

Heparin Enhances the Effects of Mesenchymal Stem Cell Transplantation in a Rabbit Model of Acute Myocardial Infarction

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Summary: Stem cell transplantation in combination with administration of bioactive compounds has shown promising results in treating myocardial infarction (MI). In the current study, we investigated the effect of combining mesenchymal stem cells (MSCs) transplantation with heparin into the infarcted heart rabbits. For this purpose, 35 male New Zealand white rabbits were randomly divided into five groups: sham, MI, MI+ MSCs, MI+ heparin and MI+MSCs+ heparin. MI was induced by 30 min ligation of the left anterior descending coronary artery. The animals of MSCs and MSCs +heparin groups were injected cell culture containing MSCs intramyocardially into the infarct area. Functional parameters of the left ventricle by echocardiography, serum levels of VEGF by enzyme-linked immunosorbent assay, size of fibrotic area by Masson's trichrome staining, evaluation of morphology by Haematoxylin-Eosin and capillary density alkaline phosphatase staining were compared between groups. Ejection fraction, fractional shortening and levels of VEGF significantly improved in MSCs and MSCs + heparin group ($P < 0.05$). The fibrotic area was significantly reduced ($p = 0.009$) in MSC + heparin treated animals in comparison with MSCs. Number of live cells and angiogenesis were increased significantly in MSCs + heparin groups in comparison with MSCs ($p < 0.05$). Although injection of MSCs significantly restored normal function of fibrotic area, we found that administration of heparin combined with MSCs to infarcted heart of animals could have better effects on LV functional parameters in fibrosis area and resulted in superior therapeutic outcome in enhancing neovascularization and improving cardiac fibrosis.

Keywords: Mesenchymal stromal cells, heparin, myocardial infarction.

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INTRODUCTION

A growing body of evidence shows benefits of transplantation of adult bone marrow-derived stem cells as a potential therapy to repair damaged myocardium following an acute infarction (Donndorf *et al.*, 2013, Hass *et al.*, 2011, Hsiao *et al.*, 2013, Li *et al.*, 2017). In MI, myocardial cells are replaced with fibrotic or scar tissue, which result in reducing normal function of the heart (Zamilpa *et al.*, 2014). These stem cells which are able to differentiate into various types of cells in vitro and in vivo, could partly repair damaged tissue and restore normal function of the heart (Donndorf *et al.*, 2013, Hsiao *et al.*, 2013).

Whereof poor retention within the myocardium (6% or less) of transplanted bone marrow-derived cells in concomitant with little functional improvement in myocardium, there is an unmet need whether some soluble protein which are contribute to transplanted

cells for restoring and recovering significant function of myocardium simply There is an unmet need to find some soluble protein contributing to transplanted cells to restore within the myocardium and improve function of myocardium significantly (Freyman *et al.*, 2006, Webber *et al.*, 2010).

For this reason paracrine factor linked to transplanted stem cell are considered as facilitators to cardiac protection, repair, and regeneration following infarction, specially, chemokines and growth factors which are secreted from mesenchymal stem cells such as vascular endothelial growth factor (VEGF). VEGF may be involved in cardiovascular signaling and it is a growth factor affecting angiogenesis and improves the outcomes of MI (Gnecchi *et al.*, 2005, Tang *et al.*, 2011, Webber *et al.*, 2010).

These paracrine endogenous factors require some adjuvants to stimulate or augment the impacts of mesenchymal stem cells on repairing damaged

myocardium following an acute infarction (Caplan *et al.*, 2006, Nagaya *et al.*, 2005).

In current study was designed to examine the paracrine effects of heparin as a coordinator with VEGF on increasing angiogenesis and myocardium blood flow. Heparin, which is used as anticoagulant, is able to attach to VEGF and increase the affinity of this protein to its receptor and enhances the mitogenic and angiogenic activity of this protein. VEGF could be considered as promising angiogenic therapy for managing myocardial infarction by promoting angiogenesis and cell survival and also improve mesenchymal stem cell sheet transplantation therapy

It seems that it is a good choice to augment the beneficial effect of stem cell transplantation (Webber *et al.*, 2010).

