"The Journal of Tehran University Heart Center"
Code: 2329-1955
Original Article

Human Amniotic Membrane Mesenchymal Stem Cells-Derived Conditioned Medium Adjusts Inflammatory and Myofibrotic Factors In Vivo

Gazaleh Asgharnezhad, Msc[1], Sachli Mohamadi, Msc[1], Mahdieh Mehrab Mohseni, PhD[1], Neda Mousvi-Niri, PhD[2], Maryam Naseroleslami, PhD[1*]

[1]*Department of Cellular and Molecular Biology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.*
[2]*Department of Biotechnology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.*

Abstract

Background: Numerous people are diagnosed with heart failure (HF) or die from it. Among different therapeutics, hAMSCs-CM therapy is one of the most effective HF treatments. As the method of action of this treatment is unclear, for the first time, we used an animal model of heart failure to perform an experiment to determine the hAMSCs-CM's method of action, focusing on TGF-β/Galactin-3, MCP1, BNP, and ALD.

Methods: Forty adult Wistar male rats were divided into 4 groups of Control, HF, Culture Medium, and Conditioned Medium (CM). All rats other than the control received an injection of isoproterenol (ISO) to make animal model of HF. Rats given CM received CM, and rats given culture medium received culture media. Then, an ELISA was used to measure the serum fibrotic factors of TGF-β/Galactin-3, MCP1, BNP, and ALD. Then, Statistical analysis was done by one-way analysis of variance as well as the Tukey test.

Results: serum TGF-β/Galactin-3, MCP1, BNP, and ALD was significantly increased in HF, CM, and culture media compared with controls (*P<0.001*), as well as significantly decreased in CM rats compared with HF (*P<0.001*). Although CM therapy could not return TGF-β/Galactin-3, MCP1, BNP, or ALD to the normal range.

Conclusion: Our results show that hAMSCs-CM changed cytokines that cause inflammation and fibrosis, like TGF-β/Galactin-3, MCP1, BNP, and ALD, in ISO-stimulated HF male rats. This helps fill in some of the gaps in HF therapy.

---------- OK ----------

Keywords: *Mesenchymal stem cells; Heart failure; Inflammation; Fibrosis*

Introduction ---- درج رفرنسها در محله های مورد نظر----

Every year, numerous people are diagnosed with heart failure (HF) or die from it as the last stage of a chronic heart condition in different countries. [1] About 26 million patients are affected by HF worldwide. [2] The CDC reported that in the United States, approximately 6,200,000 adults are suffering from this condition. [3]

During cardiac remodeling, transforming growth factor-β (TGF-β) plays a pivotal role during fibrotic tissue formation through recruiting fibroblasts and initiating transition to myofibroblasts.[4]It also increased ECM synthesis and release, which leads to extreme collagen deposition .[5, 6]

It is well documented that TGF-β signaling also is associated with galectin-3 as a known cardiac fibrosis marker with inflammatory characteristics. Galectin-3, a member of Galectin family, plays a specific role in scar and fibrotic tissue formation .[7] Galectin-3 upregulation has been reported in numerous fibrosis conditions such as chronic pancreatitis, renal, and cardiac fibrosis .[8, 9] Moreover, galectin-3 actively involves in myofibroblast proliferation .[10]

MCP-1 is a member of the member of the C-C chemokine family and is produced by endothelial and smooth muscle cells and mononuclear immune cells like monocytes and macrophages during atherosclerotic plaques in response to some cytokines, angiotensin II, homocysteine, and other atherosclerosis factors. MCP-1 works as a chemotactic factor via C-C chemokine receptor type 2 (CCR-2) on monocytes to attract monocytes into the vascular wall.

In people suffering from stable coronary heart disease as well as peripheral artery disease, MCP-1 plasma was overexpressed .[11]

Corresponding Author:** ***Maryam Naseroleslami, *Assistant Professor of Cell & Molecular Biology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. Zipcode: 1949635881, Cell: +98 09122394618. Tel: +982126602642. Fax: +982126602642. E-mail: naseroleslamimaryam@gmail.com.*

B-type natriuretic peptide (BNP) is a peptide that is saved in a few granules in the ventricles and secreted in large amounts following stimulation. The BNP levels in the serum of HF patients are 100-fold higher than in healthy people, with a half-life of approximately 20 min. BNP is processed into pro-BNP and, subsequently, into the active BNP as well as the inactive NT-proBNP .[12]

Aldosterone (Aldo) is known as a mineralocorticoid hormone that regulates blood pressure (BP) and electrolytic balance through an intracellular mineralocorticoid receptor (MR). Aldo also has effects on cardiovascular remodeling and diseases by changing cardiac hypertrophy, arterial stiffness, fibrosis, inflammation, and oxidative stress .[13] As the merely decisive HF treatment is heart transplantation, research for alternative treatments continues. One of these alternatives with a focus on tissue regeneration and decreasing fibrosis in regenerative medicine is stem cell treatment .[14, 15]

Between different stem cells, human amniotic membrane-derived MSCs (hAMSCs) are the most trusted .[16, 17] and possess appropriate proliferation rate, differentiation capacity, and immunogenic characteristics as they express a few MHCI molecules .[18] They can secrete paracrine-conditioned medium (CM), which is rich in different therapeutic agents such as cytokines and growth factors.[19, 20] Based on studies, CM does not have the MSC-associated disadvantages like tumorigenicity .[17]

Though HAMSCs-CM have been used in the treatment of diseases, their mode of action has remained unclear. For the first time, we used an animal model of heart failure to perform an experiment to determine the hAMSCs-CM's method of action, focusing on TGF-β/Galactin-3, MCP1, BNP, and ALD.

