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Improvement the Therapeutic Effectiveness of Mesenchymal Stem Cells via Heat Shock Preconditioning for Liver Fibrosis Therapy

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Abstract: The present study aims to evaluate the role of heat- preconditioning MSCs in improving the efficacy of treatment of chemically induced fibrosis. To achieve the aim of the study, heat treated stem cells were obtained by isolating adipose-derived stem cells (ADSCs) from rats, and heat treating them at a temperature of 42 °C for an hour to cause heat shock to mesenchymal stem cells (MSCs). Liver fibrosis in rats was induced by thioacetamide (TAA). A group of rats were treated with mesenchymal stem cells (MSCs) and another group with heat treated mesenchymal stem cells (MSCs). To investigate the effect of heat-treated mesenchymal stem cells (MSCs) on liver cells, the liver tissue of the study groups was examined and compared to the control group using Masson Trichrome (MT), Sirius Red dyes. The results of the histological examination showed that TAA caused pathological changes and fibrosis of the liver cells. Significant improvement in hepatocytes and a decrease in fibrosis rates for the livers of rats treated with mesenchymal stem cells (MSCs) pre-treated with heat, compared to the resulting improvement in liver cells of rats treated with mesenchymal stem cells (MSCs) without heat treatment. The study concluded that heat treatment of mesenchymal stem cells improves the therapeutic efficacy of chemically induced liver fibrosis in rats.

keywords: Adipose- derived stem cells, Heat shock, liver fibrosis.

1. Introduction

Liver fibrosis is a response resulting from chronic liver damage caused by a variety of factors, including alcohol consumption, viral hepatitis (Hepatitis c (HCV) and hepatitis B (HBV), non-alcoholic steatohepatitis (NASH), and autoimmune diseases (1,2). These factors can cause chronic inflammation that leads to fibrosis, which characterized by excessive production of extracellular matrix (ECM) and generation of fibrous scar (3). The presence of this fibrous scar disrupts the architecture of the liver leading to the loss of hepatocytes and disruption of liver function, ultimately resulting in liver failure (4). Although hepatic fibrosis is a leading cause of morbidity and mortality worldwide, the only effective treatment is liver transplantation (5). Still, transplantation is accompanied by various challenges, including a shortage of donors, elevated medical expenses, and immunological rejection (6). Therefore, finding an effective treatment strategy for treating liver fibrosis is important.

Cell therapy using different cell types, including mesenchymal stem cells (MSCs), hemopoietic cells, hepatocytes and, immune cells is considered an attractive candidate therapy for hepatic fibrosis (7). Among these different cell types, MSCs are considered to be the most prospective cells for regenerative repair due to their ability of differentiation into different cell types and self-renewal, secretion of trophic factors, and immunomodulation properties (7,8). Adipose-derived stem cells (ADSCs) are preferable to other forms of MSCs for use in regenerative medicine due to their abundance, ease of access via minimally invasive and painless methods, and their differentiation ability (9,10). Hao and his colleagues demonstrated that ADSC transplantation significantly reduced liver fibrosis in vitro and in animal model with CCl4-induced liver fibrosis (11). Nevertheless, as a result of oxidative stress and inflammation at the injury site, most transplanted MSCs fail

to survive post-transplantation, thereby reducing their therapeutic efficacy (12,13). Therefore, it is essential to protect MSCs from harsh microenvironment to enhance their survival rate and therapeutic efficacy post-transplantation.

Several preconditioning strategies have been developed to improve MSCs' survival after transplantation, thereby increasing their therapeutic outcome (14,15). Heat shock (HS) preconditioning appears as a promising approach for promoting cell survival (15,16). Previous study demonstrated that HS preconditioning could improve the immunoregulatory ability of MSCs and ameliorate acute lung injury (ALI) (17). Furthermore, another study has revealed that HS preconditioning could effectively enhance the therapeutic efficacy of MSCs in a chemotherapy-induced environment (18). Therefore, this study aimed to assess if HS preconditioning could improve the therapeutic efficacy of ADSCs for treating liver fibrosis.

