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Applying Vasopressin-Pre-Conditioned Human Adipose Mesenchymal Stem Cells Improves Heart Condition after Transplantation into Infarcted Myocardium

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ABSTRACT

Transplantation of H-AdMSCs may improve heart function after MI. AVP is a neurohypophyseal hormone that reduces cardiovascular damage. This study investigated the role of AVP preconditioning in the survival of MSCs and their effect on myocardial repair in the MI rats. H-AMSCs were isolated and incubated for 3 days. The expression of oxytocin and vasopressin receptors was evaluated by Real-time-PCR. Forty male Wistar rats were divided into 4 groups: control, sham, ASC and AVP-ASC. Ischemia was established by ligation of LAD coronary artery. Electrocardiography, fibrosis, angiogenesis, and apoptosis in myocardium were determined after 7 days. Results showed that preconditioned MSCs significantly increased cardiac function when compared with group that received non-preconditioned MSCs. This was associated with significantly reduced fibrosis, increased vascular density, and decreased resident myocyte apoptosis. Results indicate that AVP preconditioned MSCs can be consider a novel approach to management of MI.

Keywords: Myocardial infarction, Arginine vasopressin, fibrosis, apoptosis, angiogenesis, mesenchymal stem cells

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Introduction

Myocardial infarction (MI) is the most common cause of death in the world. When coronary artery collapse occurs, myocardial perfusion decreases and the oxygen supply to the heart are not well provided. In this regard, it seems that the establishment of reperfusion is essential for the preservation of life in the ischemic heart. However, reperfusion has been shown to act as a double-edged sword and, along with its protective effect, increases cell death in the heart and causes other lesions in addition to the damage caused by ischemic itself (1). Stem cell therapy, initially introduced as a new approach to the rehabilitation of damaged myocytes, is widely considered as a practical strategy for repairing damaged cardiomyocytes (2).

Adipose tissue is one of the richest sources of mesenchymal stem cells (MSCs), and numerous studies have shown that cellular adipose tissue productivity is greater than bone marrow. It has been suggested that adipose tissue-derived mesenchymal stem cells (AdMSCs) constitute about 2% of mononuclear cells from adipose tissue (3, 4). Additionally, the availability and ability to easily derivate, accompanied with non-stimulation of post-transplant host immunity and ability to transplant to any host (allograft and xenograft), AdMSCs have been introduced as a good candidate for cell therapy (5, 6).

It has been shown that MSCs (by secreting multiple angiogenic growth factors and cytokines) can alter endothelial cells and cause angiogenesis in the human body. The angiogenic factors of these cells include: Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF2), Angiopoietin 1 (Ang1), Interleukin 6 (IL6), and Hepatocyte Growth Factor (HGF). The AdMSCs secrete a large amount of VEGF, Transforming growth factor-β (TGF-β), and HGF. It has been shown that these factors play an important role in angiogenesis and in the treatment of diseases including MI and stroke (7).

It has been shown that laboratory manipulation of cells before transplantation improves their biological and functional properties through the survival of donation cells, homing, preservation, and proliferation. Treatment with materials that can increase cell resistance has a significant role in preventing this mortality (8).

Arginine Vasopressin (AVP) is a peptide with nine amino acids and has several physiological functions. AVP is synthesized in the paraventricular and supra-optic nuclei of the hypothalamus and is released in response to dehydration through the posterior part of the pituitary gland (9). Vasopressin-receptor subtypes are of the G protein-coupled receptor superfamily and are divided into three types, including: V1a, V1b, and V2 (10). AVP has also been shown to affect oxytocin receptors (OXTRs). Different doses of AVP have protective effects against ischemic lesions of cardiac reperfusion by affecting V1 cardiomyocytes receptors (11).

It has been stated that angiotensin, by acting on AT1 receptors (AT1R), plays an important role in the release of arginine vasopressin (AVP) from vasopressinergic neurons. AVP by acting on V1a receptors (V1aR) leads to vasoconstriction and increased cardiac contractions. AVP increases blood pressure with sympathoadrenal stimulation and plays a role in blood pressure regulation through baroreflexes (12).

We conducted this study to investigate the role of AVP preconditioning in the survival of mesenchymal stem cells and the effect of these cells on myocardial repair in the MI model in rats.

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Materials and methods

Study design

All protocols were approved by the Medical Ethics Commission of Lorestan University of Medical Sciences (Ethical code: IR.LUMS.REC.1396.323).

Forty male Wistar rats (250-300 g) were randomly divided into four groups (n=10): The control group did not receive any surgery or cell transplantation (Sham group). The Media group, after left anterior descending (LAD) coronary artery ligation, received 150 µL of culture medium, including Dulbecco's Modified Eagle Medium (DMEM) and 10% fetal bovine serum (FBS) without cells. The ASC group received 10^6 chloromethylbenzamido derivative of octadecylindocarbocyanine (CM-DiI) labeled MSCs in 150 µL of culture medium after coronary occlusion, and the AVP-ASC group received 10^6 CM-DiI labeled AVP preconditioned cells after coronary ligation. Seven days after surgery, electrocardiography was taken from the animals and their heart samples were taken for histological and immunohistochemical studies.

Isolation and expansion of mesenchymal stem cells

Isolation and primary culture of MSCs were performed according to the previously described techniques (13). Briefly, human MSCs were isolated from adipose tissue, that lipoaspirates from voluntary patients undergoing subcutaneous surgery, with density gradient centrifugation and cultured in low-glucose DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin for 2-3 days at 37°C, 5% CO2 without change of media. At confluence, the cells were harvested for passage with 0.25% trypsin containing 0.02% ethylenediaminetetraacetic acid (EDTA).

Multi-differentiation of MSCs

After two subcultures, the cells were characterized concurring to their adipogenic and osteogenic separation potential. For this, MSCs were brooded with adipogenic separation media comprising of DMEM supplemented with 10% FBS, 1µM dexamethasone for 21 days or osteogenic separation media composed by DMEM supplemented with 10% FBS, 0.1µM dexamethasone, 50 µg/ml ascorbate-2-phosphate and 10 mM beta-glycerol phosphate for 21 days. In both cases, the medium containing separation boosts was supplanted every three days. To assess adipogenic potential, societies were recolored with Oil Red-O. To assess osteogenic potential, societies were settled with 10% ethanol and recolored with Alizarin Red-S as already portrayed (14-16).

