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Research Article

Bone marrow mononuclear cell transplantation improves mitochondrial bioenergetics in the liver of cholestatic rats

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ABSTRACT

Mitochondrial dysfunction has been associated with liver cholestatis. Toxic bile salt accumulation leads to chronic injury with mitochondrial damage, ROS increase and apoptosis, resulting in liver dysfunction. This study aimed to analyze mitochondrial bioenergetics in rats with hepatic fibrosis induced by bile duct ligation (BDL) after BMMNC transplantation. Livers were collected from normal rats, fibrotic rats after 14 and 21 days of BDL (F14d and F21d) and rats that received BMMNC at 14 days of BDL, analyzed after 7 days. F21d demonstrated increased collagen I content and consequently decrease after BMMNC transplantation. Both F14d and F21d had significantly reduced mitochondrial oxidation capacity and increased mitochondrial uncoupling, which were restored to levels similar to those of normal group after BMMNC transplantation. In addition, F21d had a significantly increase of UCP2, and reduced PGC-1α content. However, after BMMNC transplantation both proteins returned to levels similar to normal group. Moreover, F14d had a significantly increase in 4-HNE content compared to normal group, but after BMMNC transplantation 4-HNE content significantly reduced, suggesting oxidative stress reduction. Therefore, BMMNC transplantation has a positive effect on hepatic mitochondrial bioenergetics of cholestatic rats, increasing oxidative capacity and reducing oxidative stress, which, in turn, contribute to liver function recover.

Introduction

Liver diseases have high mortality and morbidity rates worldwide, being hepatic fibrosis the most common disease that results from chronic liver disease [9,52]. Liver fibrosis is characterized by fibrogenesis dysregulation as a chronic inflammatory process result leading to extracellular matrix components accumulation and hepatic architecture disruption [8,26,23,52,36].

The bile duct ligation (BDL) is a well-known experimental model consisting in the interruption of bile flow and toxic bile

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retention in the liver, process called cholestasis, which is responsible for severe liver biochemical and structural changes [21,10,32]. From the biochemical point of view, the overall mitochondrial function is impaired, resulting in a marked metabolic disorder, characterized by reduced carbohydrate and fatty acid oxidation [3]. Therefore, mitochondria are relevant organelles in hepatic cholestasis, being involved in different stages of the disease progression [20,2].

During cholestasis onset, the hydrophobicity of bile acids components retained in the liver plays a key role in the cytotoxic action exerted on hepatocytes [4,37]. Some of the adverse effects of bile acids on mitochondrial bioenergetics could be related to the disturbance in the composition of the mitochondrial membrane, since they can be incorporated and reduce its phospholipid component [27], changing the fluidity and the mitochondrial membrane potential (Ψ_m), ATP levels, and ROS production, leading to apoptosis rate increase [24,30,17,3].

Different studies have demonstrated that in vitro incubation of isolated mitochondria with bile acids involves a decrease in the maximum ADP-stimulated mitochondrial respiration (state 3), as also demonstrated in an experimental model of cholestasis [28,45]. In addition, bile acids seem to exert a direct effect on mitochondrial respiration uncoupled from ATP production (state 4), suggesting that these components directly modulate the activity of uncoupling proteins (UCP), presented in the inner mitochondrial membrane. Previous data have demonstrated that bile acids cause the increase of UCP2 in the liver at the transcriptional level already at 4 and 8 days after BDL in rats [15].

The mitochondrial mass and function need to be regulated at the cellular level due to its vital role in energy production, metabolism and signaling. The active process of mitochondrial biogenesis must meