Research Paper: H_2O_2-Preconditioned Umbilical Cord-Derived Mesenchymal Stem Cells Ameliorate Liver Regeneration in Acute Liver Failure-Induced Mice

Fatemeh Nasiri[1], Behrooz Johari[2], Fatemeh Amiri[1], Mehryar Habibi Roudkenar[1], Sedigheh Molaei[3], Marzie Bahadori[1], Marjan Movahed[1], Abazar Yari[4], Mohammad Ali Jalili[1*]

1. Department of Biochemistry, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
2. Department of Biotechnology, Pasteur Institute of Iran, Tehran, Iran.
3. Department of Biochemistry, School of Medicine, Qom University of Medical Sciences, Qom, Iran.
4 Department of Anatomy, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran.

Mohammad Ali Jalili is assistant professor in the Department of Biochemistry, High Institute for Research and Education in Transfusion Medicine, Tehran. He received PhD of Pharmaceutical Chemistry from Marburg University-Cosmital Company in 1990. His interests is working on recombinant protein and stem cell transplant.

ABSTRACT

Introduction: Mesenchymal stem cells (MSCs) are suitable candidates for the treatment of liver diseases. However, their low survival rate limits their efficacy following transplantation. This study aimed to evaluate the therapeutic potentials of H_2O_2-preconditioned umbilical cord-derived MSCs (UCMSCs) on acute liver failure (ALF) in mice.

Methods: UCMSCs were pre-conditioned with different concentrations of H_2O_2. Cell viability was evaluated by WST-1 (water soluble tetrazolium) assay followed by exposure to lethal doses of H_2O_2. ALF was induced in NMRI mice using CCl_4 and the cells therapy was performed using H_2O_2-preconditioned and normal UCMSCs. After 24, 48, and 72 hours, regenerative potentials of different UCMSCs groups were evaluated compared to sham group (that receive no MSCs) using biochemical and histological methods.

Results: Lower liver enzymes was significantly evident in mice transplanted with H_2O_2-preconditioned UCMSCs compared with the other groups. Interestingly, histological results revealed a significant improvement in liver regeneration in these mice.

Conclusion: Preconditioning of UCMSCs with H_2O_2 not only enhances their survival but also increases the efficacy of MSCs-based cell therapy in acute liver failure.

* **Corresponding Author:**
Mohammad Ali Jalili, PhD
Address: Department of Biochemistry, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
Tel: +98 (21) 88613423
E-mail: m.jalili@ibto.ir

1. Introduction

L iver has a vital role as a central organ in metabolism, detoxification, and immunity [1]. It also has appropriate self-renewal ability through different cell sources namely mature hepatocytes, intrahepatic stem cells, and extra stem cells [2-4]. Despite these regeneration abilities, these reconstruction processes cannot function in case of serious damage. Liver diseases are generally divided into two categories: acute and chronic diseases. Infections, drugs, toxins, and chemicals are the main causes of the Acute Liver Failure (ALF) in which impairment of liver function results in severe damage and necrosis [5]. This irreversible necrosis might be complicated with hepatic encephalopathy, low consciousness, coma, and death [6, 7]. Acute liver failure have high mortality rate despite the extensive treatment of afflicted individuals [8, 9].

Liver transplantation, as the main treatment of liver diseases, has been hampered due to the low number of organ donors, side effects of the immunosuppressive drug on recipients, and complications related to operation [10, 11]. Therefore, the cell-based therapeutic approaches have been proposed as an alternative to organ transplantation [12-14]. Different tissue-derived Mesenchymal Stem Cells (MSCs) are used extensively in cell therapy [15]. However, the majority of transplanted MSCs die a couple of days after the transplantation due to unfavorable environmental conditions, including lack of oxygen and the presence of radical oxygen species [16-18]. In this regard, several studies have been conducted to address the low efficiency of MSCs for cell therapy purposes [19]. In this study, we generated an approved experimental model of acute liver failure in Naval Medical Research Institute (NMRI) mice using a well-known hepatotoxin, i.e. carbon tetrachloride (CCl$_4$). Then, the H$_2$O$_2$-preconditioned umbilical cord-derived MSCs (UCMSCs) were transplanted into the mice to evaluate their regenerative effects on acute liver disease.