Little is known about the effect of heparin on increasing the ability of transplanted MSCs in recovery of ischemic heart. In other hand, heparin is widely used for clinical approach as a common anticoagulant in patients with cardiovascular disease. Therefore, in the current study, we aimed to assess the effects of heparin added to MSCs on myocardial damage after acute infarction.

MATERIALS AND METHODS

Animal model

This investigation conformed to the “Guide for the Care and Use of Laboratory Animals” published by the United States National Institutes of Health (NIH Publication, 8th Edition, 2011).

The experimental protocol was approved by ethical committee of Iran University of Medical Sciences. Thirty five New Zealand white rabbits (weight 2500-3000 g) purchased from Razi Vaccine and Serum Research Institute were housed in controlled environment conditions ($22 \pm 2^\circ\text{C}$; light–dark cycle 7 AM–7 PM). Animals were allowed to access water and standard laboratory food ad libitum. Rabbits were randomly divided into 5 groups.

1) Sham operated group (n=7): normal rabbits, which receives injection of cell culture media (150 μl) in both sides of descending anterior artery.

2) Control group (n=7): MI induced animals, which did not receive any treatment.

3) MI+MSCs group (n=7): MI induced animals, which were treated by injecting MSCs intramyocardially into the infarct area.

4) MI+MSCs + heparin group (n=7): MI induced animals, which were treated by MSCs injection followed by subcutaneous administration of heparin (200 U/Kg for 28 days). (Diquélou *et al.*, 2005)

5) MI+ heparin group (n=7): MI induced animals, followed by subcutaneous administration of heparin (200 U/Kg for 28 days)

Induction of myocardial infarctions

Animals underwent general anesthesia (2% sodium pentobarbital; 40 mg/kg i.p.). Next, they were

intubated and ventilated by room air using a rodent ventilator (tidal volume 2–3 ml, respiratory rate 65–70 per minute, Harvard rodent ventilator model 683, Holliston, MA, USA). Left intercostal thoracotomy (between the two and three costal space) was performed under sterile condition and *left anterior descending* (LAD) was ligated between first and second diagonal branches of coronary artery with a 5-0 silk suture. Successful performance of coronary occlusion was confirmed by observation of the development of a pale color in the distal myocardium after ligation as well as dyskinesia of the anterior wall.

Human mesenchymal stem cell (hMSCs) isolation and culture

Human bone marrow samples were provided by Shariati Hospital (Tehran University of Medical Sciences). Human samples were collected under ethical permissions approved by Iran University of Medical Sciences. After separating mononuclear cells, the amount of live cells were determined using trypan blue staining. The extracts were then cultured in DMEM, 20% FBS, 1% penicillin - streptomycin and incubated at 37°C , 20% O_2 and 5% CO_2 . The culture medium was removed and replaced with fresh medium two times weekly and adherent cells were retained. At 50% to 60% confluence, the cells were harvested for subculture with 0.25% trypsin containing 0.02% EDTA (Gibco). HMSCs were isolated after changing the media (DMEM, 10% FBS, 1% penicillin-streptomycin) continuously for 3 days. The animals in MSCs and MSCs +heparin groups were injected with 150 μl of cell culture containing MSCs (1×10^6) intramyocardially into the infarct area, and the sham group was injected with an equal volume of cell culture media.

Flow cytometry

HMSCs grown up to 80% confluency were trypsinized and resuspended in 500 μl of PBS after centrifugation at $900 \times g$ for 7 min, analyzed with a flow cytometer (Partec, Germany). The antibodies used in this experiment were included : phycoerythrin (PE)-conjugated anti-CD105 (BD PharmingenTM, 562759), FITC-conjugated anti- CD90 (BD PharmingenTM, 551401), PE-conjugated anti-CD160 (BD PharmingenTM, 550257), FITC-conjugated anti-CD44 (BD PharmingenTM, 560977) for detecting MSC specific markers and fluorescein isothiocyanate (FITC)-conjugated anti-CD45 (BD PharmingenTM, 345808), FITC-conjugated anti-CD34 (BD PharmingenTM, 340668) as negative markers. Mixture of antibodies and cells were incubated for 1h on ice and in dark place.