2. Materials and methods

Chemicals

Dulbecco's modified Eagle's medium-low glucose (DMEM-Low glucose), phosphate-buffered saline (PBS), Fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA solutions were purchased from (Biowest company, Canada). Type-I collagenase was purchased from (Sigma Aldrich, USA). Thioacetamide (TAA) was obtained from (Sisco Research Laboratories, India).

Animals

Twenty-four male Sprague Dawley (SD) rats with weights 150-200 g were used in our study. They were purchased from Zagazig University, Egypt. They were provided with unlimited access to food and water and were housed at standard conditions of temperature and humidity. All procedures were authorized by Ethics Committee of Faculty of Science, Mansoura University (Code no. Sci-Ch-M-2021-159).

Preparation of ADSCs

Female SD rats were used to isolate ADSCs as previously described (19). Briefly, Adipose tissue was initially isolated from rats and digested with 0.1% collagenase type-I in PBS at 37 ˚c water bath for 1h. The digestion of cells was ended with addition of DMEM-Low glucose supplemented with 10% FBS. The ADSCs were centrifuged at 1800 rpm for 10 min, the supernatant was removed, and the cells washed with PBS twice. Then, complete medium (CM) containing DMEM-Low glucose, 10% FBS, and 1% penicillin-streptomycin were added to the cells. The resuspended ADSCs were cultured in 75-cm^2 flasks and kept in a 5% CO_2 incubator at 37 ˚c. The cells were passaged using 0.25% trypsin-EDTA when they became 80% confluent. When the cells reach 3rd passage, they can be used in this study.

Heat shock preconditioning

Cultured cells at 3rd passage were treated with HS, as reported previously (20). Briefly, the culture flasks containing ADSCs were placed into a water bath at 42 ˚c for 60 min. The flasks were then removed from the water bath, and the culture medium was immediately changed to eliminate the nonadherent cells. The cells were then kept in a 5% CO_2 incubator at 37 ˚c for 48h. On the other hand, ADSCs were cultured without any preconditioning.

Liver fibrosis induction and cells transplantation

Liver fibrosis was induced in rats using thioacetamide (TAA). SD rats were randomly divided into four groups (6 rats each):

i. **Control group:** healthy rats without any treatment.

ii. **Thioacetamide (TAA) group:** rats were injected with TAA (200mg/kg body weight) intraperitoneally twice a week for 8 weeks.

iii. **Adipose-derived stem cells (ADSCs) group:** rats were injected with TAA (200mg/kg body weight) intraperitoneally twice a week for 8 weeks, followed by a single transplantation of 1×10^6 ADSCs via the tail vein.

iv. **Heat shock preconditioning (HS) group:** rats were injected with TAA (200mg/kg body weight) intraperitoneally twice a week for 8 weeks, followed by a single transplantation of 1×10^6 HS-pretreated ADSCs via the tail vein.

Three weeks after cells transplantation, the rats were sacrificed, and liver tissues were preserved in 10% formalin for histopathological

examination.

Histopathological investigation

Liver samples of four groups were collected for histopathological analysis.

They were washed with PBS and fixed in 10% neutral buffered formalin for 24h. The samples were then embedded in paraffin. Afterward, paraffin sections 5 µm thick were cut and stained with Masson Trichrome (MT) and Sirius Red. The slides were then examined under a light microscope, and random images were taken using different magnifications (100x and 400x) and quantification of fibrosis area % was performed by computer-assisted image analysis.

Statistical analysis

For statistical analyses, GraphPad Prism 8.0.1 was utilized. Data were expressed as mean ± SD and analyzed among four groups by one way ANOVA followed by Tukey's test. (p value) < 0.05 was considered statistically significant.