2. Materials and Methods

MSC preparation

Umbilical cord-derived mesenchymal stem cells (UC-MSCs) were isolated and expanded with approved ethical process and informed consent as described previously [20].

UCMSC H$_2$O$_2$-preconditioning

UCMSCs were cultured in 96-well plates, containing DMEM low glucose medium (Sigma, USA) supplemented with 10% FBS (Invitrogen, USA), 1% penicillin, and 1% streptomycin (CinnaGen, Iran) and then incubated for 24 hours at 37°C. Next, the cells were treated with 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, and 100 µM of H$_2$O$_2$ (Sigma, USA) for 12 hours. Preconditioned-cells were recovered for 7 hours and eventually subjected to 24 hours treatment with lethal H$_2$O$_2$ doses (500 µM and 1 mM) [21].

WST-1 assay

After H$_2$O$_2$-preconditioning of UCMSCs, the cellular survival rate was evaluated utilizing water soluble tetrazolium (WST-1). About 10 µL of WST-1 solution (Roche, Germany) was added to 90 µL of the culture medium in each well, and the plate was incubated for 4 hours at 37°C and 5% CO$_2$ in darkness. The absorbance of the samples was measured with an ELISA reader, and the results were interpreted in comparison to control.

Acute liver failure experimental model

8-week-old male NMRI mice (25±2g) were prepared from Iran University of Medical Sciences and kept in standard condition in term of light, food, and water accessibilities. Animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee of the Iran University of Medical Sciences. All protocols were conducted in accordance with the Ethics Committee of Iranian Blood Transfusion Organization (IBTO). The carbon tetrachloride (CCl$_4$, Merck, Germany) dissolved in olive oil was injected into the mice intraperitoneally (IP) to induce ALF. After 24 hours, induction of ALF was confirmed by biochemical and histological techniques [22].

Cell therapy

H$_2$O$_2$-preconditioned UCMSCs, normal UCMSCs, and Phosphate-Buffered Saline (PBS) were injected into the ALF-induced mice intravenously (IV) via the tail vein. The subjected cell therapy groups and their number were described in Table 1. After 24, 48, and 72 hours, the alteration in the liver enzymes and tissue necrosis were analyzed using biochemical and histological techniques to evaluate the liver regeneration in ALF-induced mice.

Biochemical analysis

The blood samples were collected 24, 48, and 72 hours after MSCs transplantation to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as important indicators of liver damage. AST and ALT

serum levels were assayed using an automatic analyzer (BT 3000 PL/S, Italy).

Histological analysis

The liver samples were collected 24, 48, and 72 hours after MSCs transplantation, and fixed in 10% formalin (Merck, Germany). Liver tissue samples were processed according to the routine process, and 4- to 6-micron-thick sections were prepared from them after paraffin molding. These slides were stained with hematoxylin and eosin (H & E stain). The slides were then subjected to microscope observation to assess the extent of necrosis.

Statistical analysis

Quantitative data were analyzed using SPSS 19 and 1-way ANOVA test. Significant differences were determined when P value was less than 0.05.

3. Results

H_2O_2-preconditioned UCMSCs show high survival rate

In order to assess the survival of H_2O_2-preconditioned UCMSCs, WST-1 assay was performed. As shown in Figure 1, the survival of 20-25 μM H_2O_2-preconditioned UCMSCs was more than those preconditioned with other doses of H_2O_2. In other words, the percentage of viable cells significantly increased upon preconditioning with 25 μM H_2O_2 (Figure 1). Thus, it was selected as optimized H_2O_2 preconditioning dose.

CCl_4 induces ALF in mice model

Different CCl_4 doses were injected into mice to induce ALF. Biochemical and histological assays revealed that the 1.5 mg/mL of CCl_4 successfully induced ALF and provided opportunity to conduct more studies on regenerative potentialities of different UCMSCs as described previously (manuscript submitted).

