Therapeutic Angiogenesis With Autologous Hepatic Tissue Implantation and Omental Wrapping

Zhan-Qiang Shao, MD*,**; Michio Kawasuji, MD*; Kentaro Takaji, MD*;
Yukihiro Katayama, MD*; Mai Matsukawa, MD*

Background The liver produces various angiogenic and cytoprotective growth factors and the omentum has potent angiogenic properties that promote wound healing. The ability of hepatic tissue implantation plus omental wrapping to induce angiogenesis and restore cardiac function was investigated in a rat model of infarction.

Methods and Results Myocardial infarction was induced in rats using coronary artery ligation. The omentum was wrapped (omentopexy group), hepatic tissue implantation was combined with omental wrapping (hepatic tissue implantation (H) group) or no other treatment was applied (control (C) group), and then ventricular function was evaluated by echocardiography 4 weeks later. Infarct size, ventricular remodeling, vascular density and collagen density were morphometrically and histologically evaluated. The expression of angiogenic growth factors in implanted tissues was examined using RT-PCR. The H group had thicker ($p<0.05$) and less expanded infarcts ($p<0.001$), as well as higher capillary ($p<0.01$) and arteriolar ($p<0.05$) density in the infarct border zone, than the C group. Hepatocyte growth factor was obviously expressed and the expression of both basic fibroblast growth factor and vascular endothelial growth factor was increased in the H group.

Conclusions Hepatic tissue implantation combined with omental wrapping stimulated angiogenesis, attenuated left ventricular remodeling and improved cardiac function. (Circ J 2008; 72: 1894–1899)

Key Words: Angiogenic therapy; Growth factors; Hepatic tissue; Ischemic heart disease

Conventional methods for myocardial revascularization, including percutaneous coronary intervention and coronary artery bypass grafting, are often limited because of diffuse atherosclerotic lesions or small-caliber vessels. Angiogenic therapy to induce myocardial neovascularization is not dependent on vessel caliber and is an alternative treatment alone or in combination with conventional revascularization. Angiogenic therapies using angiogenic growth factor proteins or gene transfection of DNA encoding angiogenic growth factors might promote the growth of new vessels and induce collateral development for advanced ischemic heart disease!^{1–3} Recent animal studies have suggested that cell transplantation can induce angiogenesis and thus improve regional perfusion and cardiac function!^{4,5} Autologous bone marrow mononuclear cells have been transplanted as the sole approach or as adjunct therapy for patients with severe ischemic heart disease!^{6–8} Bone marrow mononuclear cells have also been transplanted into patients with acute myocardial infarction (MI)^{9–12} Stem cells derived from bone marrow can participate in repair of the diseased myocardium by promoting angiogenesis via angiogenic cytokine secretion, and not through transdifferentiation into heart muscle or incorpora-

tion into vessel walls!^{13,14}

The liver has remarkable regenerative ability and it contains parenchymal cells comprising hepatocytes and non-parenchymal cells. The latter constitutes 20% of the liver tissue and includes stellate cells, Kupffer cells, lymphocytes, and endothelial cells!¹⁵ Stellate cells produce various growth factors including hepatocyte growth factor (HGF), transforming growth factor- β 1 (TGF- β 1) and basic fibroblast growth factor (bFGF), whereas endothelial cells produce vascular endothelial growth factor (VEGF). HGF has cytoprotective and angiogenic activities, and functions in organogenesis and tissue regeneration!^{16,17} The omentum produces VEGF, the potent angiogenic properties of which have been used clinically to promote wound healing and to stimulate the revascularization of ischemic tissues!^{18,19} We postulated that hepatic tissue implantation would induce angiogenesis and restore cardiac function to the infarcted heart. The present study evaluated the ability of hepatic tissue implantation to enhance the angiogenic benefits of omental wrapping, reduce ventricular remodeling and improve cardiac function in a rat model of MI.

