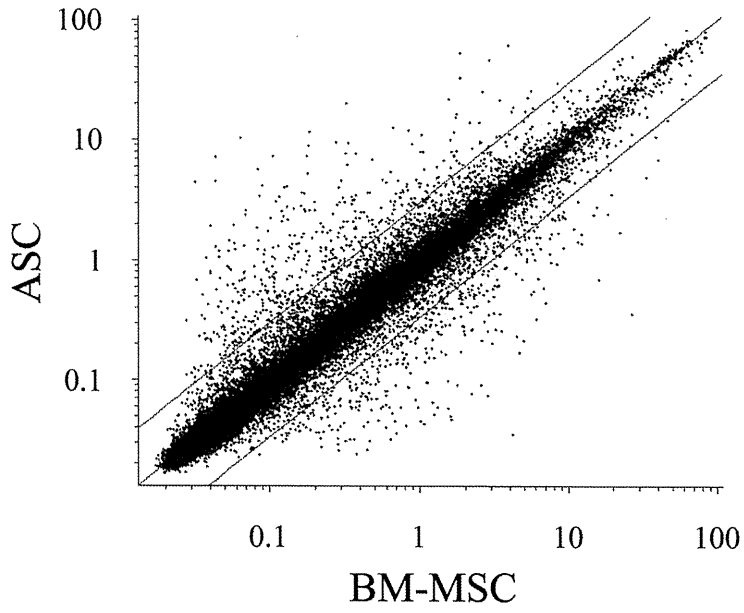

Figure 3. Gene expression profiles of adipose tissue-derived mesenchymal stem cells (ASC, vertical axis) and bone marrow-derived mesenchymal stem cells (BM-MSC, horizontal axis) by microarray. The scatter plot shows normalized microarray datasets of ASC and BM-MSC. All 31,099 gene probes are represented in these plots. The outer lines indicate a 3-fold difference; the central line represents equality.

Table 1. Genes Upregulated in ASC in Comparison With BM-MSC (>10-Fold Upregulation)

Gene name	GenBank Acc. no.	Fold change
Interleukin 1α (Il1a)	NM017019	38.1
Interleukin 1 receptor, type II (Il1r2)	NM053953	21.7
Chemokine (C-X-C motif) ligand 1 (Cxcl1)	NM030845	21.6
Lipocalin 2 (Lcn2)	NM130741	21.5
Fast myosin alkali light chain (Rgd:620885)	NM020104	20.6
Interleukin 6 (Il6)	NM012589	20.5
Chemokine (C-C motif) ligand 20 (Ccl20)	AF053312	17.6
Twist homolog 2 (Twist2)	NM021691	17.5
RAS, dexamethasone-induced 1 (Rasd1)	AF239157	17.1
Complement component 3 (C3)	NM016994	16.9
NADPH oxidase 1 (Nox1)	NM053683	16.3
Matrix metallopeptidase 9 (Mmp9)	NM031055	15.2
Colony-stimulating factor 3 (Csf3)	NM017104	14.5
Prostaglandin E synthase (Ptges)	AB048730	12.8
Adenosine A2B receptor (Adora2b)	NM017161	12.5
Oxidized low-density lipoprotein receptor 1 (Oldlr1)	NM133306	12.4
Uterine sensitization-associated gene 1 protein (Sostdc1)	AA892798	12.1
Chemokine (C-X-C motif) ligand 5 (Cxcl5)	NM022214	11.9
Neuregulin 1 (Nrg1)	U02315	11.8
CD24 antigen (Cd24)	BI285141	11.6
Cathepsin c (Ctsc)	AA858815	11.2
Lymphocyte antigen 68 (C1qr1)	BI282932	11.2
Interleukin 1 receptor antagonist (Il1rn)	NM022194	11.1
Chemokine (C-C motif) ligand 2 (Ccl2)	NM031530	10.8

ASC, adipose tissue-derived mesenchymal stem cells; BM-MSC, bone marrow-derived mesenchymal stem cells.

considered significant at $P<0.05$.