H_2O_2-preconditioned UCMSCs decrease the level of liver enzymes more efficiently

Therapeutic effect of different UCMSC groups was evaluated with the measurement of the liver enzymes in serum 24, 48, and 72 hours after transplantation. The results showed that the descending trend in AST and ALT levels was significantly different between transplanted groups. About 24 hours after transplantation, ALF-induced mice transplanted with H_2O_2-preconditioned UCMSCs (ALF-MSCs-Pre) showed a significant difference compared to the sham group that was injected with PBS (no cell therapy) (P<0.001) (Figure 2A). But the reduction in the mentioned enzymes in ALF-induced mice transplanted with normal UCMSCs (ALF-MSCs) was less than ALF-MSCs-Pre (P<0.01) (Figure 2A).

As shown in Figure 2B, AST and ALT levels of ALF-MSCs-Pre was lower than those of other groups 48 hours after cell injection, and it had significant difference with sham group (P<0.001). Transplantation of the normal UCMSCs led to less reduction in liver enzymes and showed less difference with sham (P<0.05) (Figure 2B). About 72 hours after cell therapy, both AST and ALT levels in ALF-MSCs-Pre were different from their value in sham group (P<0.001). The level of these enzymes decreased in this group, and was approximately the same as their normal basic level. However, there was no significant difference between AST level of ALF-MSCs and that of the sham. These liver enzymes were higher than ALF-MSCs-Pre compared to ALF-MSCs 72 hours post-cell therapy (Figure 2C).

Liver necrosis repair occurs more quickly in ALF-induced mice after transplantation of H_2O_2-preconditioned UCMSCs. For further studies, the H&E-stained liver sections were assayed during interval times (24, 48, and 72 h) after cell therapy. Histological assessment of the liver sections indicated that the extent of damage in the MSC-transplanted groups decreased compared to sham group at different time points after MSCs injection (Figures 3, 4). As shown in Figure 3, about 24 hours after transplantation, the extent of necrosis was less in H_2O_2-precon-

Table 1: Description of different mice groups and their related abbreviation.

Normal (n=10)	Normal Mice Injected With Normal UCMSCs
Sham (n=10)	ALF-induced mice injected with PBS (no cell therapy)
ALF-MSCs (n=10)	ALF-induced mice transplanted with normal UCMSCs
ALF-MSCs-Pre (n=10)	ALF-induced mice transplanted with H_2O_2-preconditioned UCMSCs

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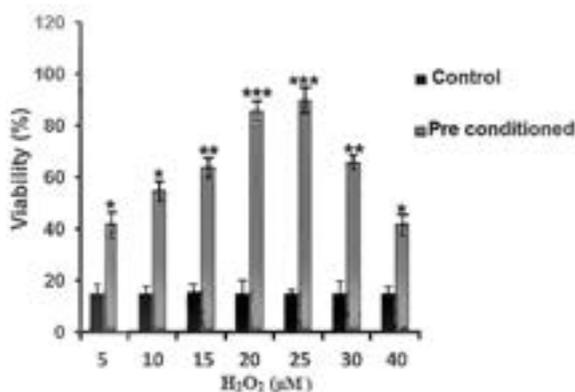
Figure 1. The viability of the preconditioned UCMSCs with various doses of H_2O_2 using WST-1. Survival of 25 μM H_2O_2-preconditioned UCMSCs was more than those preconditioned with other H_2O_2 doses. The data are expressed as the mean±SEM of three independent experiments; $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$(***) were found to be significant.

ditioned UCMSCs transplanted mice (ALF-MSCs-Pre) than that in ALF-MSC groups which were injected with normal UCMSCs without any preconditioning (no intra-lobular necrotic bridges in ALF-MSCs-Pre).

About 48 hours post-transplantation of H_2O_2-preconditioned UCMSCs, the increasing number of inflammatory cells revealed quick liver restoration in ALF-MSCs-Pre and inhibition of necrosis in comparison to ALF-MSCs that showed some scattered necrotic zones (intra-lobular necrotic bridges) (Figure 3). There was no necrosis along with decreased inflammation reaction in ALF-MSCs-Pre groups 72 hours post-transplantation. While the liver sections of ALF-MSCs indicated mild necrosis with increased inflammation reactions and partial delayed regeneration (Figure 3).