3. Results and Discussion

Heat shock preconditioning ameliorates liver fibrosis in rats

To determine whether HS could improve the therapeutic potential of ADSCs in the treatment of liver fibrosis, histopathological analysis was done using Masson Trichrome (MT) and Sirius Red staining. The liver sections from the TAA group showed thick, red-stained fibrous tissue deposition (thin black arrow), dividing hepatic parenchyma into completely separated nodules. Treatment using ADSCs and HS-pretreated ADSCs decreased red-stained fibrous tissue deposition. As expected, stained fibrous tissue in the HS group decreased much more compared to the ADSCs group **(a, c)**. In addition, quantitative data were analyzed to determine the percentage of fibrosis by the analysis of fibrotic areas of MT and Sirius Red staining. Based on quantitative findings, the fibrosis percentage in the TAA group was significantly higher than that of the control group ($p < 0.0001$) **(b, d)**; however, compared to the ADSCs and HS groups, the fibrosis percentage after stem cells transplantation was reduced considerably ($p < 0.0001$) **(b, d).** Furthermore, the fibrosis percentage of MT and Sirius Red in the HS group was significantly decreased compared to the ADSCs group ($p < 0.0001$, $p < 0.001$, respectively) **(b, d).** These findings revealed that HS preconditioning enhanced the therapeutic potential of ADSCs in the treatment of liver fibrosis.

Discussion

To date, liver fibrosis is a severe health problem that may lead to liver cirrhosis, cancer and death (21). However, liver transplantation remains the only effective therapy for liver fibrosis (7). This makes it critical to investigate potential therapy approaches. MSCs are an attractive candidate for regenerative medicine because of their immunomodulatory activity, self-replicating ability, and differentiation into different cell types (22). Yang et al. (23) revealed that ADSCs effectively alleviated liver fibrosis via inhibiting TGF-β receptors. Furthermore, Yu et al. (24) indicated that ADSCs transplantation inhibits liver fibrosis in the CCL4 rat model. Several studies were conducted on MSCs to enhance their therapeutic efficacy (7). Previous studies demonstrated that Heat shock (HS) preconditioning enhances the survival rate of MSCs, therefore increasing their therapeutic outcome (16). Lv et al. (17) demonstrated that HS preconditioning could improve the immunoregulatory ability of MSCs and ameliorate acute lung injury (ALI). In addition, Wang et al. (18) have revealed that HS preconditioning could effectively enhance the therapeutic efficacy of MSCs in a chemotherapy-induced environment.

In our study, thioacetamide (TAA) was used to induce liver fibrosis in rats, as it is widely known that TAA causes fibrosis in rats that is highly resembles to that of human (25). Histopathological examination using MT and Sirius Red staining of liver tissues after TAA administration for 8 weeks showed that TAA induced the formation of fibrous tissues dividing hepatic parenchyma into completely separated nodules; these results are consistent with Jang et

al. (8). On the other hand, administration of ADSCs could decrease fibrosis lesions deposition compared to liver fibrotic rats; this

compatible with Yang et al. (23). Interestingly, histological pictures of HS group showed improvement of liver architecture compared to ADSCs group. Also, the fibrosis percentage was significantly reduced in the HS group. These findings illustrated that HS preconditioning could enhance ADSCs' ability to liver fibrosis.

Conclusion and recommendations

Overall, this study indicated that HS preconditioning may be an effective strategy for improving ADSCs' therapeutic efficacy in the treatment of liver fibrosis. Further research is needed for studying the mechanism of how HS preconditioning could improve the therapeutic efficiency of MSCs in the treatment of liver fibrosis.