In our earlier research, we examined the mode of action of hAMSCs-CM in the HF treatment of tissue, and we anticipated that this study would fill a gap in our knowledge of the mode of action of hAMSCs-CM on serum factors in HF therapy. The results of this investigation are presented in Figure 1 as a graphical abstract.

Methods

The amniotic membranes were donated by post-partum volunteers in Shahid Akbar Abadi Hospital after signing consent based on the Ethical Committee of the Iran University of Medical Sciences (IR.IAU.PS.REC.1400.018). Fluorescence-activated cell sorting (FACS) was performed to be sure that isolated cells have an amniotic origin, according to our previous job .[21] Obtained cells were cultured with α-MEM, 10% FBS (Gibco, Australia), 100 U/mL penicillin, 2 M L-glutamine, and 100 µg/mL streptomycin for 48 h, then washed with PBS. Subsequently, it was swapped via an α-MEM without serum to harvest CM. The MSCs were incubated in a hypoxic condition for 48 hours, centrifuged (1200 rpm), filtered through a 0.22 µm filter, and CM was stored at -80 °C.

Forty adult Wistar male rats were provided by the Iran University of Medical Sciences and were accidentally categorized into four groups: 1) Control: animals without any manipulations; 2) HF: animals treated with 170 mg/kg isoproterenol (Sigma, Aldrich, USA) subcutaneously for four sequential days; 3) Culture Media: under anesthesia with ketamine (80 mg/kg) as well as Xylazine (5 mg/kg), rats were injected with 150µl of cell-free DMEM into four points of the myocardium with a 31-gauge needle 28 days after the final ISO injection; and 4) Conditioned Medium (CM): rodents received 150µl of CM under the same condition as the Culture Media category.

After treatments, the blood of all rats was collected, centrifuged (600 g for 10 min at 4 °C), and the serum was used for the evaluation of TGF-β/Galactin-3, MCP1, BNP, and ALD via ELISA kits, based on the instructions (RayBiotech, Inc.). The ELISA reactions were assessed at 450 nm by the ELISA Reader (Synergy MX BioTek). All concentration results were reported based on pg/mL.

Statistical analysis was done by one-way analysis of variance as well as the Tukey test on Prism v5.0 (GraphPad Software, La Jolla, USA). All concentrations were reported as mean±SEM, and $P<0.001$ was considered significant.

Results

Effects of hAMSCs-CM *on serum levels of fibrogenic markers*

The results of the ELISA assay showed that, 4 weeks after isoproterenol administration, serum levels of TGF-β significantly increased in HF ($P<0.001$), culture media ($P<0.001$), as well as CM groups ($P<0.05$) compared to the control group ($P<0.001$), while after hAMSCs-CM administration, TGF-β significantly reduced in the conditioned medium group compared with the HF group, but it was significantly higher than the control group ($P<0.001$) (Figure 2, A).

Furthermore, ISO-induced HF caused a significant rise in serum levels of galectin-3 in both HF, isoproterenol, and culture media ($P<0.001$). However, treatment with hAMSCs-CM significantly decreased the level of galectin-3 compared with HF ($P<0.001$) and culture media (Figure 2, B).

Furthermore, serum MCP1 was significantly increased in HF, CM, and culture media compared with controls ($P<0.001$), as well as significantly decreased in CM rats compared with HF ($P<0.001$) (Figure 2, C).

In addition, serum BNP was significantly elevated in HF, CM, and culture media compared with controls ($P<0.001$), as well as significantly downregulated in CM rodents compared with HF ($P<0.001$) (Figure 2, D).

Serum ALD was also significantly overexpressed in HF, CM, and culture media compared with controls ($P<0.001$), as well as significantly underexpressed in CM animals compared with HF ($P<0.001$) (Figure 2, E). Although CM therapy could not return TGF-β/Galactin-3, MCP1, BNP, or ALD to the normal range (As the plot of MCP1 and ALD are similar togrther, the mean±SD of MCP1 and ALD in all categories is presented in Table.1).

