INTRODUCTION

Stem cells which were isolated from adipose tissue have been suggested as a new cell source in regenerative medicine. Evidences have shown that treatment with adipose tissue-derived stem cells (ADSCs) improved heart function.1-4 The putative mechanisms involved are believed to be the ability of ADSCs to differentiate into vascular endothelial cells with its associated paracrine effects.5-10 Both cultured ADSCs and the stromal vascular fraction (SVF) form of adipose tissue-derived cells (ADCs) were found to effectively improve heart function.3

Although many other resources of stem cells including bone marrow-derived mesenchymal stem cells (BM-MSCs), mesenchymal stem cells, and myoblasts have been evaluated in the area of regenerative medicine within the field of cardiology.5,11-13 ADSCs have a variety of unique benefits. ADSCs are abundant in the body, relatively easy to harvest without concern of complications, and convenient compared to the BM-MSCs via bone marrow biopsy.14 They are also well cultured and expanded with high potency compared to other stem cell resources.7,4,15 Also,
they are multipotent cell sources.11,15-17 Most of all, they are free of ethical problems.8,11 Preclinical and clinical studies evaluating the effect of ADSCs in acute myocardial infarction (MI) have recently been performed.1-4 However, less information is available about the effect of ADSCs in reducing left ventricle (LV) remodeling and chamber size. Here, we evaluated the efficacy of cultured ADSCs in improving myocardial perfusion and function as well as the effect on the LV remodeling in a large animal acute MI model to explore its potential therapeutic application in humans.

**MATERIALS AND METHODS**

**Experimental animals and design**
This study protocol was approved by the animal experimental committee of Pusan National University Hospital. A total of 28 pigs were analyzed. Among them, 14 were in the study group (ADSC group), while the other 14 were in the control group. They were followed up for 4 weeks after the induction of MI. The perfusion defect, myocardial salvage (%), and salvage index (calculated from the perfusion defect), left ventricular ejection fraction (LVEF), and left ventricular chamber volume were evaluated using 99mTc methoxyisobutylisonitrile (MBI) scan at baseline and at the 4-week follow-up examination. Wall thickness of the left ventricle (LV), gross infarct area of the ventricles, capillary density, and immunohistochemistry (IHC) staining were also evaluated after pigs were sacrificed. This study was single-blinded, and the operator was not able to know the group difference.

**Cell harvest, isolation, and culture**
Adipose tissues were obtained by simple liposuction from the abdominal subcutaneous fat under general inhalation anesthesia isoflurane (1–3%) just 2 weeks before MI induction. Subcutaneous adipose tissues were digested with collagenase I (1 mg/mL) under gentle agitation for 60 min at 37°C. The digested tissues were centrifuged, and the pellets (SVF) were resuspended in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA, USA)-based media containing 0.2 mmol/L ascorbic acid and 10% fetal bovine serum (FBS) obtained from a bovine spongiform encephalopathy-free herd. The cell fraction was cultured overnight at 37°C/5% CO2 in the above media. After 24 h, the cell medium was changed to a keratinocyte-serum-free medium (KSF, Invitrogen, Carlsbad, CA, USA) fluorescence.

**Flow cytometry analysis**
To characterize the phenotypes of the ADCs, a flow cytometry analysis was performed. At least 50000 cells (in 100 μL of PBS, 0.5% bovine serum albumin, and 2 mmol/L ethylenediaminetetraacetic acid) were incubated with fluorescence-labeled monoclonal antibodies against human Flik-1, CD90, CD31, CD34, CD44, and CD45 (BD Biosciences, CA, USA) or the respective isotype control (1/20 dilution; 4°C, 30 min). After washing steps, the labeled cells were analyzed by flow cytometry using a FACSCalibur flow cytometer and CellQuest Pro software (BD Biosciences).