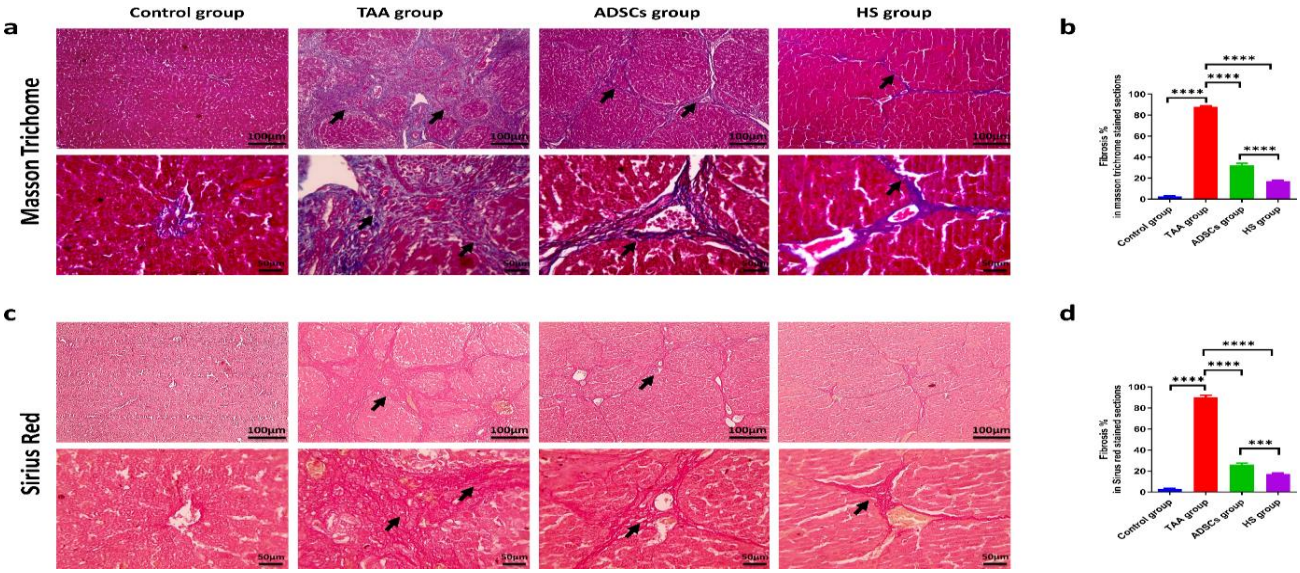

Figure1 Heat shock preconditioning ameliorates liver fibrosis in rats.

(a, c) Photomicrograph representative images illustrating histopathological analysis of hepatic tissues using Masson Trichrome (MT) and Sirius Red, respectively. (b ,d) Quantification results of MT and Sirius Red staining, respectively. Data are represented as mean ± SD (n = 6, ***p < 0.001, ****p < 0.0001).

4. References

1. Kisseleva T, Brenner D (2021). Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol [Internet].;**18 (3)**:151–66. Available from: http://dx.doi.org/10.1038/s41575-020-00372-7
2. Aydin MM, Akcali KC. (2018) Liver fibrosis. *Turkish J Gastroenterol.* ;**29 (1)**:14–21.
3. Bataller R, Brenner D a (2005). Science in medicine Liver fibrosis. *J Clin Invest.*;**115(2)**:209–18.
4. Kisseleva T, Brenner DA (2008). Mechanisms of fibrogenesis. Exp Biol Med.;**233 (2):**109–22.
5. Sun M, Kisseleva T. (2015) Reversibility of liver fibrosis. Clin Res Hepatol Gastroenterol [Internet].;39:S60–3. Available from: http://dx.doi.org/10.1016/j.clinre.2015.06.015
6. Eom YW, Kim G, Baik SK. (2015) Mesenchymal stem cell therapy for cirrhosis: Present and future perspectives. *World J Gastroenterol*.;**21 (36)**:10253–61.
7. Yang X, Meng Y, Han Z, Ye F, Wei L, Zong C (2020). Mesenchymal stem cell therapy for liver disease: full of chances and challenges. Cell Biosci [Internet].;**10 (1)**:1–18. Available from: https://doi.org/10.1186/s13578-020-00480-6
8. Jang YO, Kim MY, Cho MY, Baik SK, Cho YZ, Kwon SO (2014). Effect of bone marrow-derived mesenchymal stem cells on hepatic fibrosis in a thioacetamide-induced cirrhotic rat model. BMC Gastroenterol.;**14(1)**:1–12.
9. Zhang J, Liu Y, Chen Y, Yuan L, Liu H,

Wang J, et al (2020). Adipose-Derived Stem Cells: Current Applications and Future Directions in the Regeneration of Multiple Tissues. Stem Cells Int.; 2020.
10. Mazini L, Rochette L, Amine M, Malka G. (2019) Regenerative capacity of adipose derived stem cells (ADSCs), comparison with mesenchymal stem cells (MSCs). *Int J Mol Sci.*;**20 (10):**1–30.
11. Hao T, Chen J, Zhi S, Zhang Q, Chen G, Yu F. (2017) Comparison of bone marrow-vs. Adipose tissue-derived mesenchymal stem cells for attenuating liver fibrosis. Exp Ther Med.;**14 (6**):5956–64.
12. Liao N, Liao N, Liao N, Shi Y, Shi Y, Shi Y, et al (2020). Antioxidant preconditioning improves therapeutic outcomes of adipose tissue-derived mesenchymal stem cells through enhancing intrahepatic engraftment efficiency in a mouse liver fibrosis model. Stem Cell Res Ther.;**11(1)**:1–11.
13. Hu C, Zhao L, Duan J, Li L (2019). Strategies to improve the efficiency of mesenchymal stem cell transplantation for reversal of liver fibrosis. *J Cell Mol Med.*;**23 (3):**1657–70.
14. Schäfer R, Spohn G, Baer PC. (2016) Mesenchymal stem/stromal cells in regenerative medicine: Can preconditioning strategies improve therapeutic efficacy? Transfus Med Hemotherapy.;**43 (4):**256–67.
15. Kang SH, Kim MY, Eom YW, Baik SK (2020). Mesenchymal stem cells for the treatment of liver disease: Present and perspectives. Gut Liver.;**14 (3)**:306–15.
16. Sart S, Ma T, Li Y. (2014) Preconditioning Stem Cells for In Vivo Delivery . Biores Open Access.;**3 (4):**137–49.
17. Lv H, Yuan X, Zhang J, Lu T, Yao J, Zheng J, et al (2021). Heat shock preconditioning mesenchymal stem cells attenuate acute lung injury via reducing NLRP3 inflammasome activation in macrophages. Stem Cell Res Ther.;**12(1)**:1–16.
18. Wang Q, Li X, Wang Q, Xie J, Xie C, Fu X. (2019) Heat shock pretreatment improves mesenchymal stem cell viability by heat shock proteins and autophagy to prevent cisplatin-induced granulosa cell apoptosis. Stem Cell Res Ther.;**10 (1)**:1–12.
19. Wang Y, Lian F, Li J, Fan W, Xu H, Yang X, et al. (2012) Adipose derived mesenchymal stem cells transplantation via portal vein improves microcirculation and ameliorates liver fibrosis induced by CCl4 in rats. *J Transl Med.*;**10 (1)**:1–9.
20. Chen X, Wang Q, Li X, Wang Q, Xie J, Fu X. (2018) Heat shock pretreatment of mesenchymal stem cells for inhibiting the apoptosis of ovarian granulosa cells enhanced the repair effect on chemotherapy-induced premature ovarian failure. Stem Cell Res Ther.;**9(1)**:240.
21. Dhar D, Baglieri J, Kisseleva T, Brenner DA. (2020) Mechanisms of liver fibrosis and its role in liver cancer. Exp Biol Med.;**245 (2):**96–108.
22. Chiabotto G, Pasquino C, Camussi G, Bruno S. (2020) Molecular Pathways Modulated by Mesenchymal Stromal Cells and Their Extracellular Vesicles in Experimental Models of Liver Fibrosis. Front Cell Dev Biol.;8(December):1–13.
23. Yang N, Ma W, Ke Y, Liu H, Chu J, Sun L, et al. Transplantation of adipose-derived stem cells ameliorates Echinococcus multilocularis-induced liver fibrosis in mice. PLoS Negl Trop Dis [Internet]. 2022;**16 (1)**:1–18. Available from:http://dx.doi.org/10.1371/*journal.pntd*.0010175
24. Yu F, Ji S, Su L, Wan L, Zhang S, Dai C, et al. (2015) Adipose-derived mesenchymal stem cells inhibit activation of hepatic stellate cells invitro and ameliorate rat liver fibrosis invivo. *J Formos Med Assoc [Internet].*;**114 (2)**:130–8. Available from: http://dx.doi.org/10.1016/j.jfma.2012.12.002
25 Laleman W, Elst I Vander, Zeegers M, Servaes R, Libbrecht L, Roskams T, et al (2006). A stable model of cirrhotic portal hypertension in the rat: Thioacetamide revisited. Eur *J Clin Invest.*;**36 (4)**:242–9.