Migration of intravenously grafted mesenchymal stem cells to injured heart in rats

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Abstract: The present study aimed to determine the role of tissue injury in migration of mesenchymal stem cells (MSCs) intravenously transplanted into heart and to establish experimental basis for improving stem cell therapy in its targeting and effectiveness. MSCs were isolated from bone marrow of male Sprague-Dawley rats and purified by density centrifuge and adhered to the culture plate in vitro. Female rats were divided randomly into four groups. Myocardial ischemia (MI) transplanted group received MSCs infusion through tail vein 3 h after MI and compared with sham-operated group or normal group with MSCs infusion, or control group received culture medium infusion. MI was created in female rats by ligating the left anterior descending coronary artery. The heart was harvested 1 week and 8 weeks after transplantation. The characteristics of migration of MSCs to heart were detected with expression of sry gene of Y chromosome by using fluorescence in situ hybridization (FISH). Ultrastructural changes of the ischemic myocardium of the recipient rats were observed by transmission electron microscope (TEM). One week or 8 weeks after transplantation, sry positive cells were observed in the cardiac tissue in both of MI transplanted group and sham-operated group, the number of sry positive cells being significantly higher in MI transplanted group (P<0.01). No significant difference was found in the number of sry positive cells between 1 week and 8 weeks after transplantation. No sry positive cells were observed in the hearts of control and normal group. In addition, the ultrastructure of some cells located in the peri-infarct area of MI rats with MSCs transplantation was similar to that of MSCs cultured in vitro. These results indicate that MSCs are capable of migrating towards ischemic myocardium in vivo and the fastigium of migration might appear around 1 week after MI. The tissue injury and its degree play an important role in the migration of MSCs.

Key words: mesenchymal stem cell; tissue injury; migration; heart
Stem cells have long been regarded as undifferentiated cells capable of proliferation, self-renewal, production of a large number of differentiated progeny, and regeneration of tissues\(^{[1]}\). Bone marrow is a reservoir rich in stem cells and progenitor cells. Among these, a population of cells known as mesenchymal stem cells (MSCs) has been shown, \textit{in vivo} and \textit{in vitro}, to proliferate extensively and to differentiate along multiple lineages giving rise to muscle, brain, liver, cartilage, bone, fat, and the vessel \textit{in vitro} or \textit{in vivo}, contributing to tissue regeneration\(^{[2-5]}\). However, only a very small percentage of the intravenously infused cells or mobilized stem cell colonize in the target organ\(^{[6]}\). Better understanding of the signaling mechanism that attracts bone marrow stem cells to the target organ may enhance the prospects of intravenous delivery of stem cells (or stem cell mobilization) as a therapeutic strategy for tissue repair. In the present study, we aimed to observe the effects of different myocardial microenvironment on the migration of intravenous grafted MSCs in rats in order to understand the role of tissue injury in the migration of stem cells, which would possibly establish experimental basis for improving stem cell therapy in its targeting and effectiveness.

1 MATERIALS AND METHODS

1.1 Animals

Thirty-six female Sprague-Dawley (SD) rats (Animal Center, Xi’an Jiaotong University) weighted 200–250 g were randomly divided into myocardial ischemic (MI) and non-MI groups. Rats in MI group were divided into transplanted group \((n=12, \text{MSCs transplantation})\) and control group \((n=6, \text{culture medium transplantation})\). Rats in non-MI group were divided into sham-operated \((n=12, \text{MSCs transplantation})\) and normal group \((n=6, \text{MSCs transplantation})\). Half of the animals in each group were killed 1 week and the rest were killed 8 weeks after transplantation.

1.2 Isolation and culturing of MSCs

Femur and tibia of 1-month-old male SD rats were excised under sterile conditions. Bone marrow plugs were extracted from the bones by flushing the bone marrow cavity with Dulbecco’s Modified Eagle’s Medium-Low Glucose (DMEDM-LG) (Gibico, USA). Mononucleated cells were isolated from the perfusates by density gradient fractionation \(1.073 \text{ g/ml, Pharmacia, USA}\), washed and resuspended in DMEM-LG supplemented with 10% fetal bovine serum (Si-Ji-Qing, China), and cultured at 37ºC in a humidified atmosphere containing 5% CO\(_2\). The mesenchymal population was isolated on the basis of its ability to adhere to the culture plate. The hematopoietic cells, which constituted the majority of the cells, did not stick to the culture plate and were removed with subsequent medium changes. When the cultures reached 90% of confluence, cells were recovered by 0.25% trypsin (Amresco, USA) and passages followed. Proliferation and growth were observed with phase contrast microscope (Olympus, Japan) in primary and passage culture.