Assessment of LV functional activity (echocardiography)

Echocardiography was used to measure functional parameters of LV including ejection fraction (EF), and

fraction shortening (FS). Rabbits were anesthetized and placed in the supine position. M-Mode Echocardiography was performed in 3 axis (short, long and average of axes) using VVIPE3; General Electric instrument before and after MI induction and at 4 and 8 weeks after treatment. All measurements were analyzed by two independent observers who were blinded to the treatment status of the animals.

VEGF measurement

VEGF concentrations were determined in serum by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (ABIN365534). Briefly, 100 μ l of plasma were pipetted into an antibody coated, 96-well plate. After growth factor binding and washing, VEGF conjugate (enzyme-linked antibody specific for VEGF) was added to each well. Following more washes, a substrate solution was added to the wells and incubated for 30 minutes at room temperature. The reaction was stopped by adding 50 μ l stop solution, and absorption was measured by a microplate reader (Biotech, USA) at 450 nm. The kit detection Range was 1.56-100 pg/mL of VEGF.

Histological examination

One month after all cardiac functional recordings, the animals were sacrificed under deep anesthesia and their hearts were extracted for histological examinations. After washing in normal saline, the hearts were fixed by immersion in 4% paraformaldehyde for 24–48 h and embedded in paraffin. Transverse sections (7 μ m) of hearts were prepared using a microtome. Slices were put on gelatin coated slides and stained for following purposes:

Measuring the fibrotic area: After removing paraffin from tissue by series of hydration (using xylol) and alcohol, the prepared slides were stained with Masson's trichrome (Sigma- Aldrich Co., MO, USA) for measurement of fibrosis. Five to ten sections were obtained from the LV free wall of each rabbit. Myocardial fibrosis was calculated the ratio of fibrotic area (blue color) to total cardiac section and expressed as percent fibrosis. Quantitation of fibrous areas was performed with NIS-Elements D software.

Evaluation of morphology: The paraffin-embedded sections were stained by Hematoxylin and eosin (H&E) for 10 min. After washing with water, the slides were treated with eosin solution for 15 min. In the last stage, water is removed from slides according to the protocol.

Capillary Density (alkaline phosphatase staining): The slides were primarily treated with magnesium chloride 1% and then incubated in ALP substrate (100 mU/ml) (Alkaline phosphatase kit, Sigma-Aldrich) for 2h at room temperature. After washing with double distilled water (dH₂O), the samples were stained with green nuclear counterstain.

Alkaline phosphatase was applied for determining the rate of angiogenesis in ischemic tissue; moreover, percentage of pyknotic nuclei was detected using trichrome masson and Haematoxyline-Eosin methods, respectively.

Statistical analysis:

ANOVA test (post hoc, Tukey) was used to compare case and control groups. Kruskal-Wallis and Mann Whitney tests were performed to compare MSCs +heparin results with MSCs results. All data were presented as mean \pm standard deviation (mean \pm SD). Two-tailed $P < 0.05$ was considered statistically significant. All study data were analyzed using the SPSS version 15.0 for Windows (SPSS Inc. Chicago, IL, USA).

RESULTS

Characterization of isolated MSCs

After plating human bone marrow aspirates for 24h, adherent MSCs appeared in cell culture plate. Non-adherent hematopoietic cells were further separated from MSCs by culturing and removing media continuously for 3 days. Primarily culture of MSCs took 10-14 days. In order to characterize MSCs extracts, we use flow cytometry assay for specific markers of MSCs vs. hematopoietic cells. Flow cytometry results showed that obtained MSCs were positive for CD44 (96.8%), CD90 (98.8%), CD166 (93%) and CD105 (99.5%). Few amount of cells expressed hematopoietic markers including CD45 (3.8%) and CD34 (2.8%). We also used control isotype antibodies for preventing non-specific antigen-antibody interactions.