Methods

MI Model

Adult male Sprague-Dawley rats ($n=20$) weighing 275–320 g received humane care in compliance with the “Rules of the Animal Experimentation Committee, Kumamoto University, Graduate School of Medical Sciences” and the “Guidelines for Animal Experimentation (1987)” published by the Japanese Association for Laboratory Animal Sciences. The study protocol was approved by the Animal Experimentation Committee of Kumamoto University. Ventilated animals were anesthetized by ether inhalation

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*Department of Cardiovascular Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, **Department of Surgery, Chaoyang Second Hospital, Beijing, PRC

Mailing address: Michio Kawasuji, MD, Department of Cardiovascular Surgery, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. E-mail: kawasuji@kumamoto-u.ac.jp

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Fig 1. Morphometric study of infarcted hearts. Transverse section at mid-papillary level of hearts stained with Sirius red examined under polarized light at $\times 7$ magnification. (a) Control (C), (b) omentopexy (O), (c) hepatic tissue implantation (H).

and a subsequent intraperitoneal injection of pentobarbital. Left thoracotomy through the fourth intercostal space proceeded under sterile conditions. The left anterior descending coronary artery was ligated approximately 2–3 mm distal from its origin with a 6-0 polypropylene suture.²⁰ The peritoneal cavity was entered and the omentum was dissected from the great curvature of the stomach while preserving the arch structure of the gastroepiploic artery. The pedicled omentum was passed from the peritoneal cavity into the mediastinum. Thirty minutes after coronary ligation, the animals were assigned to 3 groups. The epicardium of the infarcted area was abraded and the infarcted area was wrapped with the pedicled omentum in the omentopexy (O) group ($n=9$). In the hepatic tissue implantation (H) group ($n=11$), 1 g of hepatic tissue resected from the left lobe of the liver was placed over the area of infarcted myocardium, followed by omental wrapping. No significant sequelae arose after hepatic tissue resection. The control (C) group ($n=6$) received no additional treatment after undergoing MI. After reaching hemodynamic stability, the chest and abdomen were closed and the animals were left to recover. All animals were killed 4 weeks after treatment except for 6 animals that used for RT-PCR analysis.

Cardiac Function Study

Cardiac function in the 3 groups was measured before and 4 weeks after acute MI using echocardiography (SONOS 4500, Philips, Eindhoven, The Netherlands). Left ventricular end-diastolic and end-systolic dimensions were measured and fractional shortening of the left ventricle was calculated.

Morphometric Study

After completing the physiological study, hearts arrested at diastole by infusing 15% KCl through the left carotid artery were excised, weighed, coded and then fixed in 10% buffered formalin. Four short-axis, 3-mm-thick slices were prepared from the ventricles, embedded in paraffin and then sliced into 5- μ m sections for subsequent morphometric and histopathological evaluation. The sections were stained with Sirius red (Polysciences, Warrington, PA, USA) and assessed using a colored-image analysis system comprising a microscope (SZH10, Olympus, Tokyo, Japan) with a video camera at $\times 7$ magnification (KY-F55MD, Olympus) (Fig 1). Ventricular morphology was evaluated using image analysis software (Mac SCOPEC, Mitani Corporation, Fukui, Japan). The bottom of the third slice from the apex of the heart that represented the mid-papillary level of the left ventricle was morphometrically analyzed. Infarct size was determined as the ratio (%) of epicardial and endocardial circumference occupied by the infarct on all heart slices and calculated as follows:²¹ Infarct size (%) = (infarct endocardial length/total

endocardial length)/2 + (infarct epicardial length/total epicardial length)/2. Infarcted wall thickness was determined as the ratio of the thickness of the infarcted wall to that of the non-infarcted septal wall. Left ventricular cavity area and total left ventricular area were measured and the expansion index was calculated as follows: Expansion index = (left ventricular cavity area/total left ventricular area) $\times$ (non-infarcted septum thickness/infarct wall thickness). Collagen positivity was calculated using the same image analysis software as areas stained with Sirius red. Collagen density (%) is expressed as the ratio of the area of collagen to that of the total myocardial tissue.