**MI induction and cell administration**
The MI model was made under the general isoflurane inhalation anesthesia (1–3%) and a ketamine intramuscular injection (20 mg/kg). All of the pigs were pre-medicated with 2.5 mg bisproanol, 100 mg aspirin, and 75 mg clopidogrel daily for 3 days before the ADSC administration. Before the procedure, 10000 units of intravenous heparin were administered. After the procedure, no medication was administered thereafter. The percutaneous approach was used via the right or left femoral artery. Transmural MI was induced with balloon occlusion [angio-plasty over-the-wire (OTW) coaxial balloon, 2.5x20 mm] at the mid left anterior descending artery (LAD) after a second diagonal branch for 3 h under electrocardiography (ECG) and hemodynamic monitoring (Fig. 1). Intravenous amiodarone was administered at a dose of 150 mg to prevent lethal arrhythmia during the procedure. Thirty minutes after the balloon deflation, 2x10^6 of ADSCs were injected via an OTW balloon for 3 min with balloon packing. To prevent slow or no-reflow phenomenon, total dose of ADSCs divided by 3 times was delivered into coronary artery. After a 5-min period to allow for the recovery of coronary flow in the two groups, a final angiograms were performed. After reperfusion period, a dose of 1110 MBq 99mTc-MIBI was injected via a femoral sheath to access the perfusion defect and jeopardize the myocardium with a scintigraphic ex-
amination. Except for the administration of ADSCs, all the man-
agements were same for the control group.

Scintigraphic evaluation
After the procedure, the pigs were directly transferred for the 99mTc MIBI-scan evaluation while still under anesthesia. Imaging was performed with each pig in a supine position under ECG and hemodynamic monitoring using a single head nuclear gamma camera (Phillips Vereta; ADAC Laboratories/Philips Medical Systems, Milpitas, CA, USA). The images were acquired with a circular 180° acquisition of 64 projections with an acquisition time of 30 s per image. From the raw data, dedicated software (AutoSPECT+InStill 5.0, Ultra Myocardial Display Version 3.41 and AutoQUANT 5.1; ADAC Laboratories, CA, USA) was used to analyze the LV parameters including LV wall motion and wall thickening. The myocardial perfusion defect (%) was evaluated at baseline and at the follow-up examination, and myocardial salvage was obtained using the following simple calculation: (initial perfusion defect-final perfusion defect)/initial perfusion defect. Myocardial salvage index was defined as the ratio of myocardial salvage (%) /initial perfusion defect (%). LVEF and LV volumes were also analyzed and compared.

Wall thickness and gross infarct area
To evaluate the gross infarct area and the degree of wall thickness, digital images of every heart slice were documented. Infarct area and wall thickness were demarcated and calculated. The mean of every infarct area on each gross specimen was acquired and presented as total infarct area/total ventricular area (%). The wall-thickness ratio was obtained from the ratio of the infarct area wall thickness to the non-infarct area wall thickness.

Immunohistochemistry staining
The myocardial tissues of the infarct border zone were removed and fixed in neutral buffered 4% (w/v) paraformaldehyde at 4°C for 24 h prior to being embedded in paraffin and sectioned. Transverse muscular sections (5 μm) were deparaffinized and then stained with hematoxylin and eosin. The slides were examined at ×200 magnification (=20 objective lenses with ×10 digital camera). To investigate the ability of ADSCs to differentiate into endothelial cells and cardiomyocytes, various IHC evaluations were performed: stromal cell-derived factor-1 (SDF-1), and CD44 as a mesenchymal origin surface marker, von Willebrand factor (vWF) and CD31 as an angiogenesis marker, and TnT as a cardiomyocyte regeneration marker.

Capillary density
To access the degree of neoangiogenesis at the infarct border zone, microvessels with a diameter <10 μm after vWF staining were counted under a microscope at ×40 magnification in 5 random fields on every slide.

Statistical analysis
All values are presented as mean±standard deviation. The differences were evaluated using the Kruskal-Wallis test for continuous data as well as paired t test. Statistical significance was noted at 2-tailed p values of ≤0.05.