Results

Proliferation and Differentiation of ASC and BM-MSC

Both ASC and BM-MSC could be expanded on a plastic dish, and they exhibited a similar fibroblast-like morphology (**Figures 1A,B**). To examine the potential of ASC and BM-MSC to differentiate into adipocytes, the cells were cultured in adipogenesis medium for 21 days (**Figures 1C,D**). Although lipid droplets were not observed in undifferentiated ASC or BM-MSC, ASC and BM-MSC cultured in adipogenesis

Table 2. Genes Upregulated in BM-MSC in Comparison With ASC (>10-Fold Upregulation)

Gene name	GenBank Acc. no.	Fold change
WNT1 inducible signaling pathway protein 2 (Wisp2)	NM031590	202.5
Complement component factor H (Cfh)	NM130409	81.9
Osteomodulin (Omd)	NM031817	67.4
Solute carrier organic anion transporter family, member 2a1 (Slco2a1)	AI407489	65.8
Dynein, cytoplasmic, intermediate chain 1 (Dncic1)	NM019234	64.8
3-α-hydroxysteroid dehydrogenase (RGD:708361)	BF545626	37.7
Preproenkephalin, related sequence (Penk-rs)	NM017139	29.3
Fc receptor, IgG, low affinity lib (Fcgr2b)	X73371	29.3
Actin, γ 2 (Actg2)	NM012893	25.9
α–2-macroglobulin (A2m)	NM012488	23.2
Lysozyme (Lyz)	L12458	22.2
Jagged 1 (Jag1)	NM019147	19.3
Phospholamban (Pln)	BI290034	17.6
Procollagen, type XI, α 1 (Col11a1)	BM388456	16.2
Gamma sarcoglycan (RGD:1359577)	AA850867	15.3
Pleiomorphic adenoma gene-like 1 (Plagl1)	NM012760	15.0
Matrix metallopeptidase 12 (Mmp12)	NM053963	14.7
Cyclin D2 (Ccnd2)	L09752	14.4
Transforming growth factor, β 2 (Tgfb2)	NM031131	14.3
Solute carrier family 29, member 1 (Slc29a1)	NM031684	14.1
Tissue inhibitor of metalloproteinase 3 (Timp3)	AA893169	13.2
Procollagen, type XI, α 1 (Col11a1)	BM389291	13.1
Down syndrome critical region gene 1-like 1 (Dscr1l1)	AI138048	12.8
Bone morphogenetic protein 4 (Bmp4)	NM012827	12.7
Matrix metallopeptidase 13 (Mmp13)	M60616	11.8
Macrophage galactose N-acetyl-galactosamine specific lectin 1 (Mgl1)	NM022393	11.2
Glycoprotein nmb (Gpnmb)	NM133298	10.7
Aquaporin 1 (Aqp1)	AA891661	10.6
Cadherin 13 (Cdh13)	NM138889	10.5
Selenoprotein P, plasma, 1 (Sepp1)	AA799627	10.5
Secreted frizzled-related protein 4 (Sfrp4)	AF140346	10.4
Cellular retinoic acid binding protein 2 (Crabp2)	U23407	10.2

ASC, adipose tissue-derived mesenchymal stem cells; BM-MSC, bone marrow-derived mesenchymal stem cells.

medium stained positively with Oil Red O in 3 weeks. To quantify lipid accumulation, the absorbance of the extracted cells was measured; however, there was no difference in the absorbance between differentiated ASC and BM-MSC. In addition, both ASC and BM-MSC differentiated identically into osteocytes (**Figures 1E,F**). ASC proliferated more rapidly than BM-MSC; the number of ASC was approximately 10-fold higher than that of BM-MSC at the 40th day (**Figure 2**). In approximately 2 weeks, ASC had expanded almost 200-fold, whereas BM-MSC had expanded nearly 30-fold.

Differences in the Gene Expression of ASC and BM-MSC

Of 31,099 genes analyzed, 571 (1.8%) were more highly (>3-fold) expressed in ASC, whereas 571 genes (1.8%) were more highly (>3-fold) expressed in BM-MSC (**Figure 3**). The genes showing the most enriched expression (>10-fold) in ASC and BM-MSC are listed in **Table 1**. Of note, the genes that were highly expressed in ASC included various types of molecules involved in inflammation, such as IL-1α and IL-6, and chemotaxis, such as chemokine (C-C motif) ligand 20 and chemokine (C-X-C motif) ligand 5 (**Table 1**). The genes that were highly expressed in BM-MSC included differentiation-associated genes, such as WNT1-inducible signaling pathway protein 2 (Wisp2), osteomodulin and jagged1 (**Table 2**). Furthermore, the differential expression patterns of 5 representative genes in ASC and BM-MSC obtained by microarray were confirmed by qRT-PCR, which gave the relative expression of IL-1α as 438.2±560.9 (ratio ASC/BM-MSC, n=5), IL-6 as 54.0±26.6, MMP9 as 3.9±2.2, VEGF 1.8±0.4, and Wisp2 as 7.0±2.2.