Transplanted MSCs and heparin administration for recovery of MI animals

Effects of transplanted MSCs alone and in combination with heparin for recovery of MI lesions were assessed by cardiac function, biochemical and histological factors.

Effect of MSCs and heparin on functional parameters

Within each group we compared fractional shortening and ejection fraction before and after MI induction and at 4 and 8 weeks after treatment (Fig1). After LAD ligation, EF and FS were decreased in all groups in comparison with sham group (table 1). MSCs transplantation significantly improved the ejection fraction (EF) in 4 and 8 weeks after treatment ($p: 0.01$, $p: 0.04$ respectively) and Fractional Shortening (FS) in 4 and 8 weeks after treatment ($p: 0.03$, $p: 0.01$ respectively) (table 1). All these beneficial effects of MSCs were markedly enhanced by heparin treatment. EF in MSCs+ heparin (55 ± 0.009) was higher than MSCs (52 ± 0.001) after 4 weeks ($p: 0.08$) and also after 8 weeks (59 ± 0.015 in MSCs+ heparin vs. 57 ± 0.003 in MSCs $p: 0.07$). Although EF had not

Table 1: Comparisons of cardiac function measured by echocardiography between groups after MI induction and at 4 and 8 weeks after treatment

		Sham	control	MSCs	MSCs+heparin	heparin	p-value
EF %	After MI	70±0.014	49±0.018	51±0.09	52±0.015	52±0.08	0.001
	4 weeks	70±0.04	46±0.002	52±0.001	55±0.009	53±0.01	0.01
	8 weeks	70±0.01	45±0.02	57±0.003	59±0.015	53±0.005	0.04
FS	After MI	45.7±0.02	22.7±0.019	23.3±0.021	23.3±0.023	23±0.009	0.001
	4 weeks	45.9±0.05	20.5±0.002	24.8±0.05	31.7±0.03	22±0.001	0.03
	8 weeks	42.1±0.001	21±0.001	29.1±0.007	33±0.006	22±0.009	0.01

EF: ejection fraction, FS: fractional shortening, MI: myocardial infarction, MSCs: mesenchymal stem cells. One way ANOVA test showed MSCs transplantation significantly improved the ejection fraction (EF) in 4 and 8 weeks after treatment (p: 0.01, p: 0.04 respectively) and Fractional Shortening (FS) in 4 and 8 weeks after treatment (p: 0.03, p: 0.01 respectively). The difference between EF in MSCs+ heparin and MSCs after 4 weeks and also after 8 weeks was not statistically significant (p: 0.08, p: 0.07 respectively). The difference between FS in MSCs+ heparin and MSCs after 4 weeks and also after 8 weeks (p: 0.04, p: 0.04 respectively).