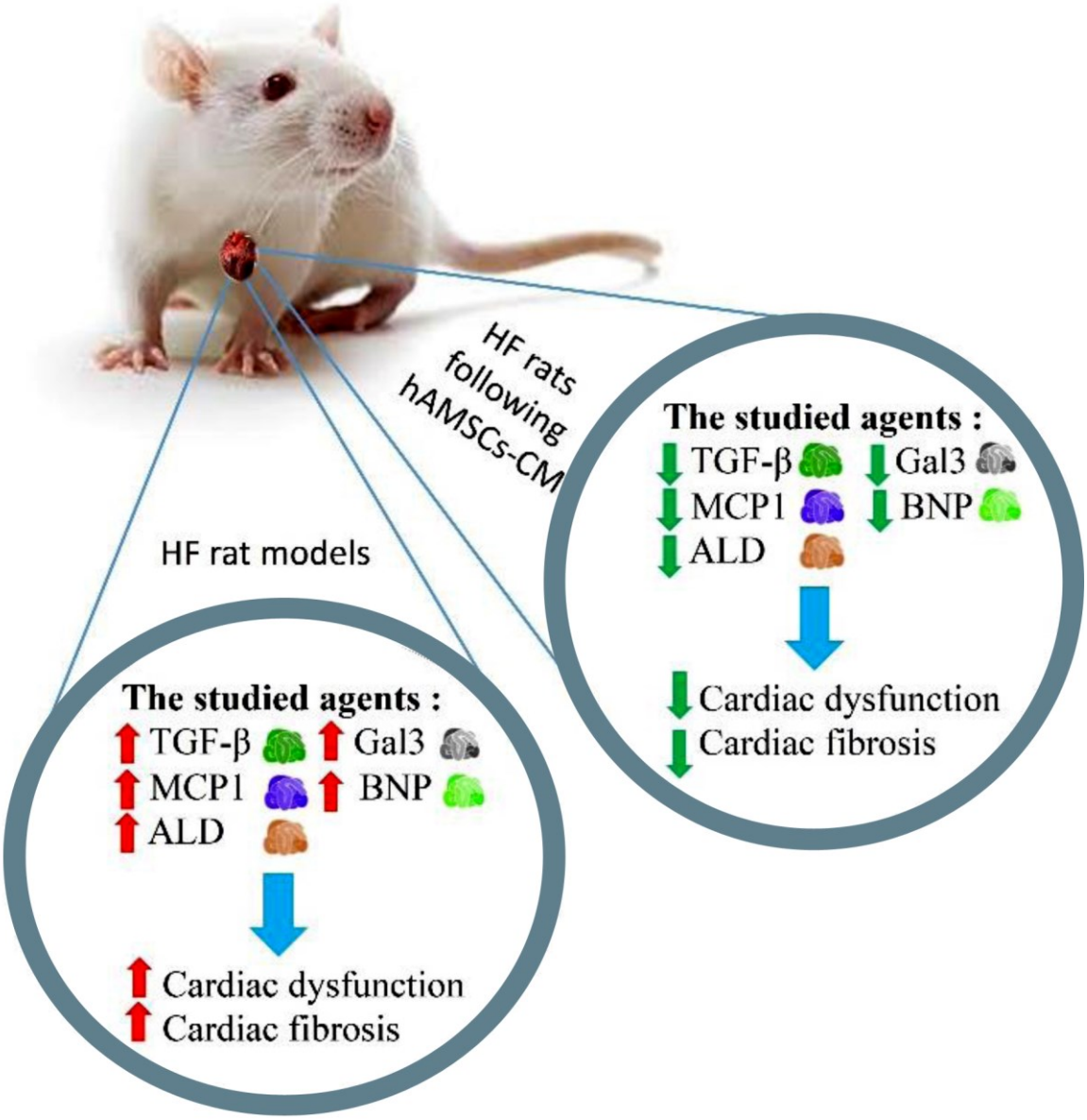
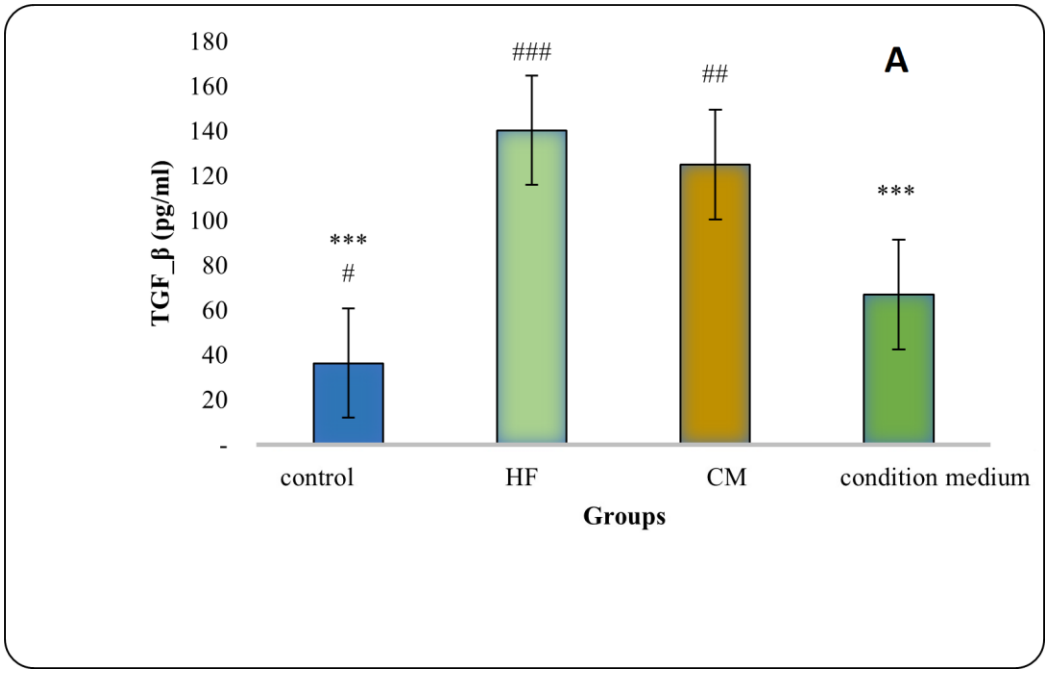
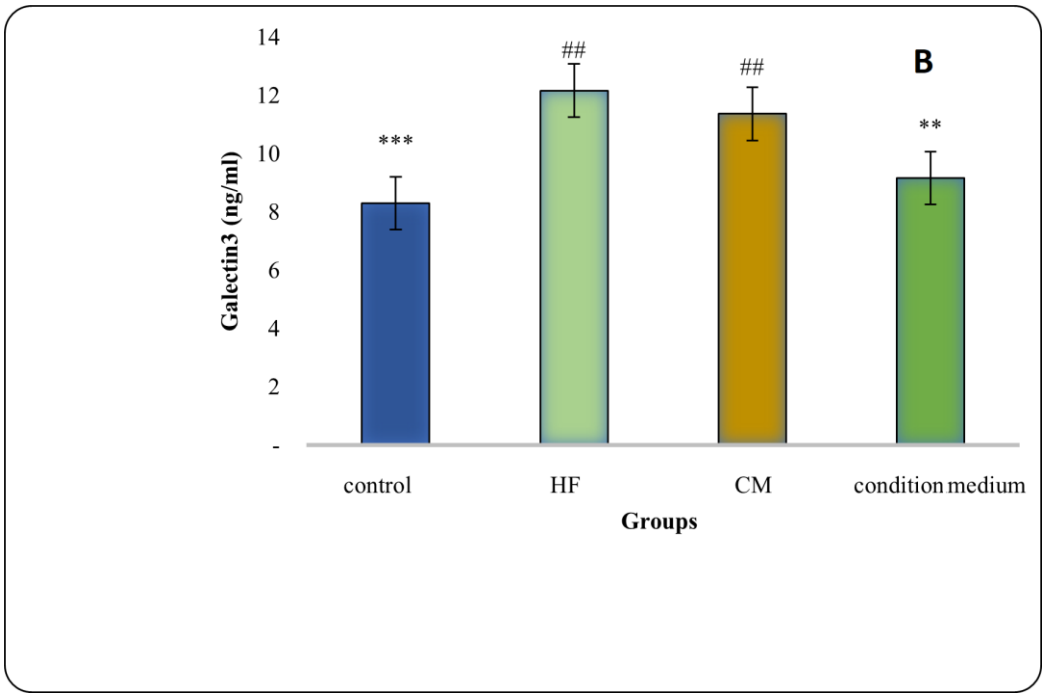