Histological Study

The sections were stained with hematoxylin-eosin. We detected angiogenic activity by staining endothelial cells with anti-von Willebrand factor antibody (Zymed, South San Francisco, CA, USA) and smooth muscle cells were stained with antibody to α -smooth muscle actin (Dako, Glostrup, Denmark). Capillaries were identified as having a diameter $< 20 \mu\text{m}$, a thin layer of endothelial cells and no smooth muscle cells. Arterioles were similarly identified as being ≥ 20 and $< 100 \mu\text{m}$, with a thin layer of smooth muscle cells.²² Ten fields in the border zone at $\times 400$ magnification were examined, and number of blood vessels presented as means per unit area (0.2 mm^2). The border zone is the region between the transmural infarct containing only necrotic tissue and outer non-infarct area containing only normal tissue. Cardiomyocyte apoptosis was determined by terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) assays using the Apop Tag® Plus In Situ Apoptosis Detection Kit (Chemicon, Temecula, CA, USA). Total and TUNEL-positive cardiomyocyte nuclei were counted in the same area of 8 fields in the border zone at $\times 400$ magnification. The amount of apoptotic cardiomyocytes is expressed as the proportion of TUNEL-positive cardiomyocyte nuclei to the total number of cardiomyocyte nuclei.

RT-PCR of Growth Factors

We killed 3 rats each from the O and H groups 1 week after treatment and sampled tissues for RT-PCR analysis of angiogenic growth factors. We reverse-transcribed 5 μg of total RNA obtained from wrapped tissue in the border zone using the SUPERScript II kit (Invitrogen, Carlsbad, CA, USA). We designed specific oligonucleotide primers for rat angiogenic growth factors using Primer Express software (Applied Biosystems, Foster City, CA, USA) as follows: bFGF forward, 5'-ATCACTTCGCTTCCCGCACT; reverse, 5'-AGTATGGCCTTCTGTCCAGG; HGF forward, 5'-CG-ACCGGCTTGCAACAGGAT; reverse, 5'-GCCAAACCC-

Fig 2. Echocardiographic study before and 4 weeks after myocardial infarction. (a) Baseline, (b) control (C), (c) omentopexy (O), (d) hepatic tissue implantation (H).

Table 1 Cardiac Function Measured by Echocardiography at Baseline and 4 Weeks After Myocardial Infarction

	Baseline	C group	O group	H group
<i>n</i>	8	6	6	8
HR (beats/min)	362±15	342±26	358±9	340±31
LVEDd (mm)	7.0±0.3*	10.0±0.7	10.9±0.6	9.5±0.7†
LVESd (mm)	2.6±0.3*	8.4±0.5	8.7±0.7	7.1±1.2†,‡
FS (%)	61.5±4.1*	15.8±1.3	19.3±5.4	25.5±10.3#

Data are means±SD. * $p<0.0001$ vs Control, O and H groups. † $p<0.01$ vs O group. ‡ $p<0.05$ vs Control group.

C, control; O, omentopexy; H, hepatic tissue implantation; HR, heart rate; LVEDd, left ventricular end-diastolic dimension; LVESd, left ventricular end-systolic dimension; FS, fractional shortening.

Fig 3. Results of morphological analysis after myocardial infarction. Infarcts are thicker and less expanded in the H group than in the control group. (a) Infarct size (%). (b) Ratio of infarcted to non-infarcted wall thickness. (c) Expansion index. C, control; O, omentopexy; H, hepatic tissue implantation.

TTTTTTCACCTCCACT; VEGF forward, 5'-ACTGCTGT-ACCTCCACCATG; reverse, 5'-ACCGCCTTGCTTGTC-ACAT. The forward and reverse primers for GAPDH were 5'-TGGCACAGTCAAGGCTGAGA and 5'-CTTCTGAGTGGCAGTGATGG, respectively. Angiogenic growth factors were amplified by RT-PCR using 0.5 μ l of reverse transcriptase reaction in a total volume of 25 μ l and Taq polymerase (Takara Shuzo, Otsu, Japan). The PCR conditions were 18 cycles of 94°C for 30s, 60°C for 1 min and 72°C for 1 min for GAPDH (control), and 28 cycles for bFGF, VEGF, and HGF.