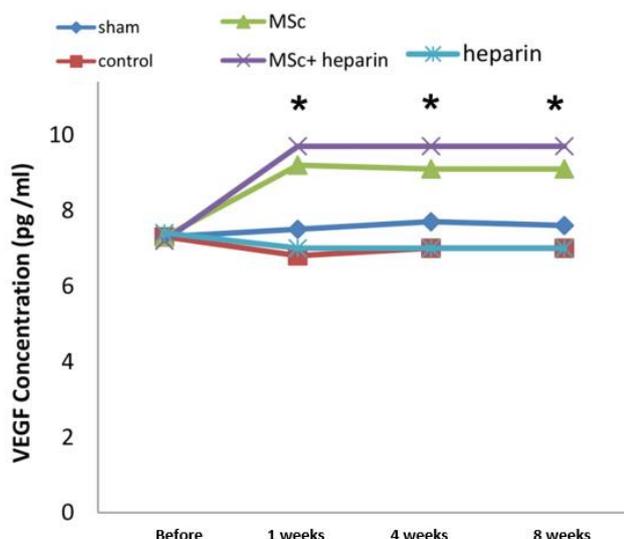


Figure 1: Comparison the amount of VEGF in serum of different study groups. *: significant differences between MSCs (p<0.001 one, four and eight weeks after treatment) and MSC + heparin (p: 0.01 1 week and p<0.001 4 and 8 weeks after treatment) treated animals vs. control group. VEGF in MSCs+ heparin had significant differences compared to MSCs after 1 week (p =0.01), after 4 weeks (p = 0.05) and after 8 weeks (p = 0.01).

statistically increased in MSCs+ heparin in comparison with MSCs after 4 and 8 weeks, these increases can be clinically remarkable (table1). Fractional Shortening in MSCs+ heparin (31.7 ±0.03) was improved significantly than MSCs (24.8±0.01) after 4 weeks (p = 0.04) and also after 8 weeks (33 ±0.006in MSCs+ heparin vs. 29.1±0.007 in MSCs p = 0.04) (table1).

Biochemical analysis:

The levels of VEGF in the serum which compared between groups are shown in figure 1. Before MI induction, blood VEGF level was assessed in all animals. The mean of VEGF level before study was 7.10± 0.05 (pg/ml). Level of VEGF in the MSCs transplantation group was significantly higher than control group, 1 week (9.2±0.17 (pg/ml) vs. 6.8±0.1 (pg/ml), P <0.001) and 4 weeks (9.1±0.17 (pg/ml) vs.

7±0.2 (pg/ml), P <0.001) after treatment. Level of VEGF in the MSCs transplantation+ heparin group was also significantly higher than control group, 1 week (9.7±0.22 (pg/ml) vs. 6.8±0.1 (pg/ml), P= 0.001) and 4 weeks after treatment (9.8±0.25 vs. 7±0.2, P<0.001).

The VEGF in MSCs+ heparin (9.85±0.05 (pg/ml)) level was significantly higher than MSCs (9.21± 0.007 (pg/ml)) after 1 week (p: 0.01) and after 4 weeks (9.88±0.02 (pg/ml) in MSCs+ heparin vs. 9.15± 0.02 (pg/ml) in MSCs p: 0.05) and also after 8 weeks (9.90±0.05 (pg/ml)in MSCs+ heparin vs. 9.21± 0.01 (pg/ml) in MSCs p: 0.01).

There were no significant differences between heparin only group and control group, 1 week (7±0.01 (pg/ml) vs. 6.8±0.1 (pg/ml), P: 0.8) and 4 weeks (7±0.1 (pg/ml) vs. 7±0.2 (pg/ml), p: 0.9).

Histological analysis:

Fibrosis in the heart tissue was measured 8 weeks after treatment using trichrome staining. The percent of fibrotic area was significantly reduced (p=0.01) in MSCs and MSCs + heparin treated animals in comparison with control group (MSCs: 15% and MSCs + heparin: 10% vs. control: 29%). The difference was also significant between MSCs and MSC + heparin groups (p= 0.04) (Fig 2 a and b). The histological evaluation using hematoxylin and eosin staining revealed that number of normal cells were increased significantly in MSCs and MSC + heparin groups in comparison with sham and control animals (p= 0.01) (Fig 3). Interestingly, numbers of viable cells in MSCs and MSC + heparin models were significantly more than control group (MSCs: 57.7 and MSCs + heparin: 51 vs. control: 29 p= 0.02).