RESULTS
Initially, a total of 109 female pigs aged 8 weeks were enrolled, but 50% of pigs (n=55) died during the acute phase of MI. Another 24% of pigs (n=26) died during the follow up examination or period. There was no significant difference in the number of dropout pigs between two groups. Finally, 28 pigs (14 in the ADSC group, 14 in the control group) survived after 4 weeks and were analyzed. At the 4-week follow-up examination, ADSC engraftment was noticed in all pigs, evidenced by CM-dil fluorescence tagging under fluorescence microscopy at ×40 magnification (Fig. 2).

Scintigraphic analysis: perfusion defects, myocardial salvage, LVEF, and LV remodeling
The degree of improvement of the perfusion defect at follow-up examination was significantly greater in the ADSC group (-13.0% in the ADSC group vs. -2.6% in the control group, p=0.019) (Table 1, Fig. 3). Sequentially, the degree of myocardial salvage and the myocardial salvage index were numerically higher in the ADSC group, but the difference was not statistically significant (myocardial salvage (%), 31.0±24.0 in the ADSC group vs. 9.8±39.3 in the

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**Fig. 1.** Porcine acute myocardial infarction (MI) model. Transmural MI was induced with balloon occlusion (angioplasty over-the-wire OTW coaxial balloon, 2.5×20 mm) at the mid left anterior descending artery (LAD) after a second diagonal branch for 3 h under electrocardiography (ECG) and hemodynamic monitoring.
control group, \( p=0.097 \); myocardial salvage index, 0.7±0.7 in the ADSC group vs. 0.4±1.3 in the control group, \( p=0.345 \).

LVEF (%) decreased from 43.9% at baseline to 35.9% at the follow-up examination in the ADSC group and from 54.7% to 38.9% in the control group. Although the initial LVEF was significantly lower in the ADSC group, the LVEF at 4 week follow up was similar among two groups. The change in LVEF (%) between the baseline and follow-up was lower in the ADSC group without statistical significance (-8.0±15.4 in the ADSC group vs. -15.9±14.8 in the control group, \( p=0.181 \)).

A volumetric evaluation showed a tendency of reduced dilatation of LV chamber size in the ADSC group. Both left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) increased at final examination in both groups, but the change of LV volume was numerically smaller in the ADSC group without significance. \( \Delta \text{LVEDV (mL)}, \ 20.4±20.3 \text{ in the ADSC group vs. } 42.9±26.0 \text{ in the control group, } p=0.109; \Delta \text{LVESV (mL)}, 14.3±15.3 \text{ in the ADSC group vs. } 32.3±27.4 \text{ in the control group, } p=0.155 \) (Table 1).

Wall thickness ratio and infarct area
Gross specimens of 24 pigs (12 in the ADSC group and 12 in the control group) were analyzed. The mean wall thickness ratio was not different between the two groups (0.6±0.2 cm in the ADSC group vs. 0.6±0.2 cm in the control group; \( p=0.375 \)). The infarct area was smaller in the ADSC group with marginal significance (13.9±10.6 cm\(^2\) in the ADSC group vs. 22.7±13.2 cm\(^2\) in the control group; \( p=0.085 \) (Fig. 4).

Immuno histochemical staining
Staining with SDF-1, CD44, vWF, and CD31 were more enhanced in the ADSC group compared to the control group (Fig. 5). However, no differences in TnT staining were observed (not shown). Capillary density was higher in the ADSC group than in the control (Fig. 6).

**DISCUSSION**

This study showed that ADSC treatment after acute MI was as-
sociated with improved LV perfusion defects and might be associated with improved LV remodeling in a porcine MI model. The major mechanism of this improvement might be the ability of ADSCs to differentiate into vascular endothelial cells and the associated paracrine effect rather than direct myocardial regeneration effect as we affirmed in this study.

Valina, et al.1 earlier evaluated the efficacy of ADSCs in an acute MI model. It was the first study of efficacy and safety of cultured ADSCs in an acute MI model compared with BM-MSCs. Using a 99mTc MIBI scan as well as the LVEF change using an LV angiogram, they showed that cultured ADSCs significantly reduced perfusion defects and improved LVEF at the 4-week follow-up examination compared to that observed at baseline, and also proved that the paracrine effect is the major underlying mechanism with IHC staining. Cultured ADSCs showed efficacy similar to that of BM-MSCs.