Statistical Analysis

All values are expressed as means±SD. Data were statistically evaluated using the analysis of variance (StatView 5.0, SAS Institute, Berkeley, CA, USA). Dichotomous variables were compared using Fisher's exact test. Differences were considered statistically significant at $p<0.05$.

Results

Cardiac Function

Fig 2 shows echocardiograms taken in M-mode before and 4 weeks after treatment. The left ventricular end-diastolic and end-systolic dimensions were increased at 4 weeks in each group. Table 1 summarizes cardiac function measured by echocardiography. Left ventricular end-systolic dimension at 4 weeks was significantly smaller in the H group than in the C and O groups. Significantly more fractional shortening was evident at 4 weeks in the H group than in the C group ($p<0.05$).

Morphological Analysis

Infarct size was significantly smaller ($p<0.05$; Fig 3a), the ratio of infarcted to non-infarcted wall thickness was significantly higher ($p<0.05$; Fig 3b) and the left ventricular expansion index was significantly lower ($p<0.01$; Fig 3c) in the H group than in the C group.

Fig 4. Histological staining of infarcted heart, implanted hepatic tissue, and omentum. (a) Hematoxylin-eosin (HE) staining, $\times 40$ magnification. (b) von Willebrand factor (vWF) staining, $\times 100$ magnification. (c) The α -smooth muscle actin (α -SMA) staining, $\times 100$ magnification. O, omentum; H, hepatic tissue; M, myocardium.

Fig 5. Effect of hepatic tissue implantation and omental wrapping on angiogenesis and arteriogenesis. (a) Capillary density expressed as number of vessels per visual field (0.2 mm²) at 4 weeks. Capillary density is higher in the H group. (b) Arteriolar density expressed as number of vessels per visual field (0.2 mm²) at 4 weeks. Arteriolar density is higher in the H group. C, control; O, omentopexy; H, hepatic tissue implantation.

Histological Analysis

Fig 4 shows sections from the H group stained with hematoxylin-eosin, as well as von Willebrand factor and α -smooth muscle actin antibodies. At 4 weeks after treatment, hepatic tissue implanted between the myocardium and omentum at the infarcted area and border zones contained many capillaries and arterioles.

Capillary density in the infarct border zone was significantly higher in the H group ($16.3 \pm 2.5/0.2 \text{ mm}^2$, $p < 0.01$) compared with the C group ($11.5 \pm 3.5/0.2 \text{ mm}^2$) (Fig 5a). Arteriolar density was significantly higher in the H group ($1.5 \pm 0.6/0.2 \text{ mm}^2$, $p < 0.05$) compared with the C group ($0.9 \pm 0.3/0.2 \text{ mm}^2$) (Fig 5b). Collagen stained with Sirius red was localized to the infarct area of the H group, but detecta-

Fig 6. Effect of hepatic tissue implantation and omental wrapping on collagen density. Collagen is less dense in the infarcted area of H group at 4 weeks. C, control; O, omentopexy; H, hepatic tissue implantation.

Fig 7. RT-PCR analysis of angiogenic growth factors at 1 week. Expression of hepatocyte growth factor (HGF) is obvious and that of basic fibroblast growth factor (bFGF) and of vascular endothelial growth factor (VEGF) is increased in H group. O, omentopexy; H, hepatic tissue implantation.

ble in the infarcted area and widely scattered in the border zones of the C and O groups (Fig 1). Collagen density (%) was significantly reduced in the H group ($10.5 \pm 3.2\%$, $p < 0.05$) compared with the C group ($14.3 \pm 2.1\%$) (Fig 6). Cardiomyocytes were TUNEL-positive in the infarct, border zones and non-infarcted tissues from all 3 groups. The ratio of TUNEL-positive cardiomyocytes in the border zone did not differ among the 3 groups.