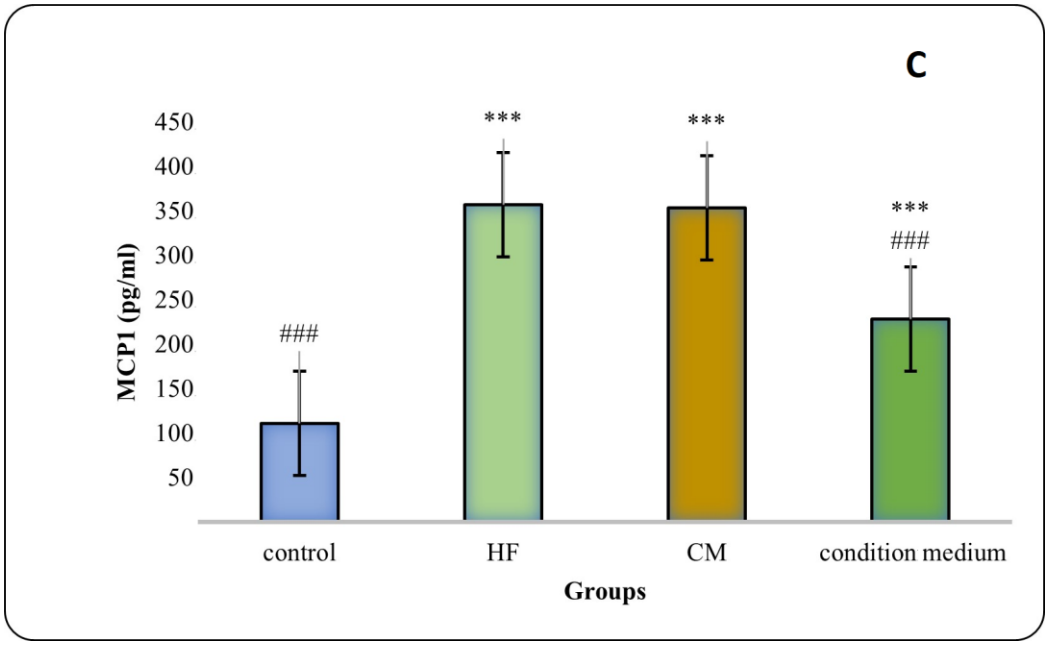
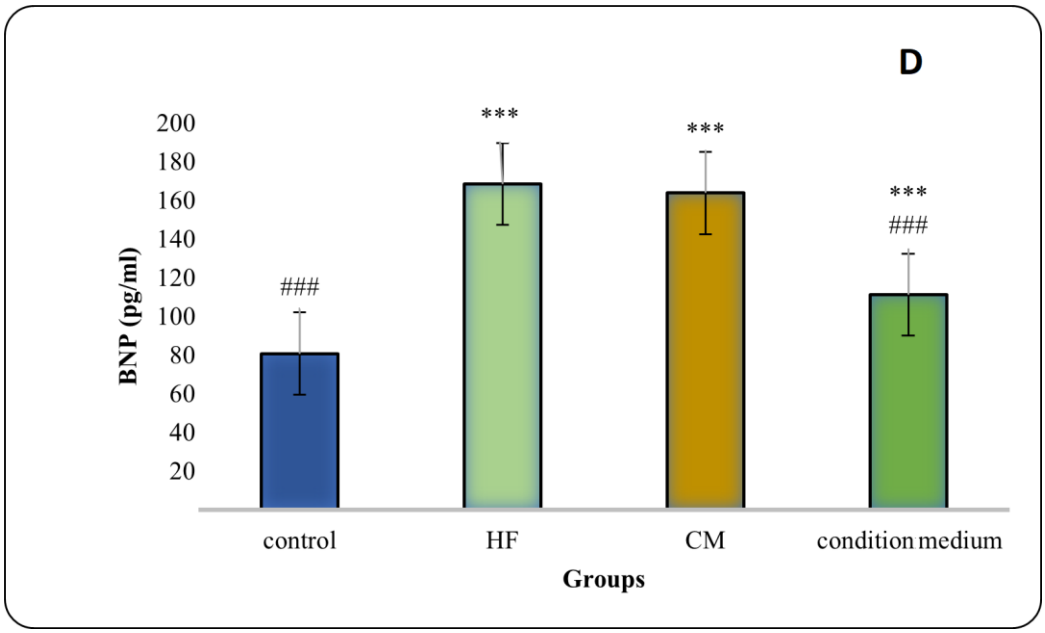