Characterization of MSCs

Immunophenotyping was performed by flow-cytometry investigation after immune-staining with monoclonal antibodies against putative human MSC markers (cluster of differentiation: CD marker) CD29, CD105, and CD90 or characteristic markers of other cell heredities: CD34 and CD45 (17).

MSCs preparation

MSCs (passage 3) at 70% confluency were randomly assigned to two experimental groups as follows: non-preconditioned (ASC group) and preconditioned with vasopressin (10 nm; AVP-ASC group) (18). For this purpose, 28 µL of vasopressin was dispensed in 7 ml culture medium containing DMEM, 10% FBS, 1% Pen-Strep and diluted 100 times. The cells were incubated for 3 days in this culture medium and then used for real time PCR (Polymerase Chain Reaction) and transplantation.

Investigation of the expression of OXTR and V1aR genes in MSCs

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Expression of the OXTR and vasopressin receptor (V1aR) genes was investigated by real time PCR for third passage MSCs. The gene expression was investigated in two groups of cells, including not preconditioned MSCs and AVP preconditioned MSCs. The reaction for each gene was triplicate and repeated twice. The primers were designed using Primer 3 software version 0.4.0 (table 1). The Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was considered as a normalizer. The rate of change in the expression of the target genes was compared to the non-preconditioned MSCs group. Finally, the data obtained from Real-time PCR are presented as fold change expression (19).

Table 1. Sequences of primers

Gene	Sequence (5′ → 3′)	
	Forward	**Reverse**
V1aR	TCAGCAGCGTGAAGTCCATT	AGGGTTTTCCGATTCGGTCC
OXTR	CGTACTGGCCTTCATCGTGT	AAGGCAGAAGCTTCCTTGGG
GAPDH	CTCTCTGCTCCTCCTGTTCG	ACGACCAAATCCGTTGACTC

Myocardial infarction model

The animals were anesthetized with ketamine 10% (50 mg/kg) and xylazine 2% (10 mg/kg) administered intraperitoneally and ventilated with room air by a small animal ventilator. Subcutaneous needle electrode was used for recording lead II of electrocardiogram (ECG). MI was induced by permanent ligation of the left anterior descending (LAD) coronary artery with a 6–0 silk suture. The successful performance of coronary occlusion was confirmed by ECG changes between 7-13 minutes after coronary obstruction (20).

Heart rate and blood flow

The number of heart beats per minute (heart rate) was recorded by electrocardiogram three times, baseline, after induction of MI and seven days later. The blood flow was measured two times in the baseline (before induction of MI) and after seven days from the region under the LAD ligation for three minutes. Blood flow is reported as a percentage.

MSCs transplantation

After preconditioning, the cells were trypsinized, and the number of living cells was estimated with trypan blue solution dye test. Then 10 µL of the mixed suspension was loaded into a counting chamber, and the number of viable cells was determined under an invert microscope. Prior to transplantation, MSCs were labeled with Cell Tracker CM-Dil (Molecular Probe, Invitrogen, USA) (21). 10^6 CM-Dil-labeled MSCs or CM-Dil-labeled AVP preconditioned-MSCs (18) in a total volume of 150 µl DMEM were injected at three focuses around the peri-infarct zone 20 minutes after post-AMI (22). The Media group received the same volume of cell-free DMEM.

Histological analysis

At 1-week post AMI, the animals were sacrificed (deeply anesthetized) to collect heart tissues. The hearts were rapidly removed and fixed in 10% formalin for 24 h, embedded in paraffin, and histologically sectioned (5 mm). Samples were mounted onto slides and stained with Hematoxylin-Eosin (H&E), and Mason trichrome for measurement of the histologic parameters (23).

H&E-stained slides were evaluated by fluorescence microscopy with a red filter for the presence of CM-DiI positive cells in the heart tissue. Invitrogen company protocol was used.

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TUNEL assay

Cell apoptosis was determined by TdT-mediated dUTP nick-end labeling (TUNEL) using the In Situ Cell Death Detection kit according to the manufacturer's instruction (24). TUNEL-positive cells were examined under light microscope at 200× magnification in 5 randomly selected fields. An apoptosis endpoint, indicative of positive staining in the apoptosis detection assay, was represented by a dark brown (DAB) signal. The results were presented as the rate of apoptotic cells.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics 27. Data were presented as mean ± SD. Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Bonferroni post hoc test, while comparisons between two experimental groups were performed by Student's t test. A $p<0.05$ was considered statistically significant.

Results

Characterization of human MSCs

Human MSCs were separated from grown-up adipose tissue. Five days after plating, MSCs illustrated the characteristic long spindle-shaped morphology (Figure 1A) and showed adipogenic and osteogenic

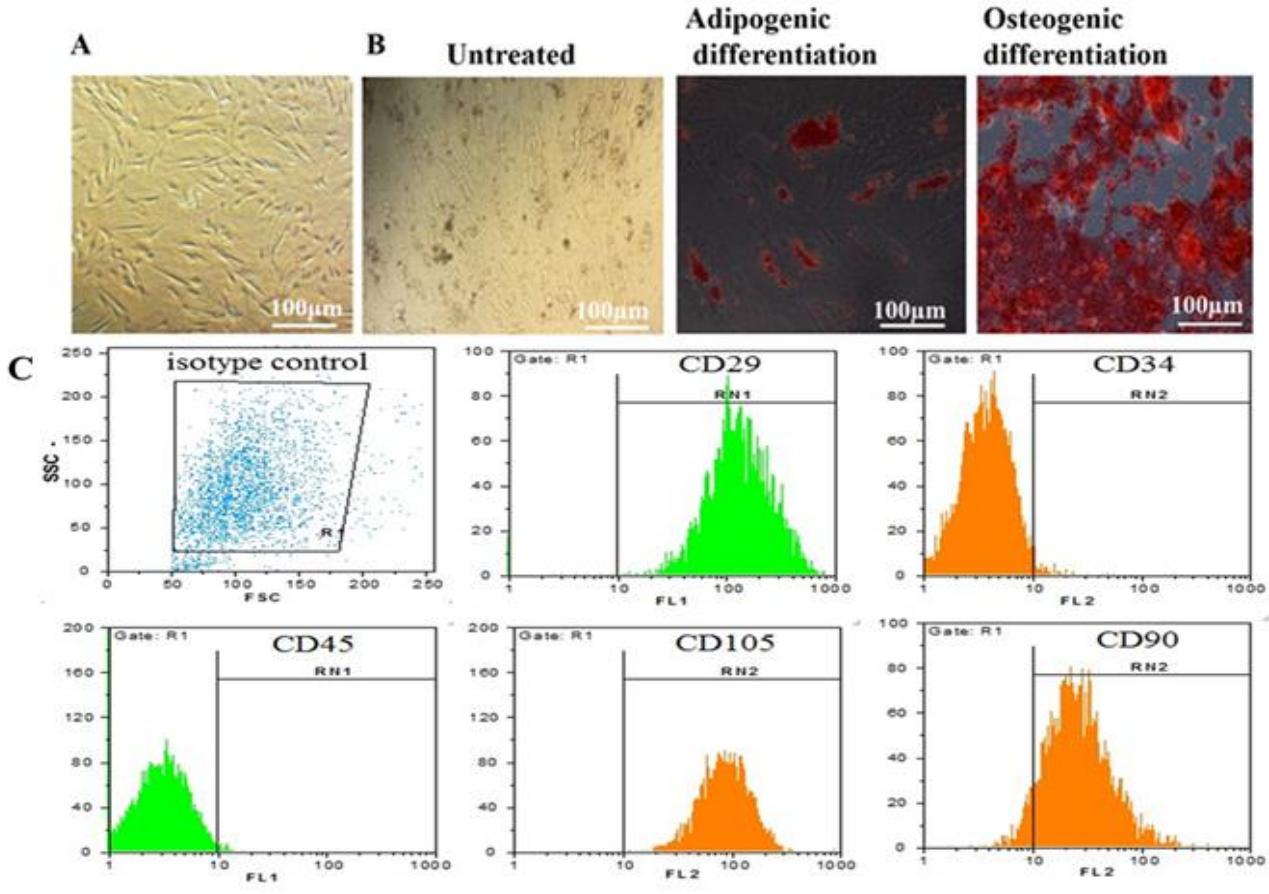


Fig.1. Characterization of human MSCs. (A) Morphological characteristics of MSCs five days after plating. (B) Representative images of MSCs at passage three, differentiated towards adipogenic and osteogenic lineages. Lipid's droplets were stained with Oil

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red-O, whereas hydroxyapatite precipitates were stained with alizarin red. (C) Flowcytometry analysis of AdMSCs at passage three, for putative human MSC markers (CD29, CD105, and CD90) and markers characteristic of other cell lineages (CD31 and CD45). Scatter blue histograms represent isotype controls. Data are representative of MSCs isolated from four different donors. Experiments were repeated three times at technical level.

differentiation when invigorated with the suitable differentiation media (Figure 1B). The flow-cytometry investigation appeared that MSCs were more than 98% positive for CD29, CD105, and CD90, and less than 1% positive for CD31 and CD45 (Figure 1C), demonstrating that the separated cells had the ordinary MSC phenotype.

AVP-preconditioned MSCs stimulate the OXTR/V1aR pathway

The expression of OXTR/V1aR levels was evaluated through Real-time PCR to demonstrate the cardioprotective effect of AVP-MSCs. The effects of AVP-preconditioned MSCs on targeted recruitment and survival of MSCs, anti-inflammation, anti-apoptosis and pro-angiogenesis increased, indicating that these cardioprotective effects conferred by AVP+MSCs were mainly attributed to the augmented stimulation of OXTR/V1aR pathway. V1aR expression significantly increased in the AVP group compared to the ASC group ($\eta 2 = 0.655$, $P=0.049$). However, OXTR expression was higher in the AVP group than in the ASC group, but this increase was not significant (Figure 2).

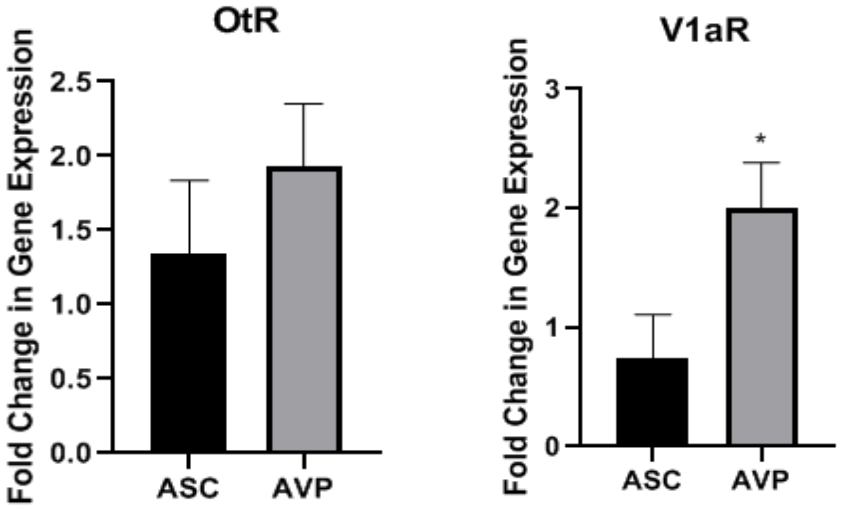


Fig.2. The expression level of OXTR and V1aR genes in MSCs groups including MSCs (ASC group) and MSCs receiving AVP (AVP group). OtR: oxytocin receptor; V1aR Vasopressin receptor 1a; AVP: Vasopressin. * P<0.05 compared to the ASC group.

AVP- preconditioning MSCs increased the production of MSCs

The recruitment and retention rates of transplanted MSCs in the peri-infarct myocardium (In-vitro stage) were investigated one week after infarction (In-vivo stage). The CM-Dil positive cells in the AVP+MSCs group were markedly higher in two-stages than that in the MSCs-only group. Together, these data indicated that AVP treatment, especially the AVP preconditioning (AVP+ MSCs), remarkably augmented targeted recruitment (Figure 3).

AVP- preconditioning MSCs improve heart rate

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Heart rate was measured in the study groups before surgery, after AMI and treatment, and seven days after treatment. As it can be seen in Table 2, the heart rate decreased after AMI and treatment in the Media group; But after seven days, it showed a significant increase (η2=0.050, P=0.030). However, the heart rate in the ASC group during ischemia increased significantly compared to the baseline (η2=0.103, P=0.042); this increase was significant after seven days (η2=0.050, P=0.009). Heart rate was more stable in AVP.ASC group and no significant difference was observed between different times in this group.

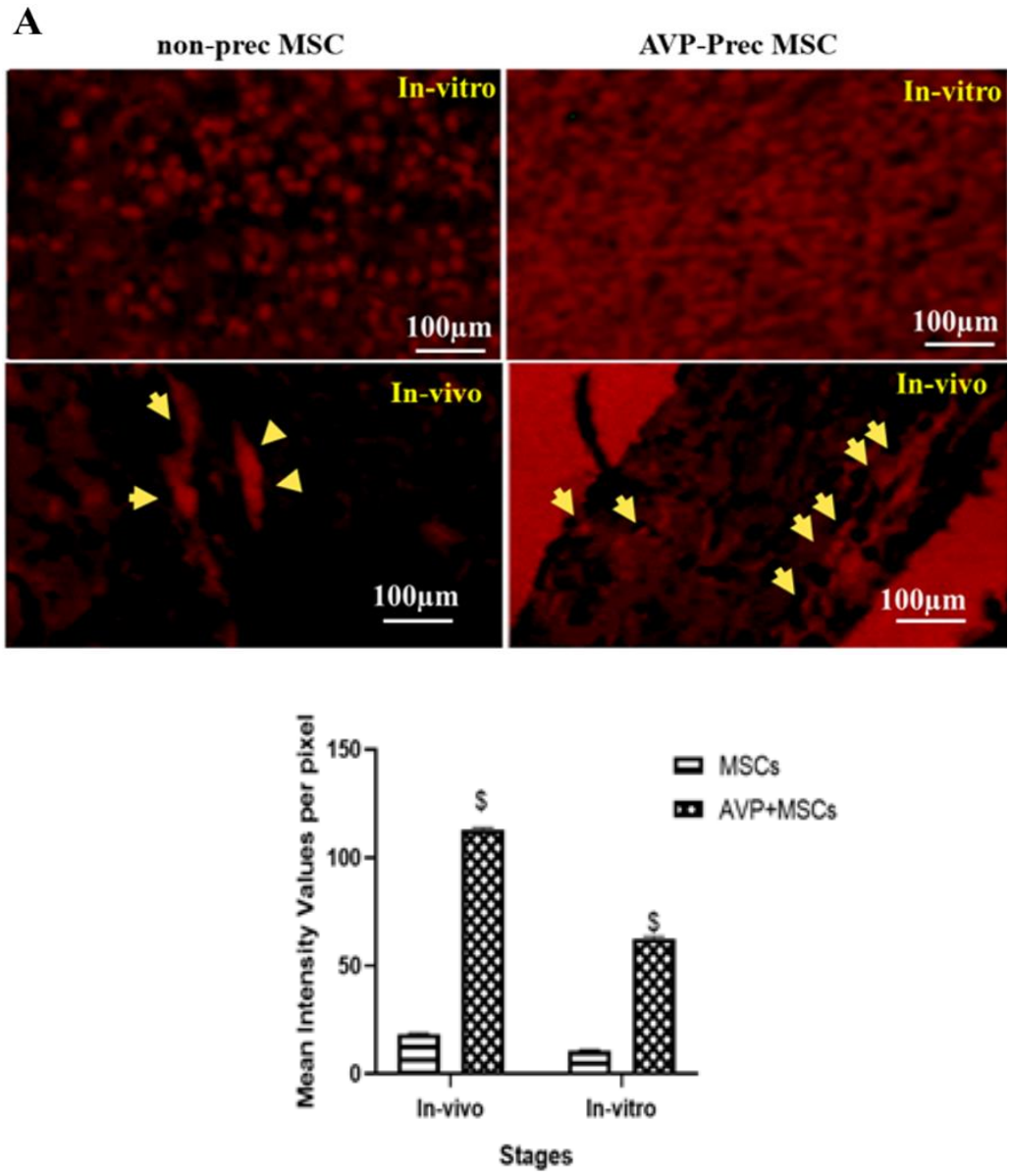


Fig.3. Mean intensity values per pixel of Fluorescently CM-DiI labeled MSCs remain in -vivo and in-vitro (the peri-infarct myocardium at the end of one –week post AMI). The number of CM-Dil positive cells in the AVP+MSCs group was markedly higher than that in the MSCs only group. **A.** non preconditioned MSCs and AVP pre-conditioned MSCs are localized in different layers in the myocardium tissue around the site of injection. **B.** Quantitative analysis of MSCs (red) fluorescence cargo (DiI), which has been

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monitored at two different time points (in-vitro and in-vivo). Fluorescence intensities are presented in the mean of red values (± SD) for MSCs and AVP+MSCs. $ P<0.05 compared to MSCs group. **AMI:** acute myocardial infarction; **AVP:** arginine vasopressin; **MSCs:** mesenchymal stem cells; **AVP+MSCs:** AVP pre-conditioned mesenchymal stem cells; **CM-Dil**: 1, 1'-dioctadecyl-3, 3, 3', and 3'-tetramethylindocarbocyanine perchlorate. Scale bar = 100 µm.

Table 2. Blood flow and heart rate results

Blood Flow	**Base Line**	**After 7 days**	
Media	100	$73.7 \pm 21.39^{**}$	
ASC	100	$97.16 \pm 21.08^{\#}$	
AVP.ASC	100	$79.06 \pm 8.3^{**}$	
Heart Rate	**Base Line**	**Ischemia**	**End point**
Media	229.96 ± 35.55	191.62 ± 56.99	$247.57 \pm 40.29^{£}$
ASC	211 ± 14.51	$218.49 \pm 30.18^{*}$	$244.13 \pm 35.14^{**}$
AVP.ASC	$207.32 \pm 34.77^{\#\#}$	$220.92 \pm 30.20^{\#\#}$	230.51 ± 19.58

The blood flow is measured two times as shown in Table 2. The results show that the blood flow of Media and AVP-ASC groups decreased significantly in the seven days after induction of AMI ($\eta 2= 0.243$, P=0.020 and P=0.003). However, the ASC group did not have a significant difference, and after seven days the blood flow reached the baseline levels.

AVP-preconditioned MSCs reduces infarct size and fibrosis

We performed histological analysis one week after cell transplantation. Masson's trichrome staining showed that transmural infarction existed in all experimental groups, while the infarct size and collagen depositions (fibrosis; mm^2) in AVP + MSCs and MSCs treated AMI rats were reduced compared with those in the AMI controls ($\eta 2=0.878$ and $\eta 2=0.575$, P=0.001). The infarct size in the AVP+MSCs group was also significantly reduced compared with Media and MSCs groups ($\eta 2=0.878$ and $\eta 2=0.575$, P=0.001), similar to the rate of collagen depositions (Figure 4A-B).

AVP-preconditioned MSCs reduced cardiomyocytes hypertrophy and inflammation

Cardiomyocytes hypertrophy in the left ventricle was calculated (Figure 5). The cardiomyocytes hypertrophy in the MSCs and AVP+MSCs groups was significantly reduced compared with Media group ($\eta 2=0.840$, P=0.001). Myocardial infarction triggers intense inflammatory response. H&E staining demonstrated massive infiltration of inflammatory cells as indicated by an increased number of neutrophils per mm2 in the peri-infarct regions of myocardium in the AMI group. Inflammatory cell infiltration was considerably reduced in AVP+MSCs group compared to the sham, Media and MSCs groups ($\eta 2=0.825$, P=0.008) (Figure 5B).

AVP-preconditioned MSCs promote angiogenesis in peri-infarct myocardium

We performed H&E staining for micro-vessels week after AMI to assess angiogenesis in the peri-infarct myocardium. As shown in Figure 6, the number of micro-vessels in the Media group decreased compared to the Sham group, although the MSCs group increased compared to the Media group, but these changes were not statistically significant. The number of micro-vessels in AVP+MSCs group was significantly higher than

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the sham and media groups (η2=0.589, P=0.001). Of particular note, AVP+ MSCs group demonstrated significant enhancement in angiogenesis compared with MSCs (η2=0.590, P=0.001).

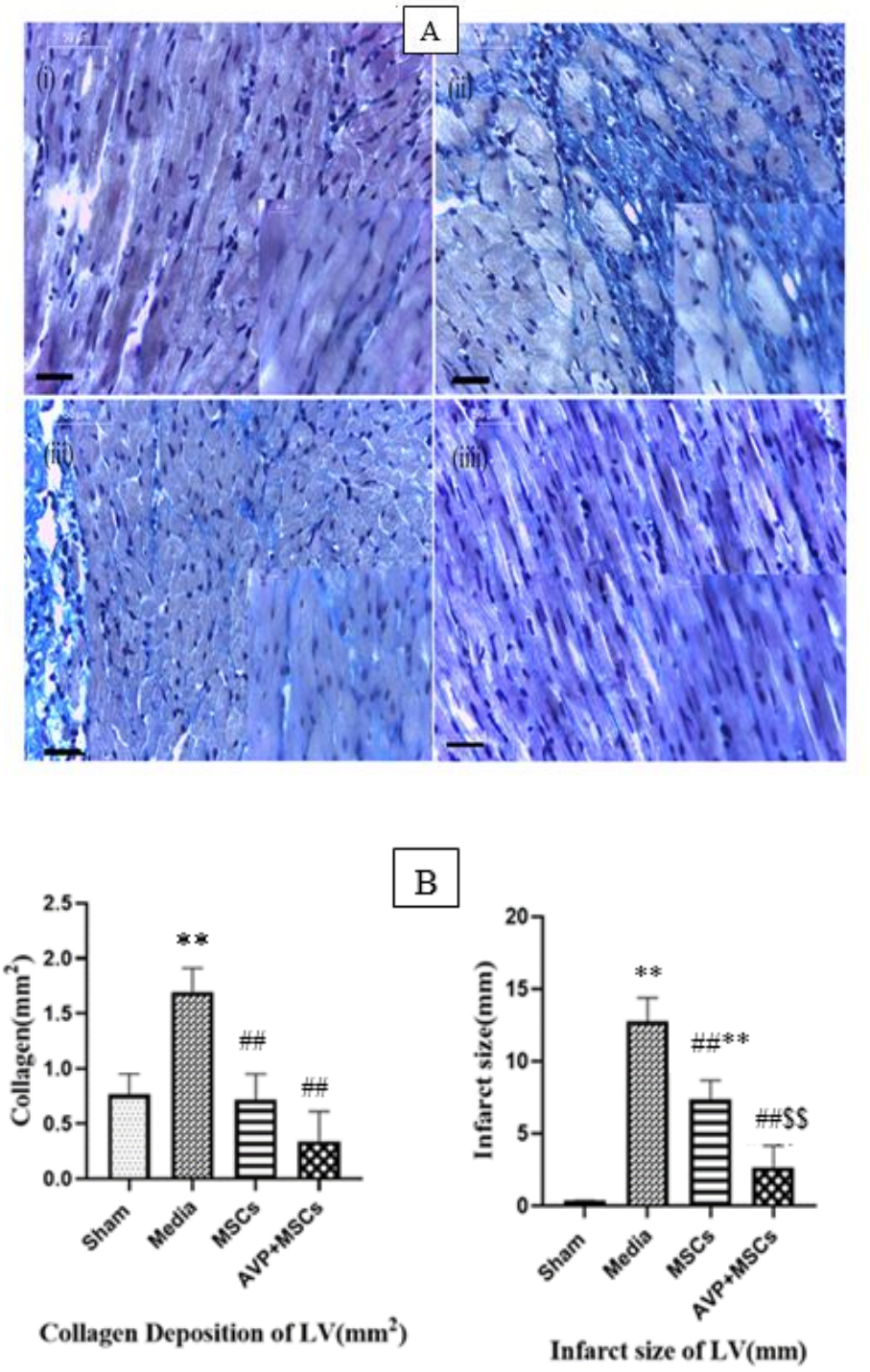


Fig.4. Infarct size and fibrosis (Collagen deposition) were measured using Masson's trichrome staining. (A) Representative images of Masson's trichrome staining in each group. (B) Quantitative data for the left ventricular infarct size and fibrosis. ** P<0.01

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compared to the Sham group (i); ## P<0.01 compared to the Media group (ii); $$ P<0.01 compared to the MSCs group (iii). iiii: AVP+MSCs group; AMI: acute myocardial infarction; AVP: Vasopressin; MSCs: mesenchymal stem cells; AVP+MSCs: AVP preconditioned mesenchymal stem cells. Scale bar =50 µm (×40) and 20 µm (×100).

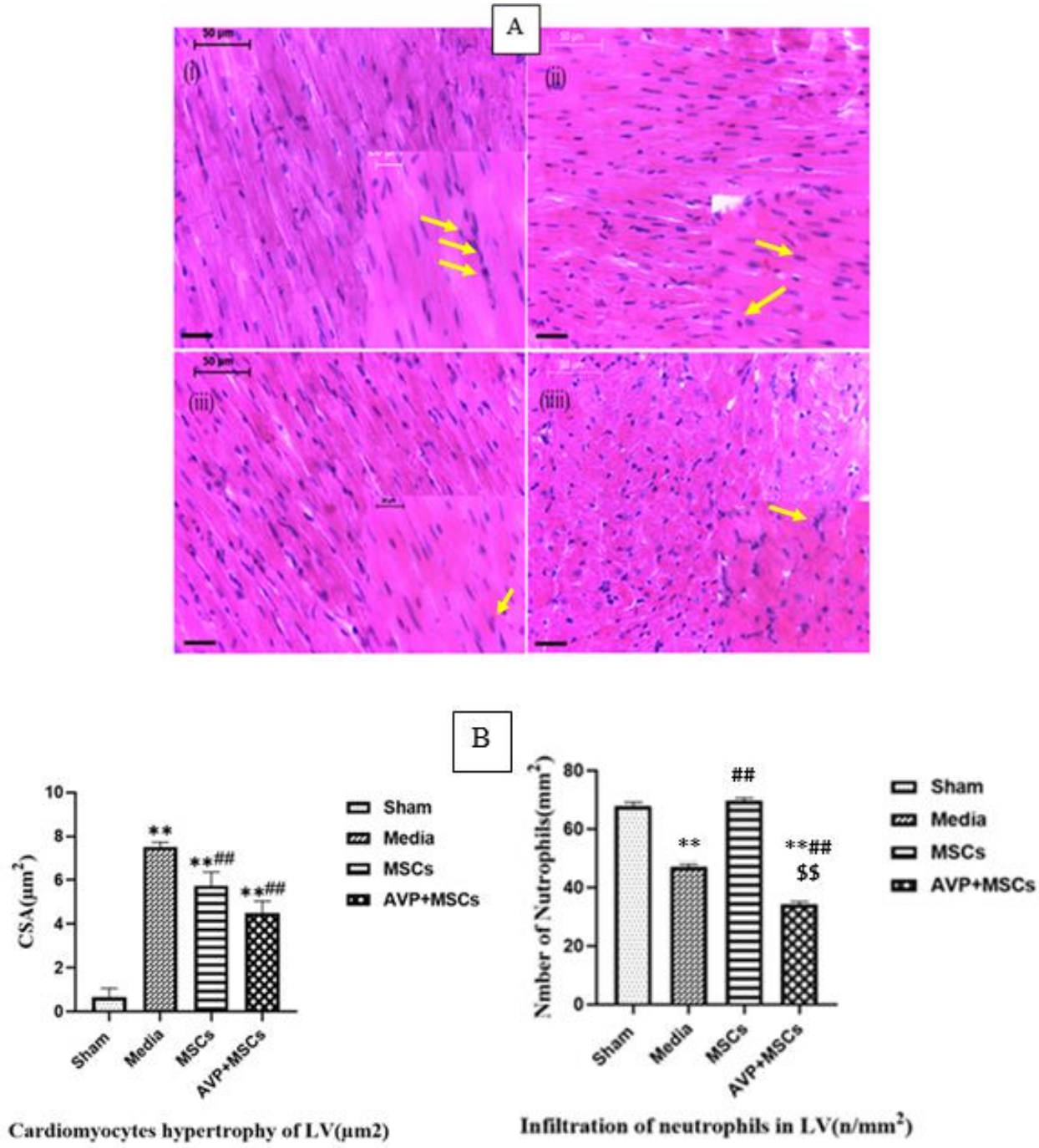


Fig.5. Cardiomyocytes hypertrophy and inflammation measured using Hematoxylin-Eosin staining measurement. (A) Representative images of Hematoxylin-Eosin staining in each group (× 200). (B) Quantitative data for the infiltration of neutrophils in peri-infarct myocardium and cardiomyocytes hypertrophy. iiii: AVP+ MSCs group. n = 10 in each group. ** P<0.01 compared to the Sham

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group (i); ## P<0.01 compared to the Media group (ii); $$ P<0.01 compared to the MSCs group (iii). iiii: AVP+MSCs group; AMI: acute myocardial infarction; AVP: Vasopressin; MSCs: mesenchymal stem cells; AVP+MSCs: AVP preconditioned mesenchymal stem cells. Scale bar =50 µm (×40) and 20 µm (×100).

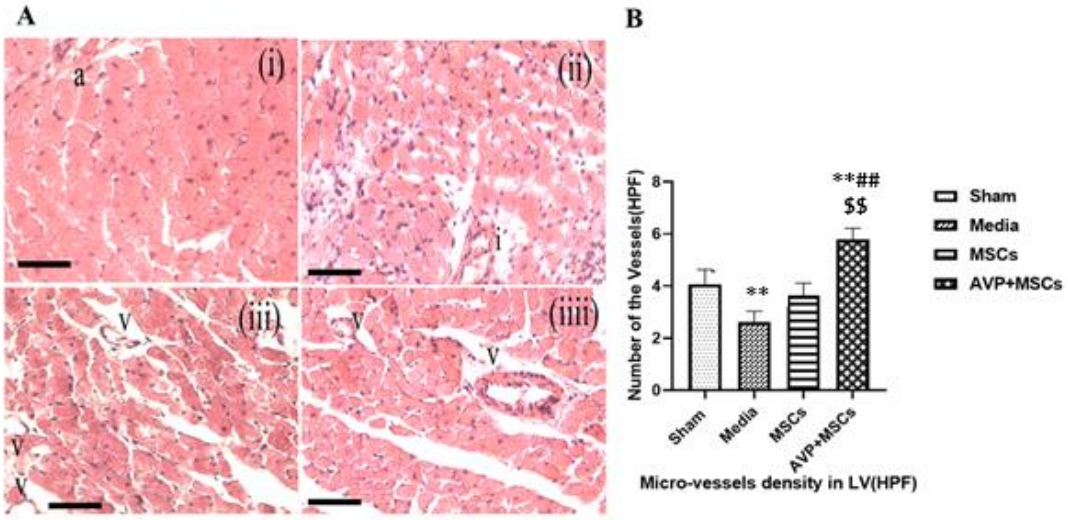


Fig.6. Angiogenesis in peri-infarct region evaluated with H&E staining (×40). (A) Representative images of H&E staining in each group. (B) Angiogenesis was evaluated with the number of micro vessels per HPF. ** P<0.01 compared to the Sham group (i); ## P<0.01 compared to the Media group (ii); $$ P<0.01 compared to the MSCs group (iii). iiii: AVP+MSCs group; AMI: acute myocardial infarction; AVP: Vasopressin; MSCs: mesenchymal stem cells; AVP+MSCs: AVP preconditioned mesenchymal stem cells; H&E: Hematoxylin and Eosin; HPF: high power field. a: arteriole; i: incomplete vessels; v: vessels. Scale bar = 50 µm.

AVP-preconditioned MSCs inhibited cardiomyocytes apoptosis in the peri-infarct region

TUNEL staining was performed to evaluate the anti- apoptotic effects of MSCs and AVP on AMI-induced cardiomyocytes apoptosis. As shown in Figure 7, AMI-induced cardiomyocytes apoptosis in the-

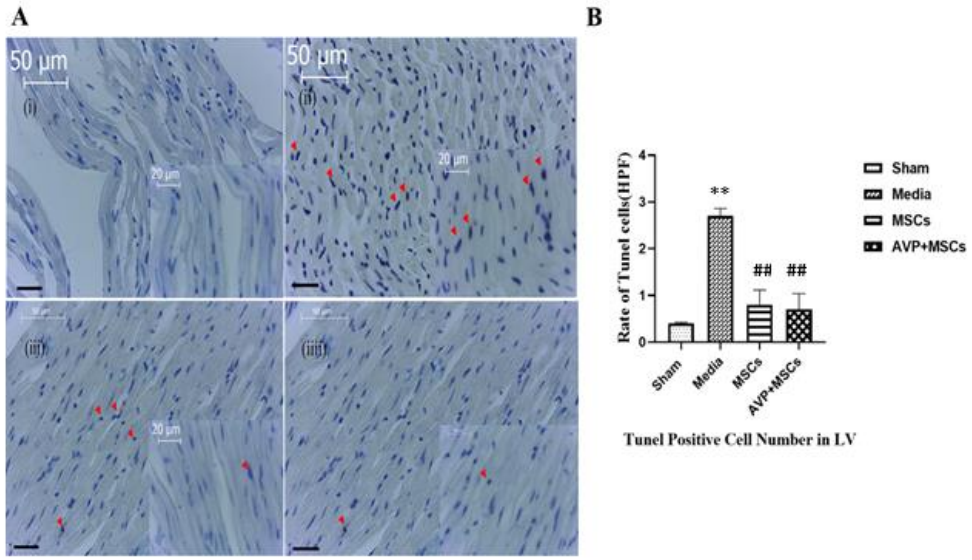


Fig.7. Apoptosis of cardiomyocyte evaluated with TUNEL (×40, ×100). (A) Representative images of TUNEL staining in peri-infarct myocardium in each group. (B) Ratio of apoptotic cells to total cells in each group. ** P<0.01 compared to the Sham group (i); ## P<0.01 compared to the Media group (ii). iii: MSCs group; iiii: AVP+MSCs group; AMI: acute myocardial infarction; AVP:

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Vasopressin; MSCs: mesenchymal stem cells; AVP+MSCs: AVP preconditioned mesenchymal stem cells. TUNEL: terminal-deoxynucleotidyl transferase-mediated dUTP nick end labeling; DAPI: 4'6-diamidino-2-phenylindole dihydrochloride. Scale bar =50 µm (×40) and 20 µm (×100).

media group and the number of TUNEL positive cells in this group significantly increased compared to the Sham group (η2=0.683, P=0.001). AVP+MSCs and MSCs treatment also significantly reduced cardio-myocytes apoptosis in the peri-infarct myocardium compared to the Media group (η2=0.680, P=0.001). There was no significant difference between the MSCs and AVP+MSCs groups when compared with sham group.

Discussion

The current study provides laboratory evidence that pre-conditioned stem cells with AVP improve cardiac repair and regeneration in the rat model of AMI. The results of the present study proved that: 1) AVP+MSCs transplantation enhanced the targeted recruitment and survival of transplanted MSCs accompanied by enhancement of heart functional recovery, attenuation of ventricular remodelling, and infarct size reduction; 2) At the cellular level, cardiac functional improvement was mostly attributable to marked inhibition of inflammation and myocardial apoptosis, enhanced angiogenesis in the infarcted myocardium; 3) At the molecular level, the beneficial effects conferred by AVP+MSCs were attributed to an augmented V1aR/OXTR stimulation.

It has been shown that following the transplantation, the majority of the transplanted stem cells fail to survive in the inflammatory microenvironment of an infarcted heart. This low engraftment rate is believed to be the blockage of cell therapy that needs to be overcome for further improving the efficacy of stem cell therapy. Laboratory manipulation of cells prior to transplantation improves their biological and functional properties through survival, homing, maintenance, and proliferation of donated cells (8). Previous studies have demonstrated that a high dose of oral AVP administration could improve the survival of MSCs and heart function through ameliorating undesirable effects of cardiac infarction (25-27). Additionally, our prior studies also indicated that activation of V1aR and OXTR receptors in H9C2 cells mediate the protective effect of vasopressin via regulating apoptosis marker that leads to cell survival under conditions of oxidative stress. The role of AVP in the improvement of heart ischemia may be related to its action on V1aR and OXTR receptors (28). Previous studies revealed that atorvastatin- pretreated MSCs not only exerted anti-apoptotic effect but also improved targeted recruitment of MSCs by increasing the expression of chemokine receptor type 4 (CXCR4) on MSCs surface (29, 30). Based on these studies, we reasoned that the conditioned administration of AVP with MSCs transplantation could have a more enhanced therapeutic effect in a rat model of AMI.

Studies revealed that the hormone is capable of stimulating cellular growth and proliferation. Despite the identification of AVP receptors in stem cells, previous animal studies have demonstrated promising results of MSCs implantation for myocardial recovery in ischemic heart diseases. Using genetic approaches locally after ischemic insult resulted in striking enhancement in the migration and survival of transplanted MSCs (9). However, the elevated OXTR/V1aR expression in the infarcted region is a major obstacle, because the manipulating of OXTR/V1aR expression with genetic approaches is currently clinically infeasible. On the other hand, findings revealed that the mechanism by which AVP increases the production of human ASCs is through activating V1a receptors and the PLC-IP3 (Phospholipase C-inositol triphosphate) pathway (9).

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AVP has anti-apoptosis, anti-inflammation, and anti-oxidation effects (28). Based on the results of previous studies, AVP could reduce apoptosis in cardiomyocytes cell lines through down-regulation of caspase-3, Bcl-2-associated X (BAX), and up-regulation of B-cell lymphoma 2 (Bcl-2) (28). Also, there was a decrease in anti-apoptosis effect of AVP when V1aR and OXTR receptors were blocked with their antagonists (28). AVP stimulates protein synthesis via the V1aR. In addition, V1 receptors play a significant role in the release of intracellular calcium (9). We obtained that AVP treatment might protected infarcted myocardium and move forward the transplanted MSCs survival and cardiac execution in rat models of AMI. Based on the real-time results on stem cells, it seems that treatment with AVP and MSCs can increase the peak level of OXTR/V1aR expression in the peri-infarct area. More importantly, enhancing the expression of OXTR/V1aR in the post-infarction heart by AVP treatment is clinically more feasible than the genetic approaches used in previous studies (31-34). Consistent with these findings, our recent study has revealed that AVP preconditioned MSCs cannot only have an anti-apoptotic effect, but also improved OXTR/V1aR expression on the cell surface of MSCs and promoted targeted recruitment of MSCs towards the injured myocardium, thereby resulting in improved cardiac performance (9, 10).

In this study, we further demonstrated that MSCs resulted in a respective small fold increases, and AVP+MSCs led to even a very low fold increase in the recruitment and survival of the transplanted MSCs in the infarcted myocardium. These generally low increments in the recruitment and survival of MSCs by AVP+MSCs may result from an up-regulation of OXTR/V1aR expression, suppression of inflammation in the infarcted area (28, 35) and an augmentation of OXTR/V1aR expression on the transplanted AVP+MSCs surface (32). Furthermore, the AVP preconditioned presented in the current study exhibited striking enhancement of therapeutic effect and great reduction of infarct size. To the best of our knowledge, this is the first report of a clinically feasible strategy that improves the recruitment, survival and efficacy of transplanted MSCs. Thus, the AVP- preconditioned MSCs may represent a big breakthrough in stem cell therapy for AMI.

In addition, our study revealed that the outstanding therapeutic benefits conferred by the synergistic effects of MSCs and AVP+MSCs are associated with anti-inflammation, anti-apoptosis activity and enhancement of angiogenesis stem cell mobilization. It was well documented that AVP by itself has anti-inflammatory and even immunomodulatory effects (36), and MSCs could also release anti-inflammatory cytokines and reduce the number of natural killer cells and neutrophils in the post-infarct heart (37). Consistent with these findings, we demonstrated that MSCs treatment decreased the number of inflammatory cells in infarcted and peri-infarct myocardium, while AVP+ MSCs could further profoundly suppress infiltration of inflammatory cells and the production of pro-inflammatory factors, and anti-inflammatory effect afforded by AVP (38). Also, the enhanced targeted MSCs recruitment by both MSCs (39) and AVP+MSCs act synergistically to suppress inflammation, thereby promoting MSCs survival.

This study demonstrated that AVP+MSCs treatment had greater anti-apoptotic effect than MSCs alone. More importantly, we found that AVP+ MSCs was the most potent anti-apoptotic treatment that enhanced the survival of cardiomyocytes in peri-infarct region**,** which could in part lead to their striking cardioprotective effects. MSCs have been shown to secret cytokines to suppress apoptosis and promote cardiomyocytes survival under ischemic conditions (40). AVP+MSCs had greater anti-apoptotic activity and

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exhibited enhanced targeted recruitment to infarct myocardium that possibly due to the improved expression of OXTR/V1aR on their cell surface (28). Furthermore, it is conceivable that the combined enhancement of anti-apoptotic effect and targeted recruitment of AVP+MSCs could synergistically promote cardiomyocytes survival in the infarct heart, thereby causes reducing infarct size, and attenuating AMI-induced adverse structural remodeling and deterioration in LV function. To the best of our knowledge**,** a similar finding has not been reported before.

In this study, we also found that AVP+ MSCs treatment promoted angiogenesis in peri-infarct regions, which could further help to repair the damaged region. The pro-angiogenesis effect of AVP is attributable to enhanced endothelial progenitor cells (EPC) targeted migration (41) into ischemic sites (42). Since AVP treatment up regulated OXTR/V1aR in ischemic cardiac tissues, the circulating OXTR/V1aR positive stem cells or the transplanted AVP+MSCs with improved OXTR/V1aR reported in the current study could be more likely recruited to the injured heart to form new vessels. Additionally, a recent study has indicated that MSCs employ the OXTR/V1aR signaling pathways to promote the migration and differentiation of endogenous stem cells (43).

More importantly, our study demonstrated that the therapeutic effects of AVP+ MSCs were almost completely improved by the OXTR/V1aR signaling, suggesting that the OXTR/V1aR signaling are the major signaling that mediates the effect of AVP+ MSCs on the enhancement of cardiac performance. At last, AVP –preconditioned with injections AVP+MSCs transplantation can targeted recruitment, survival, and therapeutic efficacy of transplanted MSCs through augmented stimulation of OXTR/V1aR pathway. Our study thus suggests AVP+ MSCs preconditioned as a promising strategy for the further improvement of the clinical therapeutic efficacy of MSCs in patients with AMI.

We showed that the AVP drug improves the stem cell capacity of cardiac tissue repair and regeneration. In the current study, we demonstrated for the first time that the Vasopressin-preconditioned human AdMSCs transplantation had a prominent increase in the targeted recruitment and survival of transplanted MSCs, and improvement of cardiac function after AMI through increasing the rate of angiogenesis, decreasing the amount of cardiomyocytes hypertrophy, reducing fibrosis, and reducing apoptosis. Thus, our study indicates the possibility that AdMSCs conditioned with AVP might be a promising therapeutic strategy with potential clinical applications for treating AMI.

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