1.3 Identification of MSCs

Analysis of cell surface molecules was made on the third passage of MSCs using flow cytometry (BD, USA). MSCs were evaluated by direct immunofluorescence, using antibodies of CD90, CD34 and CD71 (Santa Cruze, USA). Meanwhile, immunofluorescence staining was used to test cultured MSCs as well.

1.4 Establishment of myocardial ischemic model

Female SD rats were anesthetized with inhalation of ether. The chest was opened, a 5-0 silk suture was passed with a tapered needle under the left anterior descending coronary artery 1 to 2 mm from the tip of the left atrium, and the ends of the suture were tied to induce MI. Sham-operated rats received the same procedure but without left anterior descending ligation. Benzathine penicillin G 160 000 was given intramuscularly after surgery.

1.5 Transplantation of MSCs

The transplantation was performed 3 h after MI. The male MSCs suspension \(500 \mu\text{l contains }1\times10^7 \text{ cells}\) was slowly infused into the tail vein. Meantime, the normal group received MSCs transplantation and the control group was injected with equal volume of culture medium through the tail vein.

1.6 Tissue harvesting and fluorescence in situ hybridization

One week or 8 weeks after MSCs transplantation, rat hearts were removed, embedded in OCT, snap frozen in liquid nitrogen, and stored at –80ºC. Rat hearts in OCT blocks...
were sectioned, and 5 µm serial sections were collected on slides followed by fixation with 4% paraformaldehyde at 4°C for 5 min. Fluorescence in situ hybridization (FISH) was performed to detect male MSCs in female rats with a probe of rat Y chromosome \textit{sry} gene (Tianjin Hao-Yang Company). The synthesized sequence of \textit{sry} probe was 5'-ATAGT GTGTA GGTTG TTGTC CCATT GCAGC-3'. Sections were prepared by heat denaturalization at 95°C for 10 min and chilled on ice for 5 min, and incubated with the probe at 37°C for 12 h. Finally, the nuclei was marked by PI (red) and observed by laser confocal microscope (Leica, Germany).

1.7 Ultrastructural investigation
Heart tissue of the MI transplantation group and cultured MSCs \textit{in vitro} was fixed with 3.0% glutaldehyde and 1.5% paraformaldehyde, washed in PBS, post-fixed in osmium tetroxide, dehydrated in an ethanol series, embedded in epoxy resin, and then observed through transmission electron microscope (JEM-2000EX).

1.8 Statistical analysis
The number of MSCs of migration to heart was determined by the number of nuclei labeled by \textit{sry} gene of \textit{Y} chromosome. Five tissue sections (30 µm interval) around the suture area of the heart were counted per animal. All variables are expressed as mean±SD. Differences in migration between groups were compared by unpaired \textit{t} test with SPSS software 10.1 (Statistical Product and Service Solutions). All tests were 2-tailed, and significance was accepted at \( P<0.05. \)

2 RESULTS

2.1 Characteristics of cultured MSCs
Three to 4 d after MSCs seeding in culture plates, a contrast phased microscope revealed adherent cells in small colonies with typical fibroblast-shaped morphology. The primary cells reached confluence in single layer after plating for 7~10 d and were passaged for the first time. The cells were noted to have a large expansive potential after subculture. The fibroblast-like morphology was also maintained after cell passages and throughout the culture period (Fig.1).

2.2 Identification of MSCs
Flow cytometric analyses showed that the third passage of MSCs was CD90 positive (96.5%), but was negative for CD34 and CD71 (Fig.2). Cultured MSCs were positive for CD90 by immunofluorescence staining (Fig.3).

2.3 Establishment of myocardial ischemic model
One week after the ligation of left anterior descending coronary artery, the wall of left ventricle became thinner (Fig.4A). The massion-colored sections of control animals showed serious histopathological changes. Compared with normal myocardium (Fig.4B), ischemic myocardium demonstrated denaturation atrophy and lysis of cardiac myocytes with extensive interstitial fibrosis (Fig.4C).

2.4 Migration of MSCs to heart
One week after transplantation, \textit{sry} positive cells were ob-
observed in the cardiac tissue of MI transplanted group (Fig. 5) and sham-operated group, but were distributed sporadically in the myocardium of sham-operated group. In comparison with the sham-operated group, the numbers of sry positive cell increased significantly in MI transplanted group (P<0.01). However, no significant difference was found in the number of sry positive cells between 1 week and 8 weeks after transplantation (Fig. 6). No sry positive cell was observed in the hearts of control and normal group.

2.5 Ultrastructural observation
One week after transplantation, large number of villus in surface, rough surfaced endoplasmic reticuli and mitochondria in endoplasm, and large nucleus with irregular shape were observed in some cells located in the peri-infarct area.

Fig. 3. Cultured MSCs were positive for CD90. Scale bar, 200 µm.

Fig. 4. Morphological observation after myocardial ischemia. A: Transection of ischemia myocardium, arrowheads indicate suture. B: Massion coloration of normal myocardium. C: Massion coloration of infarcted myocardium. Scale bar, 200 µm.

Fig. 5. Fluorescence in situ hybridization (FISH) analysis of the sry positive cells in MI transplanted group. A: Hybridization signals (green) of sry were detected in the heart. B: Nuclei were stained with PI (red). C: Overlap of the A and B. Scale bar, 8 µm.
of transplanted group through transmission electron microscope (TEM). In comparison with the cultured MSCs in vitro, ultrastructure of some cells located in the peri-infarct area of MI rats with MSCs transplantation was similar to that of MSCs cultured in vitro (Fig. 7).

3 DISCUSSION

Myocardial infarction is a leading cause of heart failure and death in the world. Recent attempts to repair experimentally induced acute myocardial infarctions have provided encouraging but limited success in a number of animal models. The most promising results have been obtained after transplantation and mobilization of stem cells to the area of infarction\[7\]. With consideration of ethic problems and easy acquirement, bone marrow MSCs may be optimal choice in clinic\[8\]. However, only a very small percent-

Fig. 6. Characteristics of migration of MSCs to heart. One week or 8 weeks after transplantation, sry positive cells were observed in the cardiac tissue in both of MI transplanted group and sham-operated group, but the number of sry positive cells was significantly higher in MI transplanted group. *P<0.01 vs sham-operated group. n=6 per group.

age of the infused or mobilized stem cells colonize in the heart. Therefore, it has become a key issue in the development of a tool for myocardial regeneration to determine the signaling mechanism that mediates cellular migration \[8\].

In the present study, we aimed to determine the effect of tissue injury on migration of stem cell through observing the migration of intravenous grafted MSCs in different myocardial microenvironment.

We found that MSCs in culture appeared fibroblastic and homogeneous in size and morphology by the third passage. Flow cytometry and immunofluorescence staining were used to determine the surface molecules in the expanded cell population. The analyses showed that the third passage of MSCs was CD90 positive (96.5%), but was negative for CD34 and CD71. It indicated that cultured cells expressed surface markers of mesenchymal lineages, but not those of hematopoietic cell. We observed that the differences in cell morphology and characteristics initially existed, but the MSCs population became homogeneous with time in subculture and remained so for many passages. The present findings about cultured MSCs in vitro are in accordance with previous reports\[9\]. Therefore, the cultured cells based on their morphology and surface markers might be MSCs. It was reported that examination of the interaction of allogeneic MSCs with cells of the immune system indicated little rejection by T cells\[10\]. In our study, the purified MSCs could survive in exogenous host hearts without addition of any immunosuppressant. Persistence of allogeneic MSCs in vivo suggests their potential therapeutic use for multiple recipients.

Fluorescence in situ hybridization (FISH) uses fluorescent molecules to vividly paint genes or chromosomes.