RT-PCR Analysis of Angiogenic Growth Factors

Fig 7 shows RT-PCR analysis of angiogenic growth factor expression at 1 week after treatment. The omentum expressed bFGF and VEGF, but not HGF, in the O group, whereas hepatic tissue wrapped with omentum from the H Group expressed HGF and elevated levels of bFGF and VEGF.

Discussion

The major findings of the present study are as follows. Hepatic tissue implantation combined with omental wrapping stimulated angiogenesis, reduced infarct expansion, attenuated left ventricular remodeling after MI and consequently improved cardiac function in the infarcted rat heart. Angiogenesis enhanced by hepatic tissue implantation might be associated with a paracrine mechanism of releasing various angiogenic growth factors. This study is the first to use hepatic tissue implantation to promote angiogenesis and restore cardiac function in an animal model and the results demonstrate that hepatic tissue implantation into the compromised myocardium is feasible, safe and effective.

Cell therapy is becoming an important novel modality for restoring function in patients with few viable myocytes in an infarcted region.^{7,23,24} Candidates for cell transplantation include bone marrow-derived mesenchymal and non-mesenchymal stem cells, skeletal myoblasts, and cardiac stem cells. Cell transplantation is based on the concept that stem cells with myogenic and/or angiogenic potential might compensate for cardiomyocyte loss and improve myocardial blood flow. Recent studies suggest that stem cells derived from bone marrow can participate in the repair of diseased myocardium through a paracrine mechanism; namely, angiogenesis is promoted by the secretion of various angiogenic cytokines, and not through transdifferentiation into heart muscle or incorporation into vessel walls.^{13,14}

However, the omentum has angiogenic properties and it has been used clinically to promote wound healing and to stimulate revascularization of ischemic organs and tissues!^{9,25} In 1936, O'Shaughnessy introduced cardio-omentopexy, in which the ischemic heart was wrapped with a pedicled omental graft directed through the left diaphragm.²⁶ Vineberg subsequently introduced internal thoracic artery implantation, epicardiectomy and free omental grafting.²⁷ Those procedures were gradually abandoned for clinical use, because omentopexy did not result in sufficient revascularization. Zhang et al reported that VEGF is the major angiogenic factor produced by the omentum and that it might underlie the mechanism of omentum-induced angiogenesis.¹⁸ Ueyama et al described a novel revascularization technique in a rabbit model of MI, in which a gelatin hydrogel sheet incorporating bFGF was placed over the infarcted area and wrapped with pedicled omentum.²⁸

Originally identified as a mitogen for hepatocytes, HGF has mitogenic, motogenic, and morphogenic activities in various cell types via c-Met/HGF receptor tyrosine kinase.²⁹

HGF also has cytoprotective and angiogenic activities, and has a role in organogenesis and tissue regeneration!¹⁷ We postulated that hepatic tissue implantation combined with omental wrapping would participate in repair of the diseased myocardium by promoting angiogenesis and by ameliorating ventricular remodeling after MI.

The present findings showed that hepatic tissue implantation combined with omental wrapping increased capillary and arteriolar density at the infarct border zone and reduced collagen density, which morphologically appeared as reduced infarct size and a thinner infarcted wall. These activities might consequently ameliorate cardiac dysfunction after MI. The RT-PCR findings revealed the expression of mRNA for HGF, bFGF and VEGF, suggesting that implanted hepatic tissue secretes angiogenic growth factors. These growth factors enhance angiogenesis and arteriogenesis, and improve myocardial blood flow. After MI, various growth factors, angiotensin II and endothelin play important roles in the regulation of angiogenesis, collagen production, and ventricular remodeling.³⁰ The significantly reduced collagen density in the H group might be associated with the cytoprotective activity of HGF, which prevents tissue fibrosis by suppressing TGF- β and type I collagen!¹⁶

