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Transplantation of bone marrow cells decreases tumor necrosis factor-α production and blood–brain barrier permeability and improves survival in a mouse model of acetaminophen-induced acute liver disease

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Abstract

Background aims. Acute liver failure (ALF), although rare, remains a rapidly progressive and frequently fatal condition. Acetaminophen (APAP) poisoning induces a massive hepatic necrosis and often leads to death as a result of cerebral edema. Cell-based therapies are currently being investigated for liver injuries. We evaluated the therapeutic potential of transplantation of bone marrow mononuclear cells (BMC) in a mouse model of acute liver injury. *Methods.* ALF was induced in C57Bl/6 mice submitted to an alcoholic diet followed by fasting and injection of APAP. Mice were transplanted with 10^7 BMC obtained from enhanced green fluorescent protein (GFP) transgenic mice. *Results.* BMC transplantation caused a significant reduction in APAP-induced mortality. However, no significant differences in serum aminotransferase concentrations, extension of liver necrosis, number of inflammatory cells and levels of cytokines in the liver were found when BMC- and saline-injected groups were compared. Moreover, recruitment of transplanted cells to the liver was very low and no donor-derived hepatocytes were observed. Mice submitted to BMC therapy had some protection against disruption of the blood–brain barrier, despite their hyperammonemia, and serum metalloproteinase (MMP)-9 activity similar to the saline-injected group. Tumor necrosis factor (TNF)-α concentrations were decreased in the serum of BMC-treated mice. This reduction was associated with an early increase in interleukin (IL)-10 mRNA expression in the spleen and bone marrow after BMC treatment. *Conclusions.* BMC transplantation protects mice submitted to high doses of APAP and is a potential candidate for ALF treatment, probably via an immunomodulatory effect on TNF-α production.

Key Words: *acetaminophen, acute liver failure, bone marrow mononuclear cells, brain edema, cell therapy, tumor necrosis factor*

Introduction

Acute liver failure (ALF) is caused by liver cell dysfunction, leading to coagulopathy, hepatic encephalopathy and death in previously healthy patients. The main cause of this illness is poisoning with acetaminophen (*N*-acetyl-paraaminophen; APAP) through unintentional overdoses or suicide attempts (1). This drug induces severe centrolobular hepatocellular necrosis and increases serum transaminase levels, in a dose-dependent manner. The extent of APAP-induced hepatic lesions is increased by fasting and alcohol consumption, both in animals and humans (2,3).

APAP is metabolized by cytochrome P450 to form *N*-acetyl-*p*-benzoquinone imine (NAPQI). This reactive metabolite depletes glutathione and covalently binds to cysteine groups on proteins, leading to inhibition of mitochondrial respiration, mitochondrial permeability transition, hepatic necrosis and inflammatory response (4,5). The consequent massive death of hepatocytes is followed by disturbances in the hepatic cycle of urea and an increase in serum

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ammonia levels. In the brain, the ammonia excess is incorporated as glutamate by astrocytes to form glutamine, an organic osmolyte, which leads to oxidative/nitrosative stress, blood–brain barrier (BBB) disruption and increased cerebral blood flow (6,7). All of these features induce encephalopathy and brain edema, the main cause of death in humans after an APAP overdose. Furthermore, the systemic production of the pro-inflammatory cytokine tumor necrosis factor (TNF)-α is increased in patients with acute liver failure, and a growing body of evidence indicates this cytokine to be a central mediator promoting the development of encephalopathy in this condition (8).

The definitive treatment for APAP-induced acute liver failure is liver transplantation, limited by the shortage of donors and surgical risks (9). Research for therapeutic approaches has focused on the prevention of liver damage with anti-oxidants, control of brain edema and hepatic support with bio-artificial livers or hepatocyte transplantation (10). Cell-based therapies have also been investigated as treatments for liver lesions. Transplantation of bone marrow mononuclear cells (BMC) has been shown to ameliorate dysfunction in different organs, such as the heart, brain and liver. BMC are easy to isolate and comprise different cell populations that can produce anti-inflammatory molecules related with hepatic protection in acute liver failure (11,12). In this study we evaluated the therapeutic potential of BMC transplantation in a model of APAP-induced hepatotoxicity potentiated by alcohol consumption.

