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Comparative restoration of acute liver failure by menstrual blood stem cells compared with bone marrow stem cells in mice model

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Abstract

Background aims. The application of menstrual blood stem cells (MenSCs) in regenerative medicine is gaining increasing attention. The aim of this study was to investigate the therapeutic potential of MenSCs compared with bone marrow–derived stem cells (BMSCs) in an animal model of CCl_4-induced acute hepatic failure. *Methods.* Injured Balb/C mice were divided into multiple groups and received MenSCs, BMSCs or hepatocyte progenitor-like (HPL) cells derived from these cells. *Results.* Tracking of green fluorescent protein–labeled cells showed homing of cells in injured areas of the liver. In addition, the liver engraftment of MenSCs was shown by immunofluorescence staining using anti-human mitochondrial antibody. Microscopically examination, periodic acid-Schiff and Masson's trichrome staining of liver sections demonstrated the considerable liver regeneration post–cell therapy in all groups. Assessment of serum parameters including aspartate aminotransferase, alanine aminotransferase, total bilirubin, urea and cholesterol at day 7 exhibited significant reduction, such that this downward trend continued significantly until day 30. The restoration of liver biochemical markers, changes in mRNA levels of hepatic markers and the suppression of inflammatory markers were more significant in the MenSC-treated group compared with the BMSC-treated group. On the other hand, HPL cells in reference to undifferentiated cells had better effectiveness in the treatment of the acute liver injury. *Conclusions.* Our data show that MenSCs may be considered an appropriate alternative stem cell population to BMSCs for treatment of acute liver failure.

Key Words: *bone marrow stem cells, liver injury, menstrual blood stem cells, regenerative medicine*

Introduction

The adult liver has a critical role in regulating many metabolic pathways [1]. Acute liver failure (ALF) is a life-threatening condition with a high mortality rate [2]. Many patients with liver failure require a lifesaving liver transplant but are faced with major limitations including long waiting lists, lack of donors, transplant rejection and high cost [3]. In recent years, bone marrow–derived stem cells (BMSCs) are the most common type of mesenchymal stromal cells (MSCs) used in cell therapy [4–6]. Several studies showed the potential of BMSCs in regenerative medicine based on their multipotent, anti-apoptotic, immunosuppressive and paracrine properties [7,8]. Nevertheless, problems such as limited availability, invasive sample collection and low proliferation capacity limit the applicability of BMSCs [9]. The recent identification of MSC-like cells in menstrual blood (MenSCs) provides a non-invasive source of MSCs with several advantages, such as easy accessibility without need for anaesthetic, renewability as they can be sourced on a monthly basis, high proliferative capacity in culture without inducing genetic abnormalities and lack of ethical concerns [10–13]. Moreover, immunomodulatory properties of MenSCs and their therapeutic potential in different diseases emphasize the safety and efficacy of their application for cell therapy [14–18]. Our group and others showed MenSCs can differentiate into multiple mesodermal and occasionally endodermal and ectodermal lineages [19], but with different capacities depending

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on lineage compared with BMSCs [20–24]. More recently, in line with our project, *in vivo* efficiency of MenSCs in the treatment of hepatic fibrosis in the mouse model has been reported [25,26]; however, the capabilities of these cells compared with BMSCs in alleviating ALF has not been explored. We report the therapeutic potential of MenSCs compared with BMSCs in ALF by parallel biochemical, molecular and histochemical examination. We also assessed the effect of *in vitro* induction of differentiation into hepatocyte progenitor-like cells on the therapeutic capacity of MenSCs and BMSCs in the regeneration of liver tissue following ALF. In addition, we evaluated the immunomodulatory and anti-inflammatory effects of stem cells in ALF by assessing changes in levels of inflammatory factors pre- and post-transplantation.