In contrast, there were more intra-lobular necrotic bridges in liver sections of sham group that did not receive any MSCs and injected with PBS only 24 and 48 h after PBS injections (Figure 4). Persistent necrosis and intra-lobular necrotic bridges were observed in this group even after 72 hours (Figure 4). All together, the histological assessment of liver sections indicate that cell therapy with UCMSCs contributes to control of the necrosis progress and liver restoration in comparison with the sham group, but this liver restoration was clearly detectable in ALF-MSCs-Pre after 24 hours and continued within 72 hours post-transplantation.
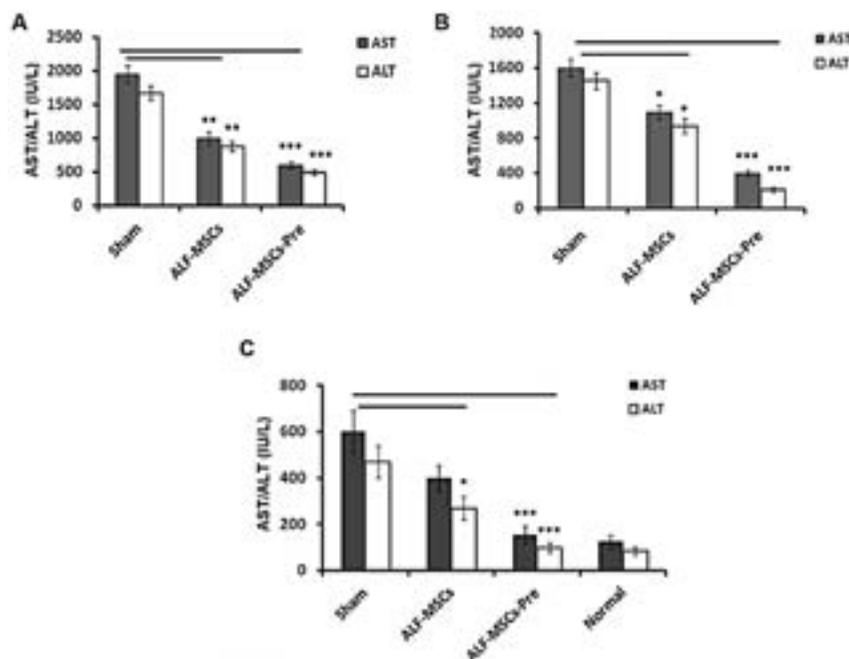
Figure 2. Biochemical assessment of AST and ALT in different mice groups 24, 48, and 72 hours post-transplantation.

(A): 24 h after transplantation, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly lower in ALF-induced mice that were transplanted with H_2O_2-preconditioned- UCMSCs (ALF-MSCs-Pre) and ALF-induced mice transplanted with normal UCMSCs (ALF-MSCs) than the sham group injected with PBS (no cell therapy); (B): AST and ALT levels of ALF-MSCs-Pre were lower than those of other groups 48 hours after transplantation; (C): 72 hours after cell therapy, the AST and ALT reduced in ALF-MSCs-Pre group, and it was closer to normal level ($P<0.05$, $P<0.01$, and $P<0.001$).
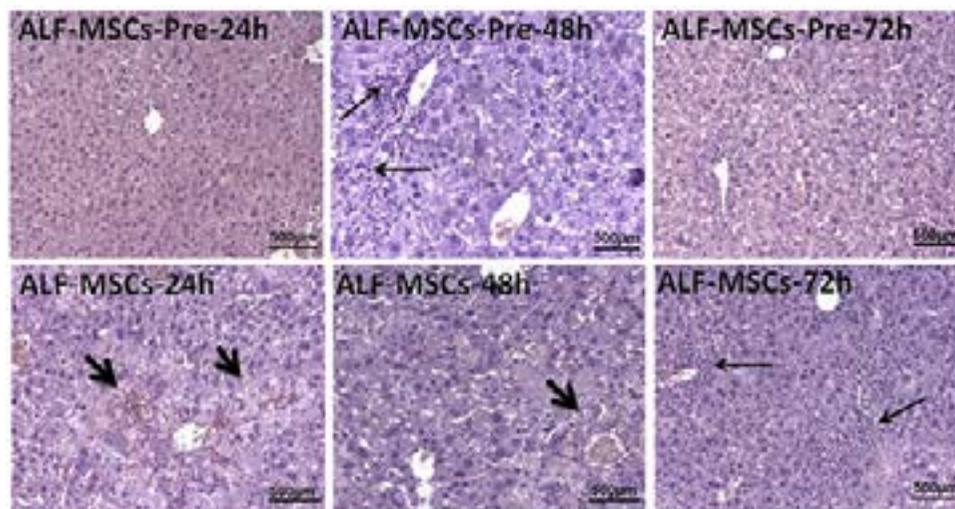
Figure 3. Histological assessment of liver sections in ALF-MSC-Pre and ALF-MSC groups 24, 48, and 72 hours after transplantation. Liver sections were stained with hematoxylin and eosin stain (H & E). After 24 hours of transplantation, mild necrosis without extensive intra-lobular necrotic bridges was observed in ALF-MSCs-Pre, but some intra-lobular necrotic bridges (bold arrows) were detectable in ALF-MSCs. After 48 hours, a number of inflammatory cells (narrow arrow) were observed due to liver regeneration in ALF-MSCs-Pre but intra-lobular necrotic bridges persisted in ALF-MSCs. There was no necrosis along with decreased inflammation in ALF-MSCs-Pre groups 72 hours post-transplantation. However, mild necrosis with increased inflammation and delayed regeneration were observed in ALF-MSCs. The liver restoration progress in ALF-MSCs-Pre was faster than that in ALF-MSCs (H & E stain, 100x, Scale bar 500μm).

4. Discussion

Acute liver failure is a clinical syndrome in which severe impairment of liver function suddenly occurs with a rapid progression as a result of acute hepatic necrosis [23]. In this regard, MSCs are employed in cell therapy and regenerative medicine [24-26].

In this study, H_2O_2-preconditioning of UCMSCs increased their viability. Employment of preconditioning enhances MSCs survival or potentiality excludes the risk and concerns associated with genetic manipulation [19]. Moreover, UCMSCs are more primitive immunosuppressive stem cells with high proliferative rate that could be harnessed in cell therapy [27]. Tang et al. (2005) pre-
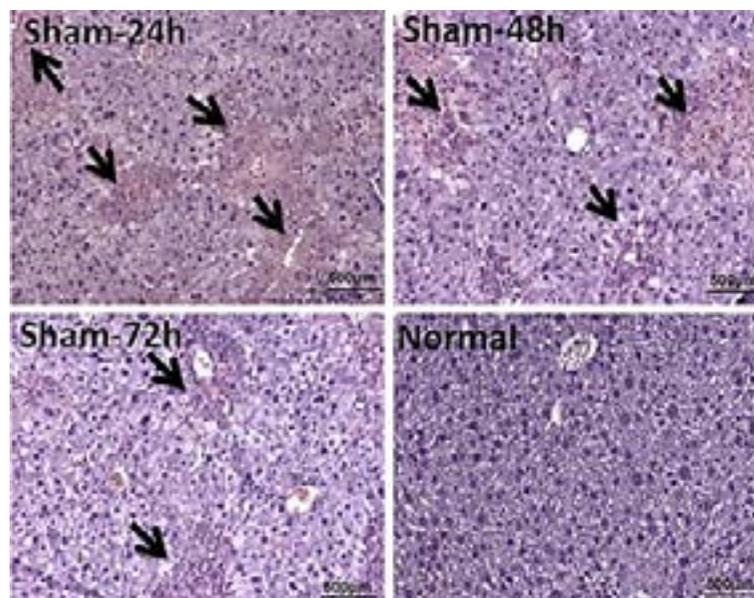
Figure 4. Histological assessment of liver sections in sham group 24, 48, and 72 h after transplantation. About 24 and 48 hours after PBS injection, more intra-lobular necrotic bridges (bold arrows) were observed in sham that did not receive any MSCs compared with cell therapy groups. Persistent necrosis in this group was considerable even after 72 hours.

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conditioned PC12 cell line with optimum concentration of H_2O_2 and demonstrated its protective effect against oxidative stress due to reducing cell apoptosis with mechanisms such as up regulation of B cell lymphoma 2 (Bcl-2) [28]. Consistent with our results, Li et al. (2009) showed that preconditioning of MSCs with H_2O_2 would lead to the expression of cytokine receptor (CXCR4) and increased cell survival [29].