To evaluate the genes upregulated in ASC, 571 genes that were more highly expressed in ASC were classified by functional annotation using gene ontology terms (**Table 3**). The 31 terms listed had a P-value <0.00001, and included mitosis (eg, pituitary tumor-transforming 1, cyclin B1, cyclin-dependent kinase 2), immune response (eg, chemokine (C-C motif) ligand 20, cathepsin C and IL-1α) and response to stress (glutathione peroxidase 2, superoxide dismutase 2 and metallothionein). In BM-MSC, 22 terms were listed for the 571 enriched genes, and included regulation of organ development (eg, Wisp2, osteomodulin and bone morphogenetic protein 4), morphogenesis (cadherin 13, elastin and Neuropillin 2) and cell migration (chemokine (C-X3-C motif) ligand 1 and chemokine (C-X-C motif) receptor 4) (**Table 4**).

Differences Between ASC and BM-MSC in Secretory Proteins Determined by ELISA

In previous reports, MSC evoked a cell protective effect and induced angiogenesis via secretion of various cytokines, includ-

Table 3. Classification of Highly (>3-Fold) Expressed Genes in ASC According to Gene Ontology Terms

Category	% of genes in category	% of genes in list in category	P value
0007067: Mitosis	1.3	11.4	4.43×10^{-24}
0000279: M phase	1.8	12.4	6.87×10^{-22}
0000278: Mitotic cell cycle	2.2	12.7	2.53×10^{-19}
0007049: Cell cycle	7.0	21.1	3.23×10^{-16}
0007059: Chromosome segregation	0.31	4.14	6.51×10^{-12}
0006260: DNA replication	1.3	7.32	9.94×10^{-12}
0007088: Regulation of mitosis	0.34	3.82	3.10×10^{-10}
0000070: Mitotic sister chromatid segregation	0.15	2.86	3.11×10^{-10}
0051301: Cell division	0.79	5.41	3.30×10^{-10}
0006955: Immune response	5.7	14.9	1.15×10^{-9}
0007017: Microtubule-based process	1.6	7.32	1.60×10^{-9}
0007093: Mitotic checkpoint	0.13	2.54	2.01×10^{-9}
0000074: Regulation of progression through cell cycle	4.5	12.7	2.77×10^{-9}
0006259: DNA metabolism	4.8	13.1	5.12×10^{-9}
0006952: Defense response	6.2	15.2	7.53×10^{-9}
0009613: Response to pest, pathogen or parasite	3.5	10.8	8.31×10^{-9}
0000075: Cell cycle checkpoint	0.44	3.82	9.71×10^{-9}
0009607: Response to biotic stimulus	6.6	15.6	1.32×10^{-8}
0043207: Response to external biotic stimulus	3.7	10.8	1.73×10^{-8}
0006950: Response to stress	9.2	19.1	3.41×10^{-8}
0031577: Spindle checkpoint	0.084	1.91	7.56×10^{-8}
0007018: Microtubule-based movement	0.87	4.77	8.72×10^{-8}
0006954: Inflammatory response	1.6	6.05	9.52×10^{-7}
0009605: Response to external stimulus	5.9	12.7	4.21×10^{-6}
0050896: Response to stimulus	16	25.8	4.24×10^{-6}
0031649: Heat generation	0.046	1.27	4.68×10^{-6}
0007052: Mitotic spindle organization and biogenesis	0.153	1.91	5.28×10^{-6}
0000226: Microtubule cytoskeleton organization and biogenesis	0.649	3.51	5.55×10^{-6}
0007010: Cytoskeleton organization and biogenesis	4.39	10.1	8.14×10^{-6}
0000067: DNA replication and chromosome cycle	0.0993	1.59	8.43×10^{-6}
0007051: Spindle organization and biogenesis	0.168	1.91	9.76×10^{-6}

ASC, adipose tissue-derived mesenchymal stem cells.

ing VEGF, HGF and SDF-1α.[4,5,10] To compare the proteins secreted by cultured ASC and BM-MSC, we used ELISA to investigate the production of several angiogenic and growth factors from ASC and BM-MSC cultures (**Figure 4**). As compared with BM-MSC, ASC secreted significantly larger amounts of not only HGF and VEGF, which are growth and angiogenic factors, but also PAI-1 and IL-6, which are adipokines. On the other hand, BM-MSC secreted significantly larger amounts of SDF-1α, which is a cell migration-related chemokine, than ASC. There was no significant difference between ASC and BM-MSC for several secreted adipokines, such as adiponectin and TNF-α.

Discussion

In this study, we examined the differences between ASC and BM-MSC in proliferation, differentiation, gene expression and secreted proteins. We showed that (1) ASC are more proliferative than BM-MSC, although there is no difference in differentiation into adipocytes or osteocytes; (2) genes associated with mitosis, inflammation and stress response are highly expressed in ASC; (3) genes associated with regulation of organ development, morphogenesis and cell migration are highly expressed in BM-MSC; and (4) ASC secrete significantly larger amounts of growth factors and inflammatory cytokines than BM-MSC, although BM-MSC secrete significantly larger amounts of chemokine than ASC.

In terms of differentiation, both ASC and BM-MSC differentiated into adipocytes and osteocytes, and there was no difference between them in adipogenesis in our quantitative analysis. A previous report demonstrated that BM-MSC had distinct osteogenic differentiation capability in comparison with ASC,[18] although we did not evaluate difference in osteogenesis between ASC and BM-MSC. Indeed, osteomodulin, which is an osteogenesis-related gene, was upregulated in BM-MSC in comparison with ASC (**Table 2**). Therefore, BM-MSC might have more osteogenic potential than ASC. These findings suggest that ASC and BM-MSC have multilineage potential and an equivalent potential to differentiate into unfavorable cells. Under these conditions, we found that ASC proliferated more rapidly than BM-MSC, and expanded 4-fold as much BM-MSC in approximately 2 weeks. Lee et al compared the proliferation and gene expression profile of human ASC and BM-MSC,[19] and also demonstrated that ASC differ from BM-MSC in terms of proliferation according to culture medium. A large number of MSC are needed for cell transplantation, so rapid proliferation of ASC ex vivo is thought to be a favorable source of transplanted cells in the acute clinical setting, although there remain prob-

Table 4. Classification of Highly (>3-Fold) Expressed Genes in BM-MSC According to Gene Ontology Terms

Category	% of genes in category	% of genes in list in category	P value
0048513: Organ development	8.86	21.9	5.02×10^{-12}
0008283: Cell proliferation	5.07	15.4	1.77×10^{-11}
0040007: Growth	2.18	9.62	4.23×10^{-11}
0009653: Morphogenesis	8.46	20.6	5.90×10^{-11}
0007275: Development	21.1	37.1	1.64×10^{-10}
0016049: Cell growth	1.53	7.56	6.82×10^{-10}
0016477: Cell migration	1.88	8.24	1.29×10^{-9}
0001558: Regulation of cell growth	1.31	6.52	8.83×10^{-9}
0007155: Cell adhesion	5.82	14.7	1.47×10^{-8}
0001501: Skeletal development	1.73	7.21	3.42×10^{-8}
0000902: Cellular morphogenesis	4.19	11.3	1.92×10^{-7}
0040008: Regulation of growth	1.64	6.52	3.21×10^{-7}
0009887: Organ morphogenesis	3.96	10.6	5.31×10^{-7}
0050678: Regulation of epithelial cell proliferation	0.0687	1.71	6.13×10^{-7}
0051674: Localization of cell	2.87	8.59	1.10×10^{-6}
0007626: Locomotory behavior	3.16	8.93	1.92×10^{-6}
0050673: Epithelial cell proliferation	0.084	1.71	2.17×10^{-6}
0006952: Defense response	6.27	13.7	2.26×10^{-6}
0009607: Response to biotic stimulus	6.59	14.1	3.12×10^{-6}
0045785: Positive regulation of cell adhesion	0.045	1.37	3.46×10^{-6}
0042127: Regulation of cell proliferation	3.32	8.93	4.56×10^{-6}
0050874: Organismal physiological process	16.7	27.1	4.75×10^{-6}

BM-MSC, bone marrow-derived mesenchymal stem cells.

lems concerning tumorigenesis and instability.