Figure 1. A graphical abstract of the current study. The HF model treated with hAMSCs-CM rats underexposed to several factors related to cardiac ferroptosis, resulting in cardiac function improvement.


A
TGF_β (pg/ml)
180
160
140
120
100
80
60
40
20
-
***
#
###
##
***
control
HF
CM
condition medium
Groups


B
Galectin3 (ng/ml)
14
12
10
8
6
4
2
-
***
##
##
**
control
HF
CM
condition medium
Groups


C
MCP1 (pg/ml)
450
400
350
300
250
200
150
100
50
###
***
***
***
###
control
HF
CM
condition medium
Groups


D
BNP (pg/ml)
200
180
160
140
120
100
80
60
40
20
###
***
***
***
###
control
HF
CM
condition medium
Groups

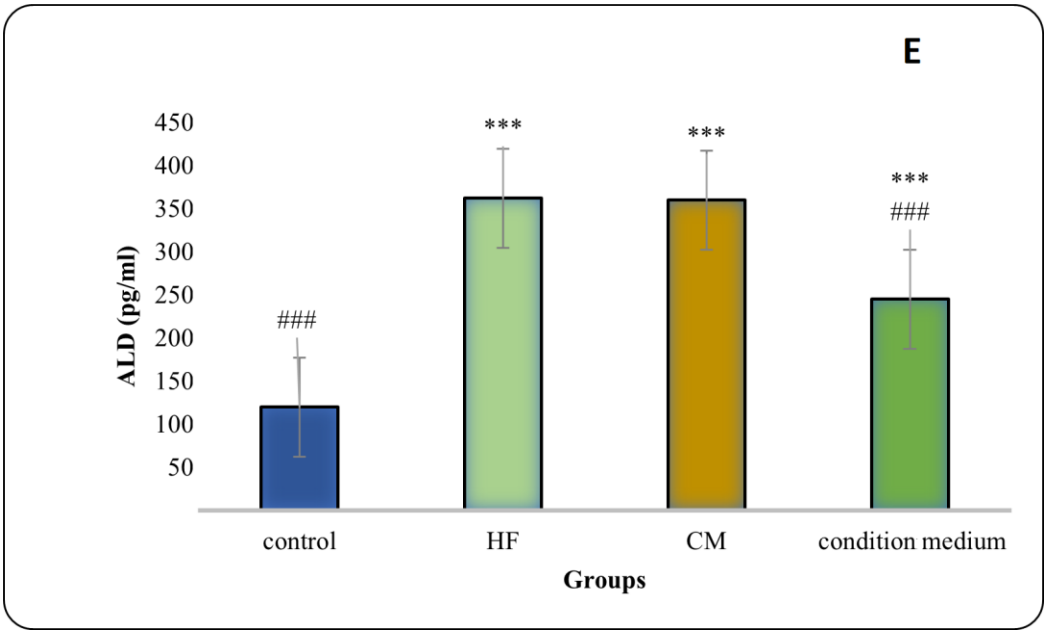


Figure 2. ELISA Assay of serum levels of
A) TGF-β,
B) Galectin-3,
C) MCP1,
D) BNP,
E) ALD; 4 weeks after intramyocardial administration of hAMSCs-CM (n = 10).
###P<0.001, ##P<0.01, #P<0.05 *vs.* control;
***P<0.001, **P<0.01 *vs.* HF.
Data are presented as mean±SEM.

Discussion

Cardiac remodeling is the most important morbidity cause in HF, which further leads to cardiac fibrosis .[22] So, prohibiting cell damage and fibrosis is the key subject in HF research .[23, 24, 25]

Recently, mesenchymal stem cells have been suggested in cardiac disease treatment. For example, hAMSCs have a high potential to differentiate into cardiomyocytes, are cost-effective, and can be easily provided .[14, 16, 26] but can lead to tumor formation. To overcome this disadvantage, hAMSCs-CM, which contains paracrine secretion of MSCs such as growth factors, cytokines, and proteins, was used by several researchers .[15, 20] Moreover, major histocompatibility complex (MHC) class I expression in hAMSCs is minimal. Hence, we have used this cell lineage, or hAMSCs-CM, for studies using animal models, and no unintended immune reactions have occurred.[27,28] Based on these outcomes, we hypothesized it could improve HF disease in rats.

Isoproterenol (ISO)-induced HF animal models have been widely used in experimental design to study HF because they mimic human HF .[22] ISO is a β-adrenergic agonist with acute positive chronotropic and inotropic effects .[25, 29,30] So, we investigated ISO-stimulated HF rats and reported serum levels of TGF-β/Galactin-3, MCP1, BNP, and ALD to evaluate the effect of hAMSCs-CM on them.

In this study, we observed that intramyocardial injection of hAMSCs-CM elevated fibrogenic cytokines, including galectin-3 and TGF-β.

In another study, we investigated the protective effects of hAMSCs labeled by superparamagnetic iron oxide nanoparticles (SPIONs) against isoproterenol (ISO)-induced myocardial injury in the presence and absence of a magnetic field. Then, we assessed myocardial fibrosis, heart function, characterization of hAMSCs, and histopathological changes by Masson's trichrome, echocardiography, flow cytometry, and H&E staining, respectively. We also measured the level of pro-inflammatory cytokines by ELISA. According to our findings, all of the tests confirmed each other and showed that the SPION-labeled MSCs in the magnetic field could be a good option to reverse the studied factors. [28]

In another project, we examined the cardioprotective effects of hAMSCs-CM in a rat model of isoproterenol (ISO)-induced myocardial damage to clarify its effect on tissue. ISO was subcutaneously injected at 170 mg/kg/day for 4 consecutive days to create the model. Echocardiography, immunohistochemistry assays, and Trichrome Masson's staining were used to evaluate the hAMSCs-CM function. Intramyocardial post-treatment with 150µl of hAMSCs-CM significantly improved the evaluated factors .[21]

Although galectin-3 has been upregulated in patients with acute and chronic HF as a clinical biomarker for HF tracing .[31], the exact mechanisms by which galectin-3 is involved in cardiac remodeling and fibrosis have not been fully understood. Some studies suggest that its expression is associated with the TGF-β/Smad signaling pathway and inflammatory conditions. There is solid evidence that increased serum levels of galectin-3 are linked to overactivation of fibroblasts and macrophages, which are responsible for adverse cardiac remodeling .[7, 32] The overexpression of galectin-3 in the LV tissue and its contribution to LV remodeling .[33] It was claimed in another study that liver myofibroblasts are activated by galectin-3 via TGF-β, which is directly linked to liver and kidney fibrosis. TGF-β activation of galectin-3 also contributes to the inflammatory response during cardiac

remodeling .[26] Interestingly, galectin-3 is related to the turnover of several ECM proteins, such as procollagen type I, type III, and MMP-2 Therefore, our results, which show a reduced serum level of galectin-3 in rats receiving the conditioned medium of hAMSCs, are of great importance.[7]

In line with our results, Tang *et al.* recently reported that bone marrow mesenchymal stem cells could mitigate renal interstitial fibrosis and decrease TGF-β1 and galectin-3. They further revealed that galectin-3 downregulation has a direct relationship with decreased renal fibrotic tissue formation, most probably through the galectin-3/Akt/GSK3β/Snail signaling pathway.[8]

Also, treatment with hMSCs-CM resulted in hindering heart tissue apoptosis through reducing oxidative stress and downregulating TGF-β signaling. TGF-β overexpression in injured cardiomyocytes regulates fibroblast function .[5] TGF-β also controlled connective tissue growth factor (CTGF) as a very influential fibrogenic factor .[23, 34] The infarct myocardium intensely requires TGF-β to go through the inflammation. Thus, TGF-β inhibition would be fatal in this circumstance.[6] Therefore, the fact that in our study, hAMSCs-CM administration failed to reduce the TGF-β level up to that of the intact animals may be a favorable aspect.

In this study, serum MCP-1 and BNP were increased in HF rats, and CM treatment decreased them. In agreement with our results, Luis et al. investigated plasma MCP-1, NT-proBNP, and Gal-3 as prediction factors of recurrent cardiovascular diseases like acute ischemic heart disease (HF, as well as death in people suffering from stable CAD and continuous or low inflammation. They reported higher plasma MCP-1 and NTproBNP in people with continuous inflammation. However, Gal-3 did not change. Plasma MCP-1 and NT-proBNP were linked with worse outcomes in patients with continuous inflammation. Furthermore, NT-proBNP was associated with a higher incidence of HF or death in people with continuous as well as low inflammation .[35]

In the current investigation, serum ALD was overexpressed in HF rats, and CM treatment reversed it. Based on the Messaoudi et al. study, Aldosterone is the key ligand of the mineralocorticoid receptor (MR). Experimental and clinical data show the harmful MR effects on cardiovascular diseases. The MR blockage in HF patients with heart failure confirms the significance of MR in the heart and blood vessels' tissue. Experimental models showed the effects of Aldosterone in the heart tissue .[36]

Furthermore, Cha et al. showed that Aldosterone stimulates galectin-3.[37] In a clinical trial, serum galectin-3 was significantly overexpressed in people with Aldosterone -producing adenoma. Following adrenalectomy, the myocardial fibrosis and serum galectin-3 were reversed to normal levels .[38] In addition, in mice and rats with Aldosterone -stimulated heart fibrosis, the galectin-3 knockout decreased fibrotic alterations and cardiac dysfunction .[39] Aldosterone upregulation also plays a key role in the inflammation to fibrosis .[40]

Conclusion

We expect that the current results have somehow filled the information gap about HF treatment, as we observed that the hAMSCs-CM injection improved cardiac fibrosis in HF rat models. It also adjusted serum TGF-β/Galactin-3 fibrogenic cytokines, MCP1, BNP, and ALD. So, our results focused on the therapeutic properties of hAMSCs-CM in ISO-stimulated HF male rats.

Acknowledgment

This research was part of the thesis of the two first authors at Tehran Medical Sciences, Islamic Azad University and they supported this research finantially.

References

1. Daltro PS, Barreto BC, Silva P, Neto PC, Sousa Filho P, Neta DS, et al. Therapy with mesenchymal stromal cells or conditioned medium reverse cardiac alterations in a high-fat diet–induced obesity model. Cytotherapy. 2017;19(10):1176-1188.
2. Hassannejad R, Shafie D, Turk-Adawi KI, Hajaj AM, Mehrabani-Zeinabad K, Lui M, et al. Changes in the burden and underlying causes of heart failure in the Eastern Mediterranean Region, 1990–2019: An analysis of the Global Burden of Disease Study 2019. Eclinicalmedicine. 2023;56:101788.
3. Roger VL. Epidemiology of heart failure: a contemporary perspective. Circulation research. 2021;128(10):1421-1434, Jain V, Minhas AMK, Morris AA, Greene SJ, Pandey A, Khan SS, et al. Demographic and Regional Trends of Heart Failure–Related Mortality in Young Adults in the US, 1999-2019. JAMA cardiology. 2022;7(9):900-904.
4. Zhou H, Yang H-X, Yuan Y, Deng W, Zhang J-Y, Bian Z-Y, et al. Paeoniflorin attenuates pressure overload-induced cardiac remodeling via inhibition of TGFβ/Smads and NF-κB pathways. Journal of Molecular Histology. 2013;44(3):357-367.
5. Dobaczewski M, Chen W, Frangogiannis NG. Transforming growth factor (TGF)-β signaling in cardiac remodeling. Journal of molecular and cellular cardiology. 2011;51(4):600-606.
6. Cinato M, Guitou L, Saidi A, Timotin A, Sperazza E, Duparc T, et al. Apilimod alters TGFβ signaling pathway and prevents cardiac fibrotic remodeling. Theranostics. 2021;11(13):6491.