To induce ALF in mice, a well-known hepatotoxin i.e. CCl_4 was used. The results showed that injection of CCl_4 would cause extensive necrosis as well as increased serum AST and ALT activity in mice (Manuscript submitted). Most of the toxic effects of CCl_4 are mediated by its metabolite i.e. 3- chloromethyl radicals [30] resulting in damage to lipids, nucleic acids, and other cell constituents, and induction of necrosis in the liver parenchymal cells. This damage finally leads to the release of liver enzymes (AST and ALT) into blood [31].

After injection of H_2O_2-preconditioned UCMSCs to ALF-induced mice, apparently hepatic necrosis slowed. We observed recovery process at a higher level and reduction of AST and LT was faster compared to control groups. Enhanced survival of H_2O_2-preconditioned UCMSCs might affect their therapeutic and paracrine potentials and increased their liver regeneration capacities due to increased secretion of growth factors and cytokines [13, 32].

Rabani et al. reported that MSCs could control and repair the fibrosis induced by CCl_4 in mice [33]. Stoke study indicates the role of human bone marrow MSCs in the improvement of liver regeneration through inhibiting the damage progression in mice models [34]. They induced ALF in mice using acetaminophen that might be a nonspecific hepatotoxin. Another study showed that adipose tissue MSCs could improve the survival of acute liver injury-induced mice model [35]. In addition, Grutadurya reported that the extent of hepatic damage decreased in rat models of acute liver injury followed by autologous transplantation of bone marrow MSCs [36]. Different tissue-derive MSCs exhibit variable potentialities despite their common characteristics. In the mentioned studies, MSCs were isolated from bone marrow and adipose tissues that might be associated with invasive sampling methods. Moreover, different experimental models and methodology were performed.

In summary, this study was conducted to evaluate the preconditioned UCMSCs potentialities for the treatment of ALF in experimental models. H_2O_2-preconditioned UCMSCs restored liver tissue in a short time of period.

H_2O_2-preconditioning of MSCs might be a practical strategy to increase the survival and potential of MSCs for the treatment of acute liver injury. It seems to improve MSC-based cell therapy in term of the quality and efficiency in the future.

Acknowledgments

Conflict of Interest

All authors certify that this manuscript has neither been published in whole nor in part nor being considered for publication elsewhere. The authors have no conflicts of interest to declare.

References

[1] Fazliana M. Studies on Labisia pumila var. alata extract with phytoestrogenic effects: Impact on biological activities and gene expression. Solna: Institute For Molecular Medicine and Surgery/ Department of Molecular Medicine and Surgery; 2010.

[2] Nejak-Bowen KN, Monga SPS. Beta-catenin signaling, liver regeneration and hepatocellular cancer: Sorting the good from the bad. Seminars in Cancer Biology. 2011; 21(1):44–58. doi: 10.1016/j.semcancer.2010.12.010.

[3] Sell S. Heterogeneity and plasticity of hepatocyte lineage cells. Hepatology. 2001; 33(3):738–50. doi: 10.1053/jhep.2001.21900

[4] Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. Gastroenterology. 2009; 137(2):466–81. doi: 10.1053/j.gastro.2009.05.044

[5] Chiang CH, Chang CC, Huang HC, Chen YJ, Tsai PH, Jeng SY, et al. Investigation of hepatoprotective activity of induced pluripotent stem cells in the mouse model of liver injury. Journal of Biomedicine and Biotechnology. 2011; 1–11. doi: 10.1155/2011/219060

[6] Kelly JH, Koussayer T, He D-E, Chong MG, Shang TA, Whisennand HH, et al. An improved model of acetaminophen-induced fulminant hepatic failure in dogs. Hepatology. 1992; 15(2):329–35. doi: 10.1002/hep.184015022.

[7] Palmes D, Spiegel H-U. Animal models of liver regeneration. Biomaterials. 2004; 25(9):1601–11. doi: 10.1016/s0142-9612(03)00508-8

[8] Braude S, Gimson AES, Williams R. Progress in the management of fulminant hepatic failure. Intensive Care Medicine. 1981; 7(3):101–3. doi: 10.1007/bf01738610

[9] Boyer TD, Sanyal AJ, Terrault NA, Lindor KD. Zakim and Boyer's hepatology: A textbook of liver disease e-book. Amsterdam: Elsevier Health Sciences; 2016.

