Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Equal distribution of mesenchymal stem cells after hepatic ischemia–reperfusion injury

Askhat Isbambetov, MD,[a,b] *Zhassulan Baimakhanov, MD,*[a] *Akihiko Soyama, MD, PhD,*[a] *Masaaki Hidaka, MD, PhD,*[a] *Yusuke Sakai, PhD,*[a] *Mitsuhisa Takatsuki, MD, PhD,*[a] *Tamotsu Kuroki, MD, PhD,*[a] *and Susumu Eguchi, MD, PhD*[a,*]

[a] *Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[b] *Department of Surgery, Syzganov's National Scientific Center of Surgery, Kazakh National Medical University, Almaty, Kazakhstan*

ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form
15 March 2016
Accepted 24 March 2016
Available online 1 April 2016

Keywords:
BM-MSCs
Transplantation
I/R injury
Rat model

ABSTRACT

Background: Liver ischemia–reperfusion (I/R) injury is one of the major causes of hepatocellular injury-related mortality and morbidity after liver transplantation. Mesenchymal stem cells (MSCs) have been shown to reduce liver I/R injury and improve regeneration. The purpose of the present study was to investigate the difference in the distribution of systemically delivered MSCs in the recipient's liver between the ischemic injury area and nonischemic area.

Material and methods: Fishers' rats (7-8 week of age) were used as donors of MSCs and recipients. Bone marrow-derived MSCs were isolated from the donor's femur. Before systemic administration, MSCs were labeled with the fluorescent dye PKH26. The rats were divided into four groups: (1) I/R injury + MSC group, (2) MSC only, without I/R injury, (3) I/R injury + saline group, and (4) the Sham group. I/R injury was performed by clamping the inflow vascular structures of the left and middle lobes of the recipient's liver for 60 min. The right lobe was considered as a nonischemic part. Subsequently, 1.5×10^6 of MSCs or saline (NaCl, 0.9%) was administrated via the rat's tail vein. Thereafter, the rats were killed after days one, three, or seven for the analyses.

Results: A fluorescent microscopy assay for labeled MSCs showed positive cells in both ischemic and nonischemic parts of the recipient's liver. The number of cells was significantly higher in the I/R injury + MSC group compared with the only MSC, without I/R injury group. Immunohistochemical staining showed that there was no significant difference in the proliferation of Ki-67-positive cells between the I/R + MSCs and I/R + saline groups. In addition, the serum transaminase levels were not different between the I/R + MSCs and I/R + saline groups.

Conclusions: After partial liver I/R injury, transplanted MSCs migrate equally to the ischemic and nonischemic parts of the recipient's liver. Considering the unique ability of the liver to regenerate, both parts of the liver presumably receive signals for regeneration.

* *Corresponding author*. Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Tel.: +81 95 819 7316; fax: +81 95 819 7319.
E-mail address: sueguchi@nagasaki-u.ac.jp (S. Eguchi).

0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2016.03.057

Introduction

Liver transplantation is currently the only approved treatment option for patients with end-stage liver diseases. However, due to the liver transplant operation procedure and its prolonged operation time, especially in cadaveric split liver transplantation or living donor liver transplantation, liver ischemic–reperfusion (I/R) injury can occur, which can lead to serious complications including graft rejection, inflammatory processes, necrosis of hepatocytes, and ultimately graft failure.[1-3] It has been reported that I/R injury accounts for 10% of early liver failure.[4]