With regards to viable cells, difference was also significant between MSCs and MSC + heparin groups (p: 0.04) (Fig 4). The amount of angiogenesis was measured by alkaline phosphatase staining. Our data showed that amount of angiogenesis in MSC + heparin and MSCs are significantly higher than control (MSCs: 25 and MSCs + heparin: 20 vs. control: 5 number/mm², p<0.001). The results showed

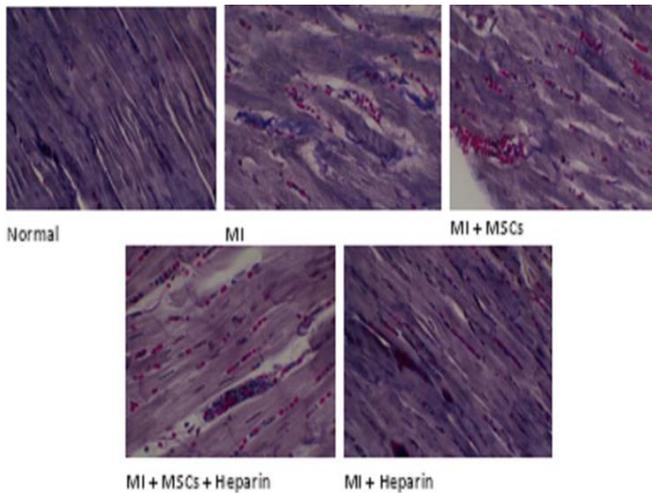


Figure 2a (40× magnification): Evaluation of fibrotic area was performed before and after intramyocardially MSCs delivery with the induction of experimental MI using Masson's trichrome staining.

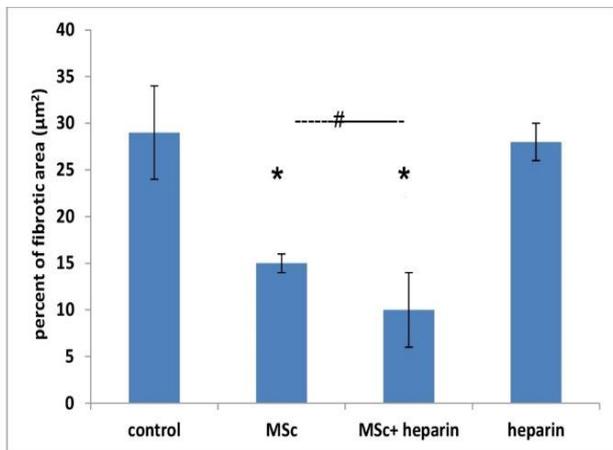


Figure 2b: one way ANOVA test showed significant decrease in area of lesion could be observed in groups treated with MSCs and MSC + heparin in comparison with control group (* p = 0.01).

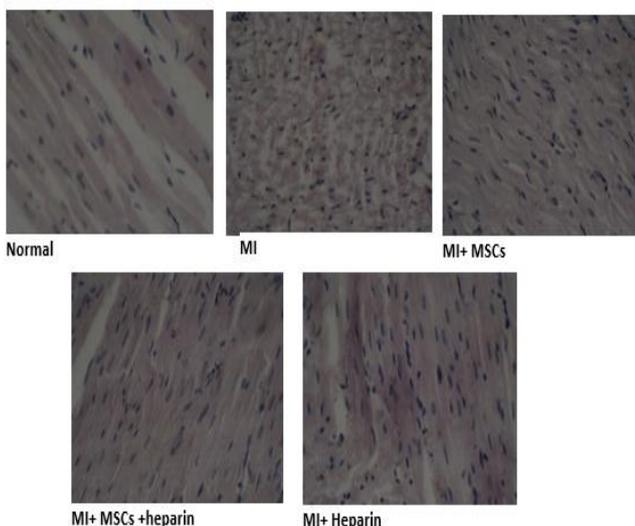


Figure 3: Histological analysis of the lesion areas in heart tissue. H&E showed replacement of damaged tissue by normal cells containing flat normal nucleus and light granules (40 × magnification).