[10] Alimoghaddam K, Mohamadnejad M, Bagheri M, Bashtar M, Ghannati H, Bonab MM, et al. Phase 1 human trial of autologous mesenchymal stem cell transplantation for the treatment of decompensated cirrhosis. Blood. 2007; 110(11):4864.

[11] Nikeghbalian S, Rasekhi A, Hosseini-Asl SM, Ramzi M, Kakaei F, Malekzadeh R, et al. [Autologous transplantation of bone marrow-derived mononuclear and CD133^ sup+^ cells in patients with decompensated cirrhosis (Persian)]. Archives of Iranian Medicine. 2011; 14(1):12-7. doi: 011141/ AIM.004.

[12] Jin SZ, Liu BR, Xu J, Gao FL, Hu ZJ, Wang XH, et al. Ex vivo-expanded bone marrow stem cells home to the liver and ameliorate functional recovery in a mouse model of acute hepatic injury. Hepatobiliary & Pancreatic Diseases International. 2012; 11(1):66–73. doi: 10.1016/s1499-3872(11)60127-6

[13] Zhang S, Chen L, Liu T, Zhang B, Xiang D, Wang Z, et al. Human umbilical cord matrix stem cells efficiently rescue acute liver failure through paracrine effects rather than hepatic differentiation. Tissue Engineering Part A. 2012; 18(13-14):1352–64. doi: 10.1089/ten.tea.2011.0516

[14] Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nature Medicine. 2000; 6(11):1229–34. doi: 10.1038/81326

[15] Prasajak P. Mesenchymal stem cells: Current clinical applications and therapeutic potential in liver diseases. Journal of Bone Marrow Research. 2014; 2(1). doi: 10.4172/2329-8820.1000137

[16] Domínguez-Bendala J, Lanzoni G, Inverardi L, Ricordi C. Concise review: Mesenchymal stem cells for diabetes. Stem Cells Translational Medicine. 2011; 1(1):59–63. doi: 10.5966/sctm.2011-0017

[17] Wei H, Li Z, Hu S, Chen X, Cong X. Apoptosis of mesenchymal stem cells induced by hydrogen peroxide concerns both endoplasmic reticulum stress and mitochondrial death pathway through regulation of caspases, p38 and JNK. Journal of Cellular Biochemistry. 2010; 111(4):967–78. doi: 10.1002/jcb.22785

[18] Robey TE, Saiget MK, Reinecke H, Murry CE. Systems approaches to preventing transplanted cell death in cardiac repair. Journal of Molecular and Cellular Cardiology. 2008; 45(4):567–81. doi: 10.1016/j.yjmcc.2008.03.009

[19] Amiri F, Jahanian-Najafabadi A, Roudkenar MH. [In vitro augmentation of mesenchymal stem cells viability in stressful microenvironments (Persian)]. Cell Stress and Chaperones. 2014; 20(2):237–51. doi: 10.1007/s12192-014-0560-1

[20] Amiri F, Halabian R, Salimian M, Shokrgozar MA, Soleimani M, Jahanian-Najafabadi A, et al. [Induction of multipotency in umbilical cord-derived mesenchymal stem cells cultivated under suspension conditions (Persian)]. Cell Stress and Chaperones. 2014; 19(5):657–66. doi: 10.1007/s12192-014-0491-x

[21] Bashiri Nahanji H, Habibi Roudkenar M, Halabian R, Jalili M, Jalili M. [Enhancement of the resistance of mesenchy-mal stem cells against killing conditions by the oxidative preconditioning (Persian)]. Scientific Journal of Iranian Blood Transfusion Organization. 2013; 10(3):256-266.

