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Intra-Myocardial Injection of Human Amniotic Membrane-Derived Stem Cells Influened Inflammatory Cytokines in HF Model of Rat

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Abstract

Heart failure (HF) is considered one of the most common heart disorders. Recent studies suggest that injections of amniotic membrane stem cells (AMSC) can improve heart function. Therefore, the current study investigated the effect of intra-myocardial injection of human amniotic membrane-derived stem cells (hAMCs) on inflammatory-related cytokines like IL-10 and IL-17 in the HF model of rats. Twenty-eight male Wistar rats were categorized into four groups: control, HF, culture medium injection group, and hAMCs injection group. After 60 days, blood samples were taken from the animals, and the expression levels of interleukins 10 and 17 were measured by the ELISA technique. The results showed that injection of hAMCs into male rats with HF caused down-regulation of IL-17 inflammatory cytokine and over-expression of IL-10 anti-inflammatory cytokine. Based on the results of this study and previous ones, we concluded that hAMCs could be considered one of the candidates in future studies on reducing inflammation in HF treatment by adjusting some inflammatory cytokines.

Keywords: Interleukin 10; Interleukin 17; Inflammation; Heart failure; Stem cell.

Introduction

Heart failure (HF) is a sophisticated disease induced by abnormal structure and function of the heart contraction or filling (1). One to three percent of adult patients suffer from heart failure, the prevalence of which increases with age (1). Significant symptoms of this disease include exercise intolerance and dyspnea. Other symptoms of HF are fatigue, peripheral edema, orthopedic problems, shortness of breath, and loss of appetite (2). Current guidelines classify HF based on left ventricular ejection fraction (LVEF) as systolic or diastolic disorder (1). The symptoms of both types of heart failure are similar, but there are differences in pathophysiology and treatment. Patients with HF with reduced ejection fraction (HfrEF) have impaired left ventricular contraction. In HF and preserved ejection fraction (HFpEF) patients, the ejection fraction is

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normal. Still, a slight impairment in the contractile function can only be detected by advanced imaging (3). However, in both forms of HF, inflammation plays a key role in cardiovascular disorders, and high levels of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IFN-γ, IL-1β, IL-6, IL-17, and IL-18 have been reported in this group of patients (4-6). Among these, IL-17 has a prominent role in causing myocardial damage, leading to fibrosis and apoptosis induction in myocytes (7). Recently, it has been suggested that this cytokine activates cardiac ventricular remodeling by activating the MAPK pathway in HF due to ischemia (8). On the other hand, one of the anti-inflammatory cytokines whose increased expression can play a protective role in HF is IL-10 (9), whose expression is negatively correlated with TNF-α expression (10). Therefore, increasing IL-10 expression can lead to decreased TNF-α expression, and finally, its protective role in HF is applied.

Despite new treatments and therapies, the mortality and complications of HF remain significant. Therefore, finding effective, easy, and accessible treatment methods for HF is of great importance. One of the options in the treatment of HF is using stem cells that can transform and regenerate the heart. Human amniotic membrane-derived mesenchymal cells (hAMCs) can transform into cardiomyocytes (11, 12). This approach has advantages such as convenience without complications for the donor and secretion of paracrine factors involved in angiogenesis and modulation of immune responses (13, 14).

Eventually, this study aimed to evaluate the effects of myocardial injection of hAMCs on inflammatory factors in HF models of rats.

Materials and Methods

Human Amniotic Membrane-derived Mesenchymal Cells Culture

hAMCs were obtained from Royan Research Institute, Tehran, Iran. First, the cells were poured into 25 cm^2 flasks. Then, 10 ml DMEM culture medium containing 1% fetal bovine serum (FBS), 2 mL L-glutamine, 100 U/mLpenicillin, and 10 µg/mL streptomycin were added. Finally, the plates were incubated in a 5% CO_2 incubator at 37 °C for 24h.