Fig. 3. Nuclear imaging. The degree of improvement of the perfusion defect at follow-up examination was significantly greater in the ADSC group on 99mTc MIBI-scan. ADSC, adipose tissue-derived stem cell; MIBI, methoxyisobutylisonitrile.

Fig. 4. Comparison of infarct area from gross specimens. Infarct territory was much smaller in the ADSC group. ADSC, adipose tissue-derived stem cell.
Our present study is based on the study of Valina, et al., and our major results support the applicability of the intracoronary injection of ADSCs in an acute MI model. In our study, perfusion defects and myocardial salvage measured by 99mTc-methoxyisobutylisonitrile-single photon emission computed tomography (MIBI-SPECT) were significantly improved, but there was no statistical significance in the LVEF change. In addition, in contrast to the study of Valina, et al., our result showed that the LVEF difference was negative in each group and no statistical difference between the two groups. There could be several explanations for this difference. First, because our study is small and low-powered one, inconclusive result might have been rendered. Second, we evaluated the LVEF change using 99mTc-MIBI-SPECT, not LV angiography. Evaluation of LV systolic function with scintigraphy might not be as accurate as LV angiography or echocardiography. Third, the animals in our study were not administered any medication after the MI; therefore, our MI model showed worsening LV systolic function, in contrast to improved LV systolic function after acute MI in other studies. Although the pattern of LVEF differed from those of other studies and there were no statistically significant differences, our study showed much lower LVEF deterioration in the ADSC group compared to that in the control group, suggesting that the use of ADSCs might diminish the degree of LV deterioration. Finally, the effect of ADSCs on improvement of LVEF might be small or less. Recent interim analysis of first-in-men study of ADSCs in ST-segment elevation myocardial infarction patients supports such suggestion. They showed that ADSCs in freshly isolated form significantly improved the LV perfusion and reduced infarct area, but there was no significant improvement of LVEF measured with various imaging methods. Also, there could be a matter of dosage. Usually up to 10 million cells can be safely delivered into each coronary vessel without significant major micro-infarction or severe embolization. However, in the Valina’s previous study comparing the efficacy between BM-MSCs and ADSCs, they used 2 millions of stem cells and showed

Fig. 5. Identification of phenotype of engrafted adipose tissue-derived stem cells in the infarct border zone by immunohistochemical staining for SDF-1, CD44, vWF, CD31 (original magnification, ×200). Staining with SDF-1, CD44, vWF, and CD31 were more enhanced in the ADSC group compared to the control group. Pattern of the TnT staining was not different between the two groups. ADSC, adipose tissue-derived stem cell; vWF, von Willebrand factor; SDF-1, stromal cell-derived factor-1.

Fig. 6. Comparison of capillary density after immunohistochemical staining for vWF (original magnification, ×200). Capillary density was higher in the ADSC group than in the control. ADSC, adipose tissue-derived stem cell; vWF, von Willebrand factor.
Intracoronary ADSC in Porcine AMI Model

The application of cultured human ADSCs in acute clinical settings is also a potential limitation since several days are needed to prepare cultured ADSCs. For this reason, the practical use of allogenic human ADSCs is under investigation. An alternative plan is the use of freshly isolated human adipose tissue-derived cells (fhADSCs). Although several studies have presented the efficacy of cultured ADSCs in the protection against MI,1,2 one study compared the effect of cultured ADSCs and fhADSCs as the SVF.3 Both types of cells were administered via intramyocardial injection after the surgical ligation of murine LAD and well engrafted into the infarcted myocardium; the cells not only survived but also improved myocardial function. With these encouraging results, the APOLLO trial, the first human phase I/IIa study evaluating the feasibility and safety of fhADSCs, was implemented and the results are pending. An interim analysis showed that the intracoronary injection of fhADSCs was feasible and safe, and showed a trend toward improved cardiac function and perfusion defects, and reduced myocardial scarring.4

In conclusion, this study demonstrated that intracoronary administration of cultured ADSCs improved myocardial perfusion defects, reduced LV chamber dilatation and infarct area in a porcine acute MI model. The underlying mechanism might involve the ability of ADSCs to differentiate into vascular endothelial cells with its associated paracrine effect.

ACKNOWLEDGEMENTS

This work was supported by clinical research grant from Biomedical Research Institute, Pusan National University Hospital (2013).

REFERENCES

1. Valina C, Pinkernell K, Song YH, Bai X, Sadat S, Campeau RJ, et al. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. Eur Heart J 2007;28: 2667-77.
2. Cai L, Johnstone BH, Cook TG, Tan J, Fishbein MC, Chen PS, et al. IFATS collection: human adipose tissue-derived stem cells induce angiogenesis and nerve sprouting following myocardial infarction, in conjunction with potent preservation of cardiac function. Stem Cells 2009;27:230-7.
3. Bai X, Yan Y, Song YH, Seidensticker M, Rabinovich B, Metzele R, et al. Both cultured and freshly isolated adipose tissue-derived stem cells enhance cardiac function after acute myocardial infarction. Eur Heart J 2010;31:489-501.
4.Houtgraaf JH, den Dekker WK, van Dalen BM, Springeling T, de Jong R, van Geuns RJ, et al. First experience in humans using adipose tissue-derived regenerative cells in the treatment of patients with ST-segment elevation myocardial infarction. J Am Coll Cardiol 2012;59:339-40.
5. Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, et al. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem 2004; 14:311-24.

6. Moon MH, Kim SY, Kim YJ, Kim SJ, Lee JB, Bae YC, et al. Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. Cell Physiol Biochem 2006; 17:279-90.

7. Cho HH, Kim YJ, Kim JT, Song JS, Shin KK, Bae YC, et al. The role of chemokines in proangiogenic action induced by human adipose tissue-derived mesenchymal stem cells in the murine model of hindlimb ischemia. Cell Physiol Biochem 2009; 24:511-8.

8. Gneccchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008; 103:1204-19.

9. Miranville A, Heeschen C, Sengenes C, Curat CA, Busse R, Bouloumié A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation 2004; 110:349-55.

10. Planat-Benard V, Silvestre JS, Cousin B, André M, Nibbelink M, Tamarat R, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation 2004; 109:656-63.

11. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284:143-7.

12. Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC. Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg 2000; 120:999-1005.

13. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve 1995; 18:1417-26.

14. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkern JE, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004; 109:1292-8.

15. Madonna R, Geng YJ, De Caterina R. Adipose tissue-derived stem cells: characterization and potential for cardiovascular repair. Arterioscler Thromb Vasc Biol 2009; 29:1723-9.

16. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katze AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7:211-28.

17. Liechty KW, MacKenzie TC, Shaaban AF, Radu A, Moseley AM, Deans R, et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nat Med 2000; 6:1282-6.

18. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410:701-5.

19. Kajstura J, Rota M, Whang B, Casparera S, Hosoda T, Bearzi C, et al. Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. Circ Res 2005; 96:127-37.

20. Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: the scientific foundations of cardiac repair. J Clin Invest 2005; 115:572-83.

21. Kuehle R, Richartz BM, Sayer HG, Kasper C, Werner GS, Höffken K, et al. Lack of regeneration of myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans with large anterior myocardial infarctions. Int J Cardiol 2004; 97:123-7.

22. Silva GV, Litovsky S, Assad JA, Sousa AL, Martin BJ, Vela D, et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. Circulation 2005; 111:150-6.

23. Yang D, Wang W, Li L, Peng Y, Chen P, Huang H, et al. The relative contribution of paracrine effect versus direct differentiation on adipose-derived stem cell transplantation mediated cardiac repair. PLoS One 2013; 8:e59020.

24. Moelker AD, Baks T, Wever KM, Spitskovsky D, Wielopolski PA, van Beusekom HM, et al. Intracoronary delivery of umbilical cord blood derived unrestricted somatic stem cells is not suitable to improve LV function after myocardial infarction in swine. J Mol Cell Cardiol 2007; 42:735-45.

25. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. Lancet 2004; 363:783-4.