[22] Amiri F, Molaei S, Bahadori M, Nasiri F, Deyhim MR, Jalili MA, et al. [Autophagy-modulated human bone marrow-derived mesenchymal stem cells accelerate liver restoration in mouse models of acute liver failure (Persian)]. Iranian Biomedical Journal. 2016; 20(3):135-44. PMCID: PMC4949977

[23] Shakil AO, Kramer D, Mazariegos GV, Fung JJ, Rakela J. Acute liver failure: Clinical features, outcome analysis, and applicability of prognostic criteria. Liver Transplantation. 2000; 6(2):163–9. doi: 10.1002/lt.500060218

[24] Cho KA, Ju SY, Cho SJ, Jung YJ, Woo SY, Seoh JY, et al. Mesenchymal stem cells showed the highest potential for the regeneration of injured liver tissue compared with other subpopulations of the bone marrow. Cell Biology International. 2009; 33(7):772–7. doi: 10.1016/j.cellbi.2009.04.023

[25] Hong ZF, Huang XJ, Yin ZY, Zhao WX, Wang XM. Immunosuppressive function of bone marrow mesenchymal stem cells on acute rejection of liver allografts in rats. Transplantation Proceedings. 2009; 41(1):403–9. doi: 10.1016/j.transproceed.2008.10.020

[26] Moslem M, Valojerdi MR, Pournasr B, Muhammadnejad A, Baharvand H. [Therapeutic potential of human induced pluripotent stem cell-derived mesenchymal stem cells in mice with lethal fulminant hepatic failure (Persian)]. Cell Transplantation. 2013; 22(10):1785–99. doi: 10.3727/096368912x662462

[27] Lu LL, Liu YJ, Yang SG, Zhao QJ, Wang X, Gong W, et al. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. Haematologica. 2006; 91(8):1017-26. PMID: 16870554

[28] Tang XQ, Feng JQ, Chen J, Chen PX, Zhi JL, Cui Y, et al. Protection of oxidative preconditioning against apoptosis induced by H_2O_2 in PC12 cells: Mechanisms via MMP, ROS, and Bcl-2. Brain Research. 2005; 1057(1-2):57–64. doi: 10.1016/j.brainres.2005.07.072

[29] Li S, Deng Y, Feng J, Ye W. Oxidative preconditioning promotes bone marrow mesenchymal stem cells migration and prevents apoptosis. Cell Biology International. 2009; 33(3):411–8. doi: 10.1016/j.cellbi.2009.01.012

[30] Nasiri F, Amiri F, Mohammadipour M, Molaei S, Habibi Roudkenar M, Jalili MA. [H_2O_2-preconditioned mesenchymal stem cell regenerative effects on acute liver failure mice (Persian)]. Scientific Journal of Iranian Blood Transfusion Organization. 2015; 12(2):111-124.

[31] Halliwell B. Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. The American Journal of Medicine. 1991; 91(3):14–22. doi: 10.1016/0002-9343(91)90279-7

[32] Xagorari A, Siotou E, Yiangou M, Tsolaki E, Bougiouklis D, Sakkas L, et al. Protective effect of mesenchymal stem cell-conditioned medium on hepatic cell apoptosis after acute liver injury. International Journal of Clinical and Experimental Pathology. 2013; 6(5):831-40. PMCID: PMC3638093

[33] Rabani V, Shahsavani M, Gharavi M, Piryaei A, Azhdari Z, Baharvand H. Mesenchymal stem cell infusion therapy in a carbon tetrachloride-induced liver fibrosis model affects

Jalili MA, et al. H₂O₂-Preconditioned Umbilical Cord-Derived Mesenchymal Stem Cells Ameliorate Liver Regeneration. Anatomical Sciences. 2017; 14(1):43-50.

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matrix metalloproteinase expression. Cell Biology International. 2010; 34(6):601–5. doi: 10.1042/cbi20090386

[34] Stock P, Brückner S, Winkler S, Dollinger M, Christ B. Human bone marrow mesenchymal stem cell-derived hepatocytes improve the mouse liver after acute acetaminophen intoxication by preventing progress of injury. International Journal of Molecular Sciences. 2014; 15(4):7004–28. doi: 10.3390/ijms15047004

[35] Mizuno H, Tobita M, Orbay H, Uysal AC, Lu F. Adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells and Cancer Stem Cells. 2013; 165–74. doi: 10.1007/978-94-017-8032-2_15

[36] Gruttadauria S, Grosso G, Pagano D, Biondi A, Echeverri GJ, Seria E, et al. Marrow-derived mesenchymal stem cells restore biochemical markers of acute liver injury in experimental model. Transplantation Proceedings. 2013; 45(2):480–6. doi: 10.1016/j.transproceed.2012.06.087

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