Animals and Induction of Heart Failure

Twenty-eight Wistar male rats (mean weight: 180-200 g) were purchased from the Department of Pharmacology, University of Tehran, Iran. The rats were placed in the animal lab in the pharmacology department under standard laboratory conditions at a temperature of 22 ± 1 °C, humidity (65 ± 5%), and a light and dark cycle of 12/12 h with free access to food and water. After 1 week, the animals were divided into4 groups as follows:

1) Control healthy group

2) HF rats; in this group, HF was induced by the subcutaneous injection of 170 mg/kg/d isoprenaline (ISO) for 4 consecutive days.

3) HF rats injected with 150µL DMEM culture medium:

4) HF rats injected by 2×10^6 hAMCs

For injection, the animals were first weighed. Measuring the animal's weight is necessary to calculate the amount of anesthetic, antibiotic, and serum to be injected into the animal. In the next step, the animal was anesthetized by injection of xylazine and ketamine, and after shaving the surgical site, it was intubated and connected to a ventilator. The animal's chest was then cut to see its heart. The pericardium was then slowly opened, and in the HF+ DMEM group, only 150 µl of culture media DMEM was injected into the myocardium. In the HF+ hAMCs group, 2×10^6 stem cells were dissolved in 0.5 ml of DMEM and injected into the myocardium. After injection, the chest was sutured, and the animals were exposed to oxygen to regain consciousness.

IL-17 and IL-10 Measurements

First, rats were anesthetized, and blood samples were taken from the hearts of rats, then the serum was prepared, and the levels of IL-17 and IL-10 factors in the serum of rats in all 4 groups were measured by enzyme-linked immunosorbent assay (ELISA). The relevant kits (Sigma, Germany) assessed these cytokines according to the manufacturer's instructions.

Statistical Analysis

Comparison between means was performed using one-way analysis of variance (ANOVA) and Tukey post hoc test at a probability level of $P<0.05$. the data was analyzed by SPSS software version 22.

Results

Characterization of MSCs and Induction of HF

MSCs were identified by expressions of hematopoietic markers (CD105, CD29, CD34) in a previous study reported by the authors (15). Also, induction of HF by ISO was associated with a decrease in EF and FS in animals, as the authors discussed in a previous article (15).

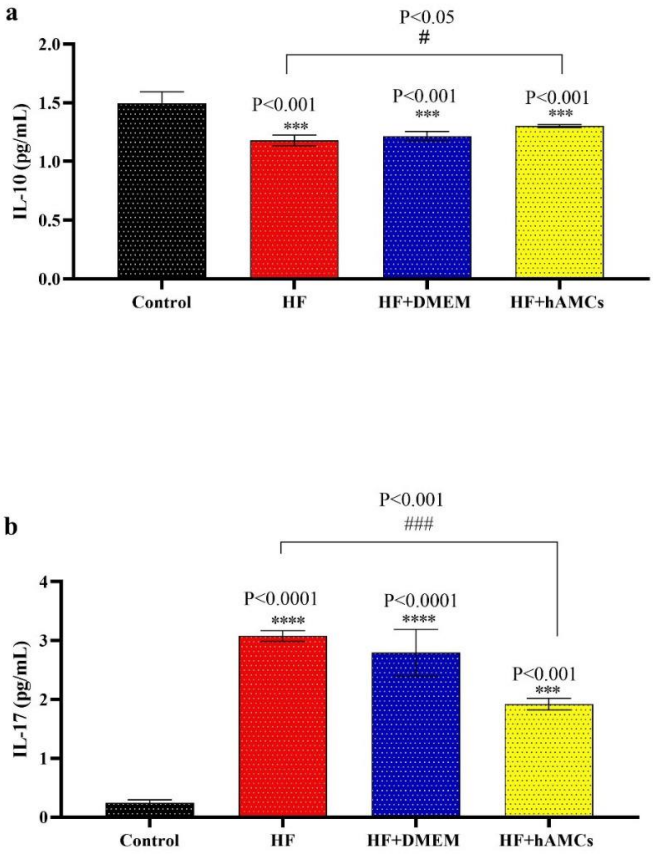

Figure 1. The serum levels of IL-10 (a) and IL-17 (b) in rats after induction of HF and injected with human amniotic membrane-derived mesenchymal cells (hAMCs)(n=7. Mean±SD). *** and **** show significant differences compared to the control at probability levels of p<0.001, and p<0.0001, respectively. # and ### show significant differences compared to the HF group at probability levels of, p<0.05, and p<0.001, respectively.