7. Frunza O, Russo I, Saxena A, Shinde AV, Humeres C, Hanif W, et al. Myocardial galectin-3 expression is associated with remodeling of the pressure-overloaded heart and may delay the hypertrophic response without affecting survival, dysfunction, and cardiac fibrosis. The American journal of pathology. 2016;186(5):1114-1127.
8. Tang H, Zhang P, Zeng L, Zhao Y, Xie L, Chen B. Mesenchymal stem cells ameliorate renal fibrosis by galectin-3/Akt/GSK3β/Snail signaling pathway in adenine-induced nephropathy rat. Stem Cell Research & Therapy. 2021;12(1):1-22.
9. Wang L, Friess H, Zhu Z, Frigeri L, Zimmermann A, Korc M, et al. Galectin-1 and galectin-3 in chronic pancreatitis. Laboratory investigation. 2000;80(8):1233-1241.
10. Martínez-Martínez E, Calvier L, Fernández-Celis A, Rousseau E, Jurado-López R, Rossoni LV, et al. Galectin-3 blockade inhibits cardiac inflammation and fibrosis in experimental hyperaldosteronism and hypertension. Hypertension. 2015;66(4):767-775.
11. Blanco-Colio LM, Méndez-Barbero N, Pello Lázaro AM, Aceña Á, Tarín N, Cristóbal C, et al. MCP-1 predicts recurrent cardiovascular events in patients with persistent inflammation. Journal of Clinical Medicine. 2021;10(5):1137.
12. Goetze JP, Bruneau BG, Ramos HR, Ogawa T, de Bold MK, de Bold AJ. Cardiac natriuretic peptides. Nature Reviews Cardiology. 2020;17(11):698-717.
13. Calvier L, Miana M, Reboul P, Cachofeiro V, Martinez-Martinez E, De Boer RA, et al. Galectin-3 mediates Aldosterone -induced vascular fibrosis. Arteriosclerosis, thrombosis, and vascular biology. 2013;33(1):67-75.
14. Markmee R, Aungsuchawan S, Narakornsak S, Tancharoen W, Bumrungkit K, Pangchaidee N, et al. Differentiation of mesenchymal stem cells from human amniotic fluid to cardiomyocyte-like cells. Molecular medicine reports. 2017;16(5):6068-6076.
15. Liguori TTA, Liguori GR, Moreira LFP, Harmsen MC. Adipose tissue–derived stromal cells' conditioned medium modulates endothelial-mesenchymal transition induced by IL-1β/TGF-β2 but does not restore endothelial function. Cell proliferation. 2019;52(6):e12629.
16. Chen T-J, Yeh Y-T, Peng F-S, Li A-H, Wu S-C. S100A8/A9 enhances immunomodulatory and tissue-repairing properties of human amniotic mesenchymal stem cells in myocardial ischemia-reperfusion injury. International journal of molecular sciences. 2021;22(20):11175.
17. Miceli V, Bertani A, Chinnici CM, Bulati M, Pampalone M, Amico G, et al. Conditioned medium from human amnion-derived mesenchymal stromal/stem cells attenuating the effects of cold ischemia-reperfusion injury in an in vitro model using human alveolar epithelial cells. International Journal of Molecular Sciences. 2021;22(2):510.
18. Naseroleslami M, Parivar K, Khoei S, Aboutaleb N. Magnetic Resonance Imaging of Human-Derived Amniotic Membrane Stem Cells Using PEGylated Superparamagnetic Iron Oxide Nanoparticles. Cell J. 2016 ,18(3):332-9.
19. Zeng Z, Xu L, Xu Y, Ruan Y, Liu D, Li J, et al. Normothermic Ex Vivo Heart Perfusion with Mesenchymal Stem Cell-Derived Conditioned Medium Improves Myocardial Tissue Protection in Rat Donation after Circulatory Death Hearts. Stem Cells International. 2022;2022.
20. Maleki SN, Aboutaleb N, Nazarinia D, Beik SA, Qolamian A, Nobakht M. Conditioned medium obtained from human amniotic membrane-derived mesenchymal stem cell attenuates heart failure injury in rats. Iranian journal of basic medical sciences. 2019;22(11):1253.
21. Naseroleslami M, Aboutaleb N. Human amniotic membrane mesenchymal stem cells exert cardioprotective effects against isoproterenol (ISO)-induced myocardial injury through suppression of inflammation and modulation of inflammatory MAPK/NF-κB pathway. Cell and Tissue Banking. 2022;23(1):67-77.
22. Kheila M, Gorjipour F, Gohari LH, Sharifi M, Aboutaleb N. Human mesenchymal stem cells derived from amniotic membrane attenuate isoproterenol (ISO)-induced myocardial injury by targeting apoptosis. Medical Journal of the Islamic Republic of Iran. 2021;35:82.
23. Lee AJ, Mahoney CM, Cai CC, Ichinose R, Stefani RM, Marra KG, et al. Sustained delivery of SB-431542, a type I transforming growth factor beta-1 receptor inhibitor, to prevent arthrofibrosis. Tissue Engineering Part A. 2021;27(21-22):1411-1421.
24. Ouyang F, Liu X, Liu G, Qiu H, He Y, Hu H, Jiang P. Long non-coding RNA RNF7 promotes the cardiac fibrosis in rat model via miR-543/THBS1 axis and TGFβ1 activation. Aging (Albany NY). 2020;12(1):996, Aujla PK, Kassiri Z. Diverse origins and activation of fibroblasts in cardiac fibrosis. Cellular Signalling. 2021;78:109869.
25. Chen MM, Lam A, Abraham JA, Schreiner GF, Joly AH. CTGF expression is induced by TGF-β in cardiac fibroblasts and cardiac myocytes: a potential role in heart fibrosis. Journal of molecular and cellular cardiology. 2000;32(10):1805-1819.
26. Frangogiannis NG. The immune system and cardiac repair. Pharmacological research. 2008;58(2):88-111.
27. Naseroleslami M, Mousavi Niri N, Hosseinian SB, Aboutaleb N. DNAzyme loaded nano-niosomes attenuate myocardial ischemia/reperfusion injury by targeting apoptosis, inflammation in a NF-κB dependent mechanism. Naunyn-Schmiedeberg's Archives of Pharmacology. 2023;396(9):2127-2136.
28. Naseroleslami M, Aboutaleb N, Parivar K. The effects of superparamagnetic iron oxide nanoparticles-labeled mesenchymal stem cells in the presence of a magnetic field on attenuation of injury after heart failure. Drug Deliv Transl Res. 2018 Oct;8(5):1214-1225. doi: 10.1007/s13346-018-0567-8. Erratum in: Drug Deliv Transl Res. 2024 May;14(5):1390-1391.
29. Huang Q-M, Long Y-L, Wang J-N, Wu J, Tang W-L, Wang X-Y, et al. Human amniotic MSCs-mediated anti-inflammation of CD206hiIL-10hi macrophages alleviates isoproterenol-induced ventricular remodeling in mice. International Immunopharmacology. 2024;129:111660.