Methods

Animals and acute liver failure induction

Animals were handled according to the Fundação Oswaldo Cruz (FIOCRUZ) guidelines for animal experimentation and the experimental protocols were approved by local animal ethics committees. Four to 6-week-old male C57Bl/6 mice weighing approximately 20 g were raised and maintained at the Gonçalo Moniz Research Center/Oswaldo Cruz Foundation (Salvador, Brazil) in rooms with controlled temperature (22 ± 2°C) and humidity (55 ± 10%) and continuous air renovation. Animals were maintained with 10% alcohol solution in water for 3 weeks. Before APAP administration, they were fasted for approximately 12 h with free access to pure water. Fresh suspensions of 16 mL/kg APAP (All Chemistry, São Paulo, Brazil) were prepared in warm saline (40°C) and given at 300 mg/kg intraperitoneally (corresponding to a lethal dose of 70%). Control mice received the same volume of saline solution.

Transplantation of BMC

Bone marrow cells were obtained from femurs and tibiae of 4–6-week-old enhanced green fluorescent protein (EGFP)-transgenic C57Bl/6 mice. The mononuclear cell fraction was purified by centrifugation in a Histopaque gradient at 1000 ***g*** for 25 min at 25°C (Histopaque 1119 and 1077, 1:1; Sigma-Aldrich, St Louis, MO, USA). The mononuclear cell fraction was collected and washed three times in Dulbecco's modified Eagle medium (Sigma-Aldrich). Cell suspensions were filtered over nylon wool and diluted in saline (5×10^7 cells/mL), and their viability was evaluated by trypan blue exclusion (Sigma-Aldrich). The cells were injected into the peripheral circulation 3 h after APAP injection (1×10^7 cells/mouse). In addition, BMC samples were analyzed by flow cytometry in a FACScalibur flow cytometer using conjugated antibodies (Becton Dickinson, San Diego, CA, USA), showing that 96.5 ± 1.3% expressed green fluorescent protein (GFP) and 96.3 ± 3.1% expressed CD45. Hematopoietic progenitor markers Sca-1, CD34 and CD117 were expressed by 0.11 ± 0.03%, 0.20 ± 0.05% and 0.17 ± 0.04% of the cells, respectively.

Tissue preparation and evaluation of liver injury

Mice from BMC- and saline-treated groups were anesthetized with ketamine (115 mg/kg) and xylazine (10–15 mg/kg) at different time-points after APAP injection. The animals were immediately perfused transcardially with 50 mL cold 0.9% saline, followed by 100 mL cold 4% paraformaldehyde (Merk, Darmstadt, Germany) in phosphate-buffered saline (PBS), pH 7.2. Livers were excised and left lobes were fixed in 10% formalin and paraffin-embedded. Four micrometer-thick paraffin-embedded sections were stained with hematoxylin-eosin (H&E). Analyses were performed on whole liver sections after slide scanning using an Aperio ScanScope system (Aperio Technologies, Vista, CA, USA). The images were analyzed using the Image Pro program (version 7.0; Media Cybernetics, San Diego, CA, USA). Hepatic injury was determined as a percentage of necrotic tissue, and a mean area of 24 676 428 μm^2/mouse was analyzed.

Quantification of EGFP cells in the liver by immunofluorescence

After transcardiac perfusion with paraformaldehyde, non-left hepatic lobes were additionally fixed in 4% paraformaldehyde at 4°C for 24 h. These samples were then incubated overnight in 30% sucrose solution in PBS at 4%, embedded in medium for congeal tissue and frozen at –70°C. Four-micrometer thick