IL-17 and IL-10

The downregulation of IL-10 and upregulation of IL-17 were seen by the induction of HF in rats, indicating an inflammation response. According to Figure 1a, HF induction reduced IL-10 expression by 31% compared to the control; however, in rats treated with hAMCs 19% reduction in expression of IL-10 was seen compared to the control. A comparison of the mean expression of IL-10 in the HF group and the HF group treated with hAMCs showed an increase in the expression of this cytokine in the hAMCs-treated group by 12%.

Figure 2b shows the extent of changes in IL-17 cytokine expression in different groups of animals. IL-17 in the HF group was increased 12.81-fold compared to the control group. Nevertheless, in the HF group treated with hAMCs, an 8-fold increase in IL-17 expression was seen compared to the control group. A comparison of the mean IL-17 expression in the HF and HF-treated groups showed approximately 40% overexpression of this cytokine in the hAMCs-treated group. These results indicate that hAMCs have anti-inflammatory effects under HF conditions.

Discussion

Based on our study, the injection of hAMCs into HF rats overexpressed IL-17 anti-inflammatory cytokine and downregulated IL-10 inflammatory cytokine. These findings suggest that hAMCs may have anti-inflammatory properties. There have been reports that amniotic membrane stem cells have therapeutic potential in cardiovascular (16), hepatic(17), and corneal diseases (18). Stem cells can be located in damaged tissues and can migrate to inflammatory tissues (19). This study also showed that myocardial injection of hAMCs reduces IL17 as an inflammatory cytokine.

Shortly, stem cell therapy is likely to be one of the

alternative treatments for HF. Several methods exist for generating HF models in laboratory animals (20). ISO-induced HF is a standard procedure in which extensive myocardial damage gradually progresses (21) while the vascular system remains intact in the heart. This is more like the natural process that occurs during HF *in vivo*.

During HF, Interleukin-17 is increased as a proinflammatory cytokine that promotes the secretion of other cytokines like IL-2, TNF-α, IL-18, IL-8, and IL-6 (22). Increases in the levels of these cytokines have been reported in individuals with cardiovascular disease (23). In the present study, with the development of HF in rats, the expression of IL-17 increased, following the above results. However, when hAMCs were injected into rats, IL-17 inflammatory cytokine downregulated and IL-10 anti-inflammatory cytokine upregulated, which aligns with the above studies. Based on the study of Jiao et al., injection of hAMCs seems to be effective in improving echocardiographic parameters due to decreased expression of inflammatory cytokines (24). In our previous study, we evaluated the role of AMSCs in protecting myocardia against myocardial injury induced by ISO. We showed inflammation inhibition by targeting the inflammatory MAPK/NFkB signaling pathway. Flow cytometry and echocardiography were used for determining hAMSCs characteristics and cardiac function, respectively and several inflammatory cytokines including TNF-α, transforming growth factor beta (TGF-β), IL-6, IL1 β, IL-8, and CRP were evaluated by ELISA test. Immunohistochemistry was used for assessing the activities of NF-kB and phosphorylated p38 MAPK. Based on the results, ISO injection led to HF and elevated the circulating inflammatory cytokines which were reversed by intramyocardial injection of AMSCs ($P > 0.05$). NF-kB and phosphorylated p38 MAPK decreased significantly ($P > 0.05$). So, our new findings are in parallel with our previous ones (15). In conclusion, according to the results of this study and previous studies, it seems that one of the mechanisms of stem cells is to regulate the expression of inflammatory factors, so considering the increase of inflammation in heart diseases, maybe these cells can be considered a candidate in future studies on the HF treatment. However, more studies are needed to incorporate this treatment into HF treatment protocols.

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