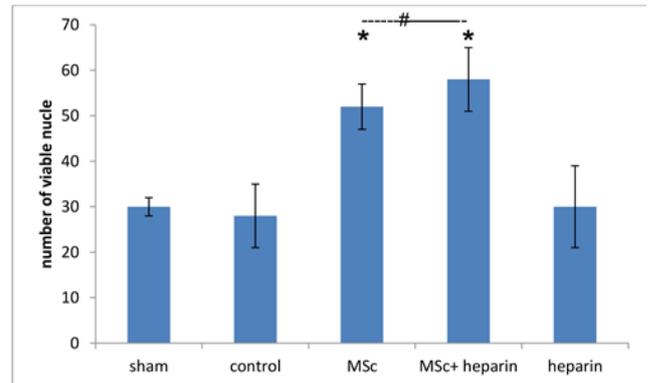


Figure 4: Comparison the number of viable cells between treated and non-treated animals (one way ANOVA test).

a) Amount of viable cells was increased significantly in MSCs and MSCs + heparin group in regard to control (*: p: 0.02). Number of viable cells was increased significantly in MSC + heparin compare to MSCs group. # p: 0.04 by Mann Whitney U test

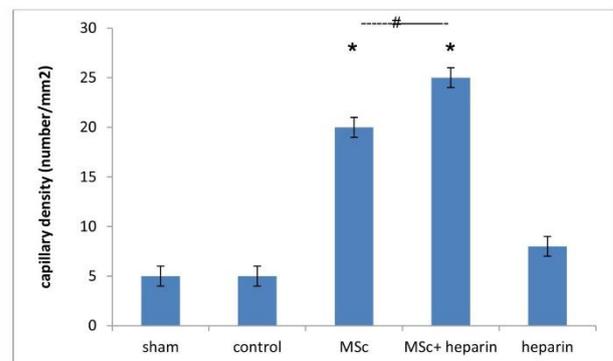


Figure 5: Analysis the amount of angiogenesis in different study groups.

Comparison different groups of study after 8th weeks by one way ANOVA test showed significant increase in amount of angiogenesis for rabbits treated with MSCs and MSC + heparin compared to control(*p <0.001). Capillary density was increased significantly in MSC + heparin compare to MSCs group. # p: 0.01 by Mann Whitney U test.

increase in amount of angiogenesis in MSC + heparin group compared to MSCs after 8 weeks (P: 0.01) (Fig 5).

DISCUSSION

Based on evidence gained from several studies demonstrating the capability of mesenchymal stem cell to partially restore cardiac function after ischemic damage in animal models and clinical results is mandatory to make a step forward in improving cardiac cell therapy as a relevant therapeutic tool (Donndorf *et al.*, 2012). Further efforts will most likely need to be taken to find ways to augment or facilitate stem cell effects on myocardial regeneration. Paracrine action of engrafted MSCs in ischemic myocardium is noteworthy for reduced remodeling and recovery of cardiac performance. The release of VEGF leads to efficient vascular regeneration and resultant blood flow and also attenuates the apoptotic pathway

(Elnakish *et al.*, 2012, Tang *et al.*, 2005, Tang *et al.*, 2011).

VEGF has been known as an endothelial cell mitogen, regulator of various cellular stress responses, as well as survival, proliferation, migration, and differentiation. Generally, VEGF has angiogenic and nonangiogenic role in cardiovascular system (Augustin *et al.*, 2013, Nichols *et al.*, 2014).

In current study, our aim was to examine the heparin in collaborating with VEGF to emboss its paracrine effects on increasing angiogenesis and resultant blood flow. Heparin, which is used as anticoagulant, is able to attach to VEGF and increase the affinity of this protein to its receptor and seems that it is a good choice to augment the beneficial effect of stem cell transplantation (Gnecchi *et al.*, 2005, Tang *et al.*, 2011).

According to this goal, VEGF level was measured in all groups. Our findings showed that VEGF was significantly increased in MSCs injected group in comparison with control after 1, 4 and 8 weeks. Interestingly, VEGF in MSCs+ heparin was significantly higher than MSCs after 1, 4 and 8 weeks.