Methods

Ethics

All donors of menstrual blood and bone marrow specimens signed the informed consent approved by the medical ethics committee of Avicenna Research Institute. All animals received human care in compliance with the Guide for Care and use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). The investigators who measured and interpreted the results of this study did not inform the design details and groups assignment. All injections and surgeries were performed by Dr. Taghizadeh, the surgeon of our study team.

Isolation and culture of MenSCs and BMSCs

MenSCs were separated from samples of five healthy female donors aged 20–35 years using a Diva cup during the first 2 days of the menstrual cycle. In brief, menstrual blood mononuclear cells were separated by Ficoll-Hypaque (GE-Healthcare) density gradient centrifugation. The cells were retrieved and washed, and the cell pellet was subsequently cultured in complete Dulbecco's Modified Eagle's Medium/F12 (Sigma-Aldrich) supplemented with 10 % fetal bovine serum (FBS), 2 mmol/L L-glutamine, 100 × non-essential amino acids, 100 U/mL penicillin, 100 mg/mL streptomycin and 25 mg/mL Fungizone and maintained at 37°C in a 5% humidified CO_2 incubator. The first colonies appeared 4–7 days later. After 70–80% confluence had been reached, adherent cells were passaged using Trypsin/EDTA (Gibco).

In parallel, BM-derived MSCs were obtained from bone marrow aspirates (5–10 mL) of five female donors aged 18–30 years. The specimens were aspirated from iliac crests at the Bone Marrow Transplantation Center, Shariati Hospital, Tehran University of Medical Sciences. The isolation procedure of BMSCs was performed as described in our previous study [20,22]. Cells were used between passages 3 and 5.

Characterization of MenSCs and BMSCs

Evaluation of the expression of CD105 and CD146 (MSC markers), OCT-4 (embryonic stem cell marker) and CD45 (hematopoietic cell marker) was done by flow cytometric analysis as described previously [20,23]. Briefly, aliquots of 10^5 cells/100 µL were washed in cold phosphate buffered saline (PBS) + 2% FBS and incubated separately for 40 min with PE-conjugated mouse anti-human CD105 (clone 43A3; BioLegend), CD146 (clone P1H12; BD Pharmingen) and CD45 (clone HI30; BD Pharmingen). For analysis of OCT-4 expression, the cells were washed with permeabilization buffer (0.5% saponin in PBS), treated with primary antibody, rabbit anti-human OCT-4 antibody (Abcam), for 40 min and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin (Ig; Sigma). The appropriate isotype controls IgG were used as negative controls (BD Pharmingen). Finally, cells were analyzed using a Partec flow cytometer and SPSS 13 software.

For further comparison of both types of MSCs, their differentiation ability into osteoblasts and adipocytes were confirmed by using alizarin red staining (Sigma-Aldrich) for detecting mineralized calcium, and oil red O staining to identify fat vacuoles, respectively. Chondrogenic differentiation was assessed by immunohistochemistry using primary monoclonal mouse anti-human collagen type II (clone 5B2.5, 1:500; Abcam) and secondary antibody FITC-labeled goat anti-mouse IgG (Abcam) using previously described protocols [20,24] provided in the online supplement.

In vitro *differentiation of MSCs into hepatocyte-like cells*

Both MenSCs and BMSCs were differentiated into hepatocyte-like cells according to the protocol reported previously [27]. In brief, cells at passage 3–4 were cultured in serum-free Dulbecco's Modified Eagle's Medium supplemented with 10 ng/mL basic fibroblast growth factor (b-FGF) and 20 ng/mL epidermal growth factor (EGF) for 2 days. Differentiation was induced by adding hepatogenic medium consisting of 40 ng/mL hepatocyte growth factor, 10^{-7} mol/l dexamethasone, 1% insulin–transferrin–selenium+1 (ITS+1), and 50 mg/mL nitrilotriacetic acid (NTA) for 14 days, followed by treatment with 10^{-7} mol/L dexamethasone, 1% ITS+1 and 20 ng/mL oncostatin