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Research Paper

Comparative study of effects of bone marrow cell vs. Ad_5-HGF administration via non-infarct-related artery injection in myocardial infarction in swine

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Abstract

Objective: To evaluate the effect of transplanting bone marrow-derived mesenchymal stem cells (BM-MSCs) or adenovirus$_5$-hepatocyte growth factor(Ad_5-HGF) via non-infarct-related artery injection in swine myocardial infarction models. **Methods**: BM-MSCs were obtained from swine bone marrow and expanded *in vitro* to a purity of >50%. A myocardial infarction(MI) was created by ligating the distal left anterior descending artery in swine. Either BM-MSCs (5×10^6/ml) or Ad_5-HGF (4×10^9 pfu) were transfused via the right coronary artery (non-infarcted artery) four weeks after MI. Gate-controled cardiac perfusion imaging was performed at the end of four and seven weeks after LAD ligation, to evaluate heart function and cardiac perfusion. Morphologic and histologic characteristics of the hearts were also studied. **Results**: (1)The gate-controlled cardiac perfusion imaging showed that the improvement in LVEF was greater in both treatment groups than in control group at the 4th weeks. (2)In both treatment groups, capillary density was significantly higher than that of control group($P < 0.05$). **Conclusion**: BM-MSCs or Ad_5-HGF transplantation via non-infarcted artery administration can stimulate angiogenesis and improve heart function, but there was no difference in therapeutic efficacy between BM-MSCs and Ad_5-HGF.

Keywords: Bone marrow-derived mesenchymal stem cells; hepatocyte growth factor; angiogenesis

INTRODUCTION

With recent progress in cardiac catheterization and surgical techniques, mortality from acute myocardial infarction (AMI) has significantly decreased, while the morbidity associated with congestive heart failure occurring later is gradually increasing. Immediate percutaneous transluminal angioplasty (PTCA) and coronary artery bypass grafts (CABG) could reopen infarct-related vessels and save lives, but ischemia/reperfusion injuries would further aggravate ischemic myocardium, which can induce cardiomyocyte apoptosis, resulting in occurrence of inevitable heart failure [1-3]. Experiments from animals showed that stem cell transplantation and gene therapy (vascular growth factor) could increase angiogenesis and improve heart function. Besides the still disputed theory of cardiomyogenic differentiation, the mechanism of stem cell transfer to improve the heart function mainly depends on its excreting cell growth factors, such as vascular endothelial growth factor(VEGF), to neovascularize and improve the perfusion of myocardium [4]. Vascular growth factor as HGF can induce angiogenesis[5,6]. However, HGF also has the effect of mobilizing stem cells[7,8]. Therefore, in the present study, we compared the impact of transferring adenovirus$_5$-hepatocyte growth factor(Ad_5-HGF) vs. bone marrow-derived mesenchymal stem cells

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(BM-MSCs) to determine the better therapeutic potential for postinfarction heart failure.

Stem cells or genes transfer via infarct-related vessels are usually options considered. However, since the infarction-related vessels are occluded in many ischemic heart disease patients, and sometimes these patients are not suitable for PTCA or CABG, the second aim of the present study was to establish the effects of stem cell or vascular growth factor transference via non-infarct-related vessels.

MATERIALS AND METHODS

Myocardial infarction model of experimental animal

This study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University. Eighteen young male Suzhong swine, weighing 24.2 ± 2.6 kg (Jiangsu Academy of Agri cultural Sciences), were randomly divided into 3 groups(n = 6 in each group). Swine were anestheized with a combination of ketamine (50 mg/Kg) and xylazine (10 mg/Kg). AMI was induced by ligating the distal left anterior coronary artery (LAD)[9,10]. Electrocardiography was used to monitor heart rate, rhythm, and ST-segment changes during the surgical procedure. Seven weeks after LAD ligation, all animals were killed after their heart function was evaluated. The myocardium samples collected from all animals were analyzed by ELISA and immunohistochemistry.

Isolation and cell culture of BM-MSCs[11]

Swine BM-MSCs were isolated as described previously for rat bone marrow and collected with added heparin (heparin 500 U/ml BM). Mononuclear cells were isolated by centrifugation through 1.073 g/ml Ficoll at 1 500 rpm for 15mins. The cells were rinsed twice with PBS and seeded at 1 to $2 \times 10^5/cm^2$ in complete medium(low-glucose DMEM, 10% FBS, 5% HS, 100 U/ml penicillin and streptomycin) at 37℃ with 5% CO_2. The medium was changed after 24 h, and 60%~70% confluent BM-MSCs were passaged. The cells were washed three times to remove medium with FBS and collected before transplantation. The cells were suspended in 4 ml IMDM for transplantation.

Cell or gene transplantation

Intracoronary cells or gene transfer was done four weeks after LAD ligation, swine were treated with Ad_5-HGF(4×10^9 pfu, 4ml), BM-MSCs(5×10^6/ml,4 ml) or IMDM (4 ml as control) via the right coronary artery. All infusions were carried out through cardiac catheterization. Ad_5-HGF was kindly provided by Prof. Zhuze Wu from Chinese Academy of Military Medical Sciences.

^{99}mTc-MIBI gate-controled cardiac perfusion imaging

Four and seven weeks after ligation, swine were anesthetized with ketamine (30 mg/Kg) and their heart function and cardiac perfusion were evaluated by ^{99}mTc-MIBI gate-controlled cardiac perfusion imaging.

Detection of HGF expression by ELISA

200 mg of myocardium from the transitional zones of each animal was pulped, and the supernatant was collected. Enzyme-linked immunosorbent assay (ELISA) was carried out using mouse monoclonal antibody against human HGF, horseradish peroxidase-labeled goat anti mouse immunoglobulin, and a spectrophotometric o-phenylenediamine color-developing system. The optical density (OD) was measured at 490 nm (Microplate Manager 450). A standard curve using recombinant human HGF was constructed for calculation of the expression of HGF.

Immunohistochemistry analysis[12]

Four weeks after cell or gene transplantation, the myocardium samples collected from all animals were fixed in 4% formaldehyde and embedded in paraffin, then cut into 10 μm-thick sections. The sections were stained with hematoxylin and eosin for cell and blood vessel identification. Immunohistochemical detection of specific antigens was performed with α-SMA antibody, and then the sections were dyed with 3, 3'-diaminobenzidine (DAB) and redyed with brazilin. Results were observed with an optical microscope and photomicrographs were taken using a digital image analysis system. α-SMA$^+$ arterioles in each tissue section were counted by an observer who was blinded to identity of groups (× 400). The number of α-SMA$^+$ arterioles in 5 high-power fields in each section were averaged, and expressed as α-SMA$^+$ arterioles/mm^2(the area of 400 high-power fields is equal to 0.152 mm^2). The averages of 5 fields for 3 samples from each animal were used for comparison.

Statistical analysis

Data were expressed as mean±SD. SPSS 11.5 software was used for all analysis. "$P < 0.05$" was considered significant. Differences in quantitative data were assessed by using the ANOVA and the

pair-sample t test.

RESULTS

HGF expression

HGF expression was examined by ELISA. Compared with the control, higher expression of human HGF was observed in the myocardium of Ad_5-HGF group via coronary transfer(109.3 ± 7.8 vs. 6.2 ± 2.6, $t = 30.685$, $P < 0.01$).

Cardiac perfusion and heart function

The 4th week and 7th week after LAD ligation, cardiac perfusion in myocardial ischemic area was significantly improved in BM-MSCs and Ad_5-HGF transfer groups after transplantation treatment, but it was unchanged in control group. There was no significant difference in heart function in three groups at the 4th week after operation. There was also no significant difference of LVEF between the 4th week and 7th week after operation in control group. The improvement in LVEF was greater in two transplantation therapy groups in the 7th week than in control or the 4th week after operation, but there was no significant difference of LVEF improvement between two transplantation therapy groups(***Tab 1***).

Neovascularization

In BM-MSCs and Ad_5-HGF groups, there were a lot of α-SMA$^+$ and Ⅷ$^+$ factor (vWF$^+$) arterioles surrounding the transitional zones of myocardial infarct area (***Fig 1***). The densities of capillary and new mature vessels were lower than those in BM-MSCs and Ad_5-HGF transfer groups, but there was no significant difference of capillary vessel density between the two transplantation groups. The density of new mature vessels in Ad_5-HGF group was higher than that in BM-MSCs group(***Tab 2***).

DISCUSSION

Tab 1 **The comparison of LVEF between transplantation and control groups** ($\bar{x} \pm s, n = 6$)

Group	4th week	7th week	The LVEF value of improvement(%)
BM-MSCs	33.6 ± 2.1[a]	45.0 ± 2.7[bde]	11.7 ± 3.6[g]
Ad_5-HGF	32.2 ± 1.8	43.9 ± 4.3[cf]	11.5 ± 1.5
Control	32.3 ± 2.1	30.4 ± 2.8	

Data were presented as mean±SD. [a]$P > 0.05$, Ad_5-HGF and BM-MSCs group vs. control group at the 4th week; [b]$P < 0.01$, BM-MSCs group vs. control group at the 7th week; [c]$P < 0.01$, Ad_5-HGF group vs. control group 7th week; [d]$P > 0.05$, Ad_5-HGF group 7th week vs. BM-MSCs group 7th week; [e]$P < 0.01$, BM-MSCs group at the 7th week vs. 4th week; [f]$P < 0.01$, Ad_5-HGF group at the 7th vs. 4th week; [g]$P > 0.05$, the LVEF value of improvement in BM-MSCs group vs. that in Ad_5-HGF group.

Tab 2 **The comparison of neovascularization between transplantation and control groups** ($\bar{x} \pm s, n = 6$)

Density of angiogeneis	Control group (/mm^2)	BM-MSCs group(/mm^2)	Ad_5-HGF group (/mm^2)
Capillary density	55.5 ± 4.7	102.4 ± 8.6[a]	105.3 ± 7.7[bc]
Density of mature vessel	16.4 ± 3.5	52.1 ± 4.1[d]	66.0 ± 3.3[ef]

Data were presented as mean±SD. [a]$P < 0.01$, BM-MSCs vs. control group; [b]$P < 0.01$, Ad5-HGF vs. control group; [c]$P > 0.05$, Ad_5-HGF and BM-MSCs group; [d]$P < 0.01$, BM-MSCs vs. control group; [e]$P < 0.01$, Ad_5-HGF vs. control group; [f]$P < 0.01$, Ad_5-HGF vs. BM-MSCs group; capillary vessel(vWF+); mature vessel(α-SMA+).

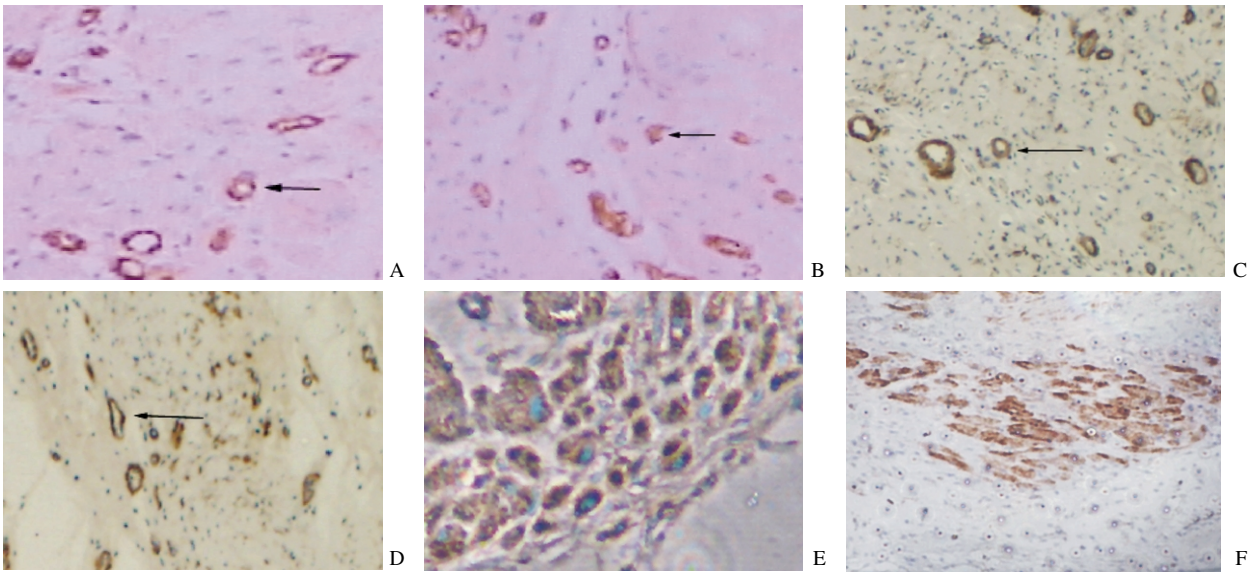

Fig 1 Immunohistochemical detection of vWF$^+$, α-SMA$^+$, BrdU$^+$ and MHC$^+$ antigens. A-B: vWF$^+$ arterioles were seen in the infarct transitional zones and infarct zones in Ad_5-HGF and BM-MSCs group; C-D:α-SMA$^+$ arterioles were seen in the infarct transitional zones and infarct zones in Ad_5-HGF and BM-MSCs group; E:BrdU$^+$ cells were seen in infarct areas in BM-MSCs group; F:MHC$^+$ cells were seen in infarct areas in BM-MSCs group(DAB, ×400; optical microscope).

Despite advances in medical and surgical treatment for heart failure, there has been no meaningful change in the total morbidity[13]. Therefore, new therapeutic strategies based on cells and genes have been proposed[14,15]. Many animal studies and some clinical trials have proved the benefit of BM-MSCs and vessel growth factor (such as HGF) to heart failure after myocardial infarction [16-19]. Besides the still disputed theory of differentiation to the myocardial cells, the mechanism of BM-MSCs is mainly regarded as that it could excrete all kinds of cell factors, such as vascular endothelial growth factor (VEGF) and HGF, to induce vasculogenesis and improve perfusion. However, HGF not only has the effect of inducing vasculogenesis, but also may mobilize stem cells. We did a clinical trial about the role of Ad_5-HGF in our hospital in cooperation with the Chinese Academy of Military Medical Sciences. In order to determine a better clinical therapy, we compared two transplantation therapy methods in these animal models.

Our study demonstrated that BM-MSCs or Ad_5-HGF transplantation via intra-arterial administration through a coronary artery supplying noninfarcted myocardium could ameliorate myocardial ischemia and improve postinfarction heart function. Abundant vWF^+ and α-SMA^+ arterioles were seen in the infarct transitional and infarct zones in both Ad_5-HGF and BM-MSCs groups. We also found vWF^+ and α-SMA^+ arterioles in the infarct transitional and infarct zones in control group, but the densities of these neovascularized areas appeared significantly lower compared with those in transplantation groups. There was no difference in the density of vWF^+ capillary between the two transplantation therapy groups. However, there were more mature vessels (α-SMA^+) in Ad_5-HGF group. The function of heart was evaluated using gate-controlled cardiac perfusion imaging, and we found that heart functions were significantly improved in BM-MSCs and Ad_5-HGF groups three weeks after treatment, but there was no significant difference in these two groups. Compared with control group, the heart functions were significantly improved in the BM-MSCs and Ad_5-HGF groups.

Our results did not suggest that either method of Ad_5-HGF or BM-MSCs transfer was better. Besides the higher density of functional α-SMA^+ vessel in the Ad_5-HGF group, there was no significant difference in LVEF improvement which would indicate a better prognosis and some other benefits of Ad_5-HGF and BM-MSCs. We found no evidence of myocardial perfusion improvement from increased α-SMA^+ blood vessels, because the method of gate-controlled cardiac perfusion imaging was not capable of quantifying the degree of myocardial perfusion improvement. Furthermore, we chose to administer via the artery supplying noninfarcted myocardium, which might have some effects on BM-MSCs transferring to myocardium. Hence, we could not conclude that Ad_5-HGF transfer would be better than BM-MSCs transfer. Comparing Ad_5-HGF treatment with BM-MSCs treatment via intra-coronary injection, we could only say that there was a higher induced density of functional α-SMA^+ blood vessel in Ad_5-HGF group by immunohistochemistry analysis.

Our study indicated that both BM-MSCs and Ad_5-HGF administrations via a coronary artery supplying noninfarcted myocardium can ameliorate myocardial ischemia and improve postinfarction heart function. This result was similar to the findings when transfer occurred through the artery supplying infarct area, or when both BM-MSCs and Ad_5-HGF were injected into the ventricular wall of the ischemic area. In addition, we found $BrdU^+$ and MHC^+ (the heavy chain of myoglobin) cells in the infarct transitional zones (***Fig 1 E and F***). All these findings demonstrated that BM-MSCs or Ad_5-HGF administration via coronary artery supplying noninfarcted myocardium can be beneficial in heart failure after myocardial infarction.

In conclusion, our study proved the feasibility of BM-MSCs or/and Ad_5-HGF transfer via coronary artery supplying noninfarcted myocardium, and also indicated that any kind of transfer might stimulate angiogenesis and improve postinfarct heart function. However, we could not ascertain that therapy, or/and we must acquire more quantitative data to make sure of it.

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