In our preliminary experiments, the infarcted heart was simply wrapped with the pedicled omentum, which weakly adhered to the pericardium without significant blood vessel communication with the myocardium. Ueyama et al reported that omental wrapping alone does not significantly reduce infarct size or restore cardiac function in a rabbit infarction model!²⁸ Moreover, to implant hepatic tissue on the epicardial surface of the infarcted heart without wrapping is technically difficult. Therefore, we removed the epicardium of the infarcted area, implanted hepatic tissue onto the myocardium, and then wrapped it with the omentum. These procedures allowed dense adhesion of the omentum, implanted hepatic tissues and the myocardium. Histological analysis of sections from the H group revealed the hepatic tissues implanted between the myocardium and omentum, and that these tissues contained many capillaries and arterioles. These results demonstrate that the hepatic tissue survived for 4 weeks after implantation and our findings suggest that the implanted hepatic tissue functioned for at least 1 week after the procedure, because RT-PCR of the implanted tissue confirmed the expression of HGF. The infarcted heart cannot supply sufficient blood flow to the implanted hepatic tissue, but the pedicled omentum might improve the survival of implanted hepatic tissue by providing a new blood supply and thus enhance the benefits of hepatic tissue implantation. Harvesting the pedicled omentum and a piece of hepatic tissue through a small laparotomy would be a simple clinical procedure.

Study Limitations

This study has limitations as a means of assessing angiogenic therapy. The acute infarction model used does not represent the widespread chronic myocardial ischemia found in the clinical setting. However, this model enabled evaluation of the effects of hepatic tissue implantation under pro-angiogenic conditions during the healing period after MI. Furthermore, myocardial blood flow was not assessed. Histological findings of increased vascular density do not constitute evidence of increased myocardial perfusion. We plan to assess myocardial blood flow in a large animal model of chronic ischemia.

In conclusion, this study showed that hepatic tissue

implantation combined with omental wrapping stimulated angiogenesis, reduced thinning of the infarction wall, attenuated left ventricular remodeling after MI and consequently improved cardiac function. Angiogenesis enhanced by hepatic tissue implantation might be associated with the release of various angiogenic growth factors. We are the first to demonstrate that implanted hepatic tissue promotes angiogenesis and restores cardiac function in an animal model of MI.