Tang YL *et al.* concluded that the enhancement of cardiac function in MSCs therapy may be attributed to the capacity of self-renewal of MSCs that can maintain the long-lasting effect of angiogenesis and cardiomyocyte recovery by inhibition of apoptosis via MSCs transplantation and VEGF protein secreted by transplanted cells likely are the causes of this advantage. (Elnakish *et al.*, 2012)

In our study, we found angiogenesis was increased in ventricular myocardium of animals who received MSCs and the capillary density was significantly higher in animals who received MSCs+ heparin in comparison with MSCs alone.

Our findings prove that MSCs transplantation, as pivotal contributing factor in making new vessels in one hand and the effect of VEGF in neovascularization in other hand seemingly leads to notable improvement of infarcted heart. It seems that heparin Facilitate this effect of MSCs transplantations. Interestingly, histological analysis confirmed this effect of MSCs transplantation alone or together with heparin in recovery of ischemic heart by increasing angiogenesis.

Similar to these results, histological findings showed highlighted increase in amount of angiogenesis, decrease in fibrotic area and increase in number of viable cells in group with MSCs injection compared to control and also in MSCs+ heparin in comparison with MSCs alone.

Induction of angiogenesis which is usually triggered by growth factors released by cells is an important process required for functional tissue formation can improve myocardium perfusion. Though hypoxia which generated by coronary artery stenosis can induce VEGF and angiogenesis in ischemic myocardium, it is insufficient to allow recover

cardiomyocytes (Elnakish *et al.*, 2012). Glycosaminoglycan such as heparan sulfate in the extracellular matrix aid in proper functioning of these growth factors (Arslan *et al.*, 2014, Mammadov *et al.*, 2011). Mammadov *et al.* (2011) designed and synthesized a self-assembling peptide molecule that is functionalized with biologically active groups to mimic heparin. By these peptide nanofibers, angiogenesis was induced without the addition of exogenous growth factors and bioactive interactions between the nanofibers and the growth factors enabled robust vascularization in vivo as well (Mammadov *et al.*, 2011). While, Webber *et al.* (2010) designed and synthesized heparin-binding peptide amphiphilic (HBPA) nanofiber networks which serve as a synthetic extracellular matrix and biomimetic delivery vehicle for paracrine factors had a marked ability to augment function and healing in ischemic tissues (Webber *et al.*, 2010).

Its use in these studies demonstrates the marked biological activity of the paracrine factors released by stem cells coupled with their material to augment function after an MI or enhance vasculature following critical ischemia (Mammadov *et al.*, 2011, Webber *et al.*, 2010, Zhang *et al.*, 2009). But we were looking for to prove that heparin, which is used as an anticoagulant to treat and prevent deep vein thrombosis, pulmonary embolism, and arterial thromboembolism and also used in the treatment of heart attacks and unstable angina and it appears to be relatively safe for use during pregnancy and breastfeeding (Xu *et al.*, 2011) can be used to induce various desired physiological responses of growth factors like VEGF for tissue repair in infarcted cardiac area.

Therefore, our data indicated that cell therapy and to large extent heparin administration could reduce the surface area of injury. In MSCs transplantation and MSCs + heparin groups, the numbers of cells with euchromatin nucleus in ischemic tissue were increased. Interestingly these results indirectly confirmed pervious report about the role of VEGF secreted by MSCs in repairing infarcted heart tissue (Tavakoli *et al.*, 2013).

To best of our knowledge, this is the first report on the role of heparin administration combined with stem cell therapy in the restoration of MI heart. However, we suggest that follow ups of our study should be carried out beyond 8 weeks and the status of VEGF receptors should be analysed as well as inflammatory markers such as IL6, TGF α , TNF α and bFGF in association with tested factors on recovery of MI animals.

Our studies to date using exogenous heparin indicate a marked ability to augment function of mesenchymal stem cell to heal the damaged tissue after infarction. The mechanisms seem to be connected with the paracrine effects of VEGF secreted by MSCs as an endothelial cell mitogen, regulator of various cellular

stress responses, as well as survival, proliferation, migration, and differentiation and totally angiogenic and nonangiogenic effects of VEGF in cardiovascular system.

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