30. Qian JF, Liang SQ, Wang QY, Xu JC, Luo W, Huang WJ, Wu GJ, Liang G. Isoproterenol induces MD2 activation by β-AR-cAMP-PKA-ROS signalling axis in cardiomyocytes and macrophages drives inflammatory heart failure. Acta Pharmacol Sin. 2024,45(3):531-544.

31. Lin Y-H, Lin L-Y, Wu Y-W, Chien K-L, Lee C-M, Hsu R-B, et al. The relationship between serum galectin-3 and serum markers of cardiac extracellular matrix turnover in heart failure patients. Clinica chimica acta. 2009;409(1-2):96-99.

32. Chumakova S, Urazova O, Shipulin V, Vins M, Pryakhin A, Sukhodolo I, et al. Galectin 3 and non-classical monocytes of blood as myocardial remodeling factors at ischemic cardiomyopathy. IJC Heart & Vasculature. 2021;33:100766, de Boer RA, Yu L, van Veldhuisen DJ. Galectin-3 in cardiac remodeling and heart failure. Current heart failure reports. 2010;7(1):1-8.

33. Thandavarayan RA, Watanabe K, Ma M, Veeraveedu PT, Gurusamy N, Palaniyandi SS, et al. 14-3-3 protein regulates Ask1 signaling and protects against diabetic cardiomyopathy. Biochemical pharmacology. 2008;75(9):1797-1806.

34. Slawik J, Adrian L, Hohl M, Lothschütz S, Laufs U, Böhm M. Irregular pacing of ventricular cardiomyocytes induces pro-fibrotic signalling involving paracrine effects of transforming growth factor beta and connective tissue growth factor. European Journal of Heart Failure. 2019;21(4):482-491.

35. Blanco-Colio LM, Méndez-Barbero N, Lázaro AMP, Aceña Á, Tarín N, Cristóbal C, et al. MCP-1 Predicts Recurrent Cardiovascular Events in Patients with Persistent Inflammation. Journal of clinical medicine. 2021;10(5):1137.

36. Messaoudi S, Azibani F, Delcayre C, Jaisser F. Aldosterone , mineralocorticoid receptor, and heart failure. Molecular and cellular endocrinology. 2012.

37. Cha J-H, Wee H-J, Seo JH, Ahn BJ, Park J-H, Yang J-M, et al. AKAP12 mediates barrier functions of fibrotic scars during CNS repair. PloS one. 2014;9(4):e94695.

38. Liao C-W, Lin Y-T, Wu X-M, Chang Y-Y, Hung C-S, Wu V-C, et al. The relation among Aldosterone , galectin-3, and myocardial fibrosis: a prospective clinical pilot follow-up study. Journal of Investigative Medicine. 2016;64(6):1109-1113.

39. Sygitowicz G, Maciejak-Jastrzębska A, Sitkiewicz D. The diagnostic and therapeutic potential of galectin-3 in cardiovascular diseases. Biomolecules. 2021;12(1):46.

40. Lin Y-H, Chou C-H, Wu X-M, Chang Y-Y, Hung C-S, Chen Y-H, et al. Aldosterone induced galectin-3 secretion in vitro and in vivo: from cells to humans. PloS one. 2014;9(9):e95254.

Table 1. Analytical analysis of MCP1, ALD, TGF-β, Galectin-3, and BNP by group

Group	MCP1	ALD	TGF-β	Galectin-3	BNP
Control	111±2.18	119.78±2.51	36.22±6.29	8.27±0.61	80.78±3.08
HF	356.56±1.84	362.11±3.47	146.22±19.16	11.86±0.44	168.44±5.74
CM	353.55±4.91	359.67±2.91	126±7.82	11.50±0.75	163.78±6.47
Condition medium	228.35±6.75	245±5.86	68.11±6.38	9.17±0.38	111.11±0.84

,