In this study, we carried out a comprehensive analysis in rat ASC and BM-MSC using microarrays. Interestingly, there was a considerable difference between the gene profile of our data and that of Lee et al,[19] who demonstrated that highly expressed genes in ASC accounted for less than 1% of all genes, and keratin 18, thrombospondin 1 and heat shock protein were included in the list of genes upregulated in ASC as compared with BM-MSC. Their human study was of 16–84-year-old patients undergoing arthroplasty and abdominoplasty, whereas we used 6-week-old rats. It is possible that differences in species and culture conditions, as well as age, contributed to these differences in gene expression.

We demonstrated that many of the genes that were highly expressed in ASC could be classified into categories such as mitosis, cell cycle and inflammatory cytokines, suggesting that ASC are more proliferative than BM-MSC. Thus, ASC transplant may not be superior to BM-MSC in terms of improvement of cardiac function in acute myocardial infarction, although it might be expected that ASC would contribute more to cell proliferation because of their secretion of VEGF and HGF. Also, ASC might initiate a stronger inflammatory response, because of the significantly increased upregulation of genes associated with inflammation as compared with BM-MSC. On the other hand, many of the genes that were highly expressed in BM-MSC were classified into categories such as organ development and morphogenesis. BM-MSC upregulated the expression of genes associated with cardiogenesis and angiogenesis, such as Wisp2, jagged1 and insulin-like growth factor binding protein 4 (IGFBP4). In particular, jagged1 and IGFBP4 have been reported to induce cardiogenesis and angiogenesis, respectively, via activation of notch signals and inhibition of Wnt signals.[20,21] Indeed, a previous report demonstrated that BM-MSC transplantation into the infarcted heart induces cardiogenesis and angiogenesis.[22–24] On the other hand, ASC are also reported to be able to differentiate into cardiomyocytes.[25] Therefore, ASC and BM-MSC both might improve cardiac function by supplementing cardiomyocytes, as well as in a paracrine manner, although we did not investigate differences in differentiation into cardiomyocytes between them.

BM-derived mononuclear cells and MSC have been used for therapeutic angiogenesis in ischemic disease.[26,27] MSC are thought to be more effective than mononuclear cells as a source of transplanted cells because MSC secrete larger amounts of growth factors.[26] Recent studies suggest that MSC exert tissue regeneration not only by differentiation into specific cell types, but also through paracrine actions, secreting various kinds of angiogenic and cytoprotective factors,[5,10] as shown in the present study. A recent report has shown that the combination of VEGF and MSC can enhance angiogenesis after acute myocardial infarction in rats.[28] Additionally, a previous study demonstrated that BM-MSC activate cardiac progenitor cells, which have the ability to differentiate into cardiomyocytes, in a paracrine manner in vitro and in vivo.[29,30] HGF and SDF-1α improve cardiac function via the activation of cardiac progenitor cells.[31] In our study, both ASC and BM-MSC secreted various cytokines and chemokines that are related to angiogenesis and cardiogenesis.

Although ASC are used as an adequate transplanted cell type for the treatment of ischemic limb disease,[32] ASC secrete larger amounts of not only inflammatory cytokines, such as IL-6, but also PAI-1 which promotes coagulation. In our gene analysis, several genes associated with other inflammatory cytokines and chemokines were upregulated in ASC. Not only the gene analysis but also the ELISA results suggested that ASC evoke more inflammation and thrombogenesis than BM-MSC. Therefore, ASC transplantation might be a more useful