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References

1. Pecher P, Schumacher A. Angiogenesis in ischemic human myocardium: Clinical results after 3 years. *Ann Thorac Surg* 2000; **69**: 1414–1419.
2. Reul M, Laham RJ, Parker JA, Post MJ, Ware JA, Simons M, et al. Long-term effects of surgical angiogenic therapy with fibroblast growth factor 2 protein. *J Thorac Cardiovasc Surg* 2002; **124**: 28–34.
3. Simons M, Annex BH, Laham RJ, Kleiman N, Henry T, Dauerman H, et al. Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: Double blind, randomized, controlled clinical trial. *Circulation* 2002; **105**: 788–793.
4. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkoff D, Wang J, et al. Neovascularization of ischemic myocardium by human bone-marrow derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001; **4**: 430–436.
5. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001; **410**: 701–705.
6. Hamano K, Nishida M, Hirata K, Mikamo A, Li TS, Harada M, et al. Local implantation of autologous bone marrow cells therapeutic angiogenesis in patients with ischemic heart disease: Clinical trial and preliminary results. *Jpn Circ J* 2001; **65**: 845–847.
7. Tse HF, Kwong KL, Chan JFK, Lo G, Ho CL, Lau CK. Angiogenesis in ischemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 2003; **361**: 47–49.
8. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003; **361**: 45–46.
9. Streuer BF, Brehm M, Zeus T, Köstering M, Hernandez A, Sorg RV, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002; **106**: 1913–1918.
10. Assmus B, Schächinger V, Teupe C, Britten M, Lehman R, Döbert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 2002; **106**: 3009–3017.
11. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: The BOOST randomized controlled clinical trial. *Lancet* 2004; **364**: 141–148.
12. Tatsumi T, Ashihara E, Yasui T, Matsunaga S, Kido A, Sasada Y, et al. Intracoronary transplantation of non-expanded peripheral blood-derived mononuclear cells promotes improvement of cardiac function in patients with acute myocardial infarction. *Circ J* 2007; **71**: 1199–1207.
13. Heil M, Schaper W. Influence of mechanical, cellular, and molecular factors on collateral artery growth (arteriogenesis). *Circ Res* 2004; **95**: 449–458.
14. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchus S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ Res* 2004; **94**: 678–685.
15. Taub R. Liver regeneration: From myth to mechanism. *Nat Rev Mol Cell Biol* 2004; **5**: 836–847.
16. Nakamura T, Matsumoto K, Mizuno S, Sawa Y, Matsuda H, Nakamura T. Hepatocyte growth factor prevents tissue fibrosis, remodeling, and dysfunction in cardiomyopathic hamster hearts. *Am J Physiol Heart Circ Physiol* 2005; **288**: H2131–H2139.
17. Bussolino F, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, et al. Hepatocyte growth factor is a potent angiogenic factor with stimulates endothelial cell motility and growth. *J Cell Biol* 1992; **119**: 629–641.
18. Zhang QX, Mogovern CJ, Mack CA, Budenbender KT, Ko W, Rosenthal TK. Vascular endothelial growth factor is the major angiogenic factor in omentum: Mechanism of the omentum-mediated angiogenesis. *J Surg Res* 1997; **67**: 147–154.
19. Shrager JB, Wain JC, Wright CD, Donahue DM, Vlahakes GJ, Moncure AC, et al. Omentum is highly effective in the management of complex cardiothoracic surgical problems. *J Thorac Cardiovasc Surg* 2003; **125**: 526–532.
20. Shao ZQ, Takaji K, Katayama Y, Kunitomo R, Sakaguchi H, Lai ZF, et al. Effects of intramyocardial administration of slow-release basic fibroblast growth factor on angiogenesis and ventricular remodeling in a rat infarct model. *Circ J* 2006; **70**: 471–477.
21. Alhaddad IA, Tkaczewski L, Siddiqui F, Mir R, Brown EJ Jr. Aspirin enhances the benefits of late reperfusion on infarct shape: A possible mechanism of the beneficial effects of aspirin on survival after acute myocardial infarction. *Circulation* 1995; **91**: 2819–2823.
22. Min JY, Yang Y, Sullivan MF, Ke Q, Converso KL, Chen Y, et al. Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. *J Thorac Cardiovasc Surg* 2003; **125**: 361–369.
23. Menasché P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, et al. Myoblast transplantation for heart failure. *Lancet* 2001; **357**: 279–280.
24. Perin EC, Dohmann HFR, Borojevic R, Silva SA, Sousa ALS, Mequita CT, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart disease. *Circulation* 2003; **107**: 2294–2302.
25. Levashev YN, Akopov AL, Mosin IV. The possibilities of greater omentum usage in thoracic surgery. *Eur J Cardiothorac Surg* 1999; **15**: 465–468.
26. O'Shaughnessy L. Surgical treatment of cardiac ischemia. *Lancet* 1937; **232**: 185–194.
27. Vineberg AM. Revascularization of the entire heart by internal mammary artery implantation, epicardiectomy and free omental graft. *Can Med Assoc J* 1966; **94**: 378–385.
28. Ueyama KJ, Bing G, Tabata Y, Ozeki M, Doi K, Nishimura K, et al. Development of biologic coronary artery bypass grafting in a rabbit model: Revival of a classic concept with modern biotechnology. *J Thorac Cardiovasc Surg* 2004; **6**: 1608–1615.
29. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, et al. Molecular cloning and expression of human hepatocyte growth factor. *Nature* 1989; **342**: 440–443.
30. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res* 2002; **53**: 31–47.