Fig. 7. Ultrastructural observation. A: Mature myocardial cell. B: Large number of villus in surface, rough surfaced endoplasmic reticuli in endoplasm, and large nucleus with irregular shape were observed in some cells located in the peri-infarct area. C: Cultured MSCs in vitro. Scale bar, 4 µm.
This technique is particularly useful for gene mapping and for identifying chromosomal abnormalities and individual’s cells\cite{10}. FISH involves the preparation of short sequences of single-stranded DNA, called probes, which are complementary to the examined DNA sequences. These probes hybridize, or bind, to the complementary DNA and, because they are labeled with fluorescent tags, show the location of those sequences of DNA. Because the distribution of intravenously transplanted male MSCs in the heart of female rats was unclear, positive findings may be on occasion. However, this study could find low expression of sry gene in sham-operated transplanted rats. It indicates that FISH is sensitive and efficient for the identification of the female cells in male hosts.

Migration to the heart refers to the phenomenon by which intravenous stem cells specifically engraft in the myocardium and not in other organs. In our experiment we found that sry positive cells were present in the cardiac tissue of transplantation group or sham-operated group, but were distributed sporadically in the myocardium of sham-operated group. No sry positive cell was seen in the cardiac tissues of the hearts of control or normal group. These findings suggest that MSCs are preferentially attracted to and retained in the injured tissue, colonize there, and might contribute to healing of the heart. It indicates that tissue injury may play an important role in migration of MSCs to heart. The present findings about donor cell entrapment in the heart are in accordance with previous reports\cite{12,13}. It is possible that the intense inflammatory reaction after myocardial infarction causes a local accumulation of mast cells and may initiate migration of MSCs to myocardium\cite{14}. The injured tissue may express appropriate receptors or ligands to facilitate trafficking and adhesion of stem cells to the site of injury where initiation of differentiation results in the generation of cells of the appropriate lineage\cite{15,16}. However, it is still unclear what environmental cues initiate migration of MSCs to injured heart. We also observed that the number of sry positive cells increased significantly in transplanted group compared to that in sham-operated group (P<0.05). Apparently, severe tissue damage, which leads to high expression of migration signals, plays a critical role in the event. Because intravenously injected cells may become trapped in other organs (liver, spleen, lung, etc), only a small portion of cells enter the coronary circulation and migrate into ischemic myocardium\cite{17,18}. This was consistent with our observations that a few sry positive cells were observed even in transplanted group. In addition, we observed no significant difference in the numbers of sry positive cells between rats of 1 week and 8 weeks after transplantation. It indicates that the fastigium of migration might appear around 1 week after transplantation. The possible explanation is that migration signals are released soon after myocardial injury, especially in the period of acute inflammation. Chu et al.\cite{19} found that the number of Lac Z+ human neural stem cells in the rat brain with focal ischemia increased between 7 and 14 d after intravenous transplantation. It is necessary to confirm the exact fastigium of migration through particular time design.

In addition, we observed that some cells located in the peri-infarct area of ischemia myocardium possessed large number of villus in surface, rough surfaced endoplasmic reticuli and mitochondria in endoplasm, and large nucleus with irregular shape through TEM 1 week after transplantation. The ultrastructure of “some cells” was similar to that of cultured MSCs, which demonstrated large number of villus in surface and rough surfaced endoplasmic reticuli and mitochondria in endoplasm as well. Thus, it is inferred that the “some cells” may be transplanted MSCs migrating to ischemia myocardium via the blood circulation.

In the present study, we observed the effects of different myocardial microenvironment on the migration of intravenously transplanted MSCs in rats, which would possibly establish experimental basis for the improvement of targeting and effectiveness of stem cell therapy. Our findings revealed that tissue injury and its degree may play an important role in the migration of MSCs. However, the contribution of intravenously transplanted MSCs to cardiac regeneration is limited\cite{20,21}. Better understanding of the signaling mechanism that attracts bone marrow cells to the ischemic heart may enhance the prospects of MSCs becoming a therapeutic strategy for myocardial repair. It will be one of the future tasks to find the most practical and specific way of evolving and targeting the healing potency of stem cells for tissue regeneration\cite{22,23}.

REFERENCES