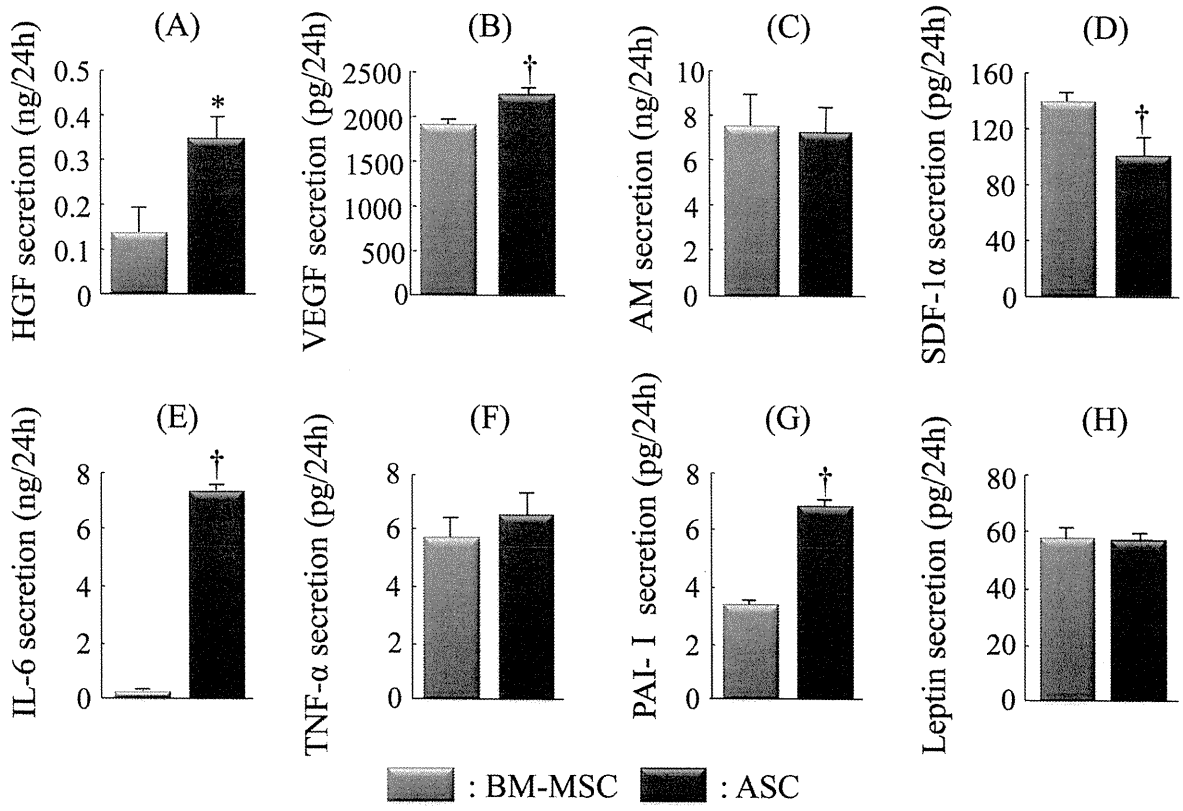

Figure 4. Secretory proteins from adipose tissue-derived mesenchymal stem cells (ASC: red) and bone marrow-derived mesenchymal stem cells (BM-MSC: blue). Conditioned media from ASC and BM-MSC were collected after incubation for 24h in complete medium. Hepatocyte growth factor (HGF, **A**), vascular endothelial growth factor (VEGF, **B**), adrenomedullin (AM, **C**), stem cell-derived factor-1α (SDF-1α, **D**), interleukin-6 (IL-6, **E**), tumor necrosis factor-α (TNF-α, **F**), plasminogen activator inhibitor-1 (PAI-1, **G**), and leptin (**H**) in conditioned media were measured by enzyme-linked immunosorbent assay. Values are mean±standard error of the mean. *P<0.05, †P<0.01 vs. BM-MSC.

treatment for chronic ischemia without severe inflammation.

In this study, we investigated ASC and BM-MSC obtained from young, 6-week-old rats, and we did not examine differences among various generations of rats. A previous report showed that MSC are subject to molecular genetic changes, such as alterations in p53, HGF and VEGF, during aging.[33] Our results might reflect the character of MSC obtained from young rats, contributing to difference from results in humans.[18] We need to further investigate differences between ASC and BM-MSC not only derived from rats but also derived from humans of various ages.

Conclusion

We have demonstrated difference in proliferation and gene expression between ASC and B-MSC, and accordingly, we suggest the importance of selecting the appropriate cell type for transplantation according to the therapeutic indication.

Acknowledgments

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