Mesenchymal stem cells (MSCs) have been shown to have a big potential in the field of liver regeneration and clinical applications, including organ transplantation.[5-8] Some reports have shown that bone marrow-derived (BM) MSCs contribute to liver regeneration at the area of I/R in cases of I/R injury.[9-12] Reza *et al.*[12] showed that human adipose-derived MSCs (HADMSC) attenuate I/R injury and promote liver regeneration in a rodent model. The mice were subjected to 60 min of liver I/R injury with or without 70% partial hepatectomy. Thereafter, the animals were treated with adipose-derived MSCs. Liver I/R injury was evaluated according to the serum levels of aspartate aminotransferase (AST), serum interleukin-6, and histopathology analyses. Liver samples were stained for specific markers of liver regeneration. However, the direct role of MSCs has not yet been studied, and it remains unclear whether additional therapeutic effects of MSCs have an impact on the non-ischemic part of the liver. To investigate this, we used a partial liver I/R injury rat model developed in our laboratory, which includes the I/R injury area and non-I/R area. Partial liver I/R injury was performed by clamping the inflow vascular structures of the left and middle lobes of the recipient's liver for 60 min, comprising 70% of the ischemic area of the whole liver. The right and caudate lobes were considered to be the non-I/R injury part of the liver, comprising 30% of the total area. The main goal of our study is to investigate and confirm whether the administrated MSCs migrate mainly to the affected parts of the liver by I/R injury or spread evenly throughout the whole liver, as an additional factor reinforcing the regenerative properties of the liver.

Materials and methods

Animals

F344 wild-type rats (male rats as the donors and female rats as the recipients, 7-8 wk of age, and body weight approximately 160-200 g) were purchased from Japan SLC (Hamamatsu, Japan). All rats were housed in a temperature-controlled environment with a 12-h light/dark cycle and access to standard rat chow and water *ad libitum*. For all procedures, the rats were anesthetized with 2%-2.5% isoflurane inhalation. All experiments were performed according to the Guidelines for Animal Experimentation at Nagasaki University.

BM-MSCs isolation

Rat MSCs were isolated using a modified protocol as described by a previous report.[13] Briefly, MSCs were isolated from the femurs of male rats by flushing the femurs with alpha Minimum Essential Medium (aMEM; Gibco, Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine saline, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (Penicillin-Streptomycin-Glutamine 100X, Gibco). Isolated MSCs were seeded onto 10 mL tissue culture dishes (Gibco) and cultured with aMEM supplemented with 10% fetal bovine saline. When the cells reached 70%-80% confluency, they were harvested with 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA; Gibco). All MSCs used for the *in vitro and in vivo* experiments were at passage 5 or 6.

Staining BM-MSCs with the fluorescent dye PKH26 red

To trace BM-MSCs after transplantation, the cells were labeled with the fluorescent dye PKH26 (Sigma–Aldrich, St. Louis, MO), which is a lipophilic membrane binding dye that can irreversibly bind into lipid regions of the cell membrane. Labeling was performed after the manufacturer's instructions using a final PKH26 concentration of 2×10^{-6} M for 1×10^{7} cells/mL with a 5-min incubation time. The labeled MSCs were cultured and evaluated for 2 wk *in vitro*. For *in vivo* experiments, labeling of the MSCs was performed on the day of transplantation.

Groups selected for research and operational procedures

The rats were randomly divided into four groups: (1) I/R injury + MSC group, (2) only MSC, without I/R injury, (3) I/R injury + saline, and (4) Sham group. Each group consisted of 15 rats (with five rats killed at each time point).

After anesthesia, laparotomy was performed through a midline incision. Partial liver I/R injury was induced by clamping with plastic microvascular clips (Biover Disposable Microvascular Clamps, Birmingham) the portal vein, hepatic artery, and bile duct of the middle and left lateral liver lobes, comprising 70% of the I/R injury part of the liver (Fig. 1). The right and caudate lobes were considered to be the non-I/R injury part of the liver, comprising 30% of the total area. After 60 min of clamping, the clips were removed. Approximately 15 min after initiating reperfusion, 200 μL of MSCs (1.5×10^{6} cells) or NaCl 0.9% were administrated via the tail vein of the rat with a 29-gauge needle. The abdomen was closed with Prolene 6-0 continuous sutures. Thereafter, the rats were killed at one, three, and seven days after the operation for further analysis. Before killing, the serum and whole liver were collected. Parts of the liver tissues were immediately fixed in 4% paraformaldehyde phosphate buffer (Wako Pure Chemical Industries, Ltd.) and liquid nitrogen for further analysis.

Fluorescent assay of the liver tissue samples after transplantation

Five-mm thick cryostat sections of the liver were fixed in chloroform and acetone for 10 min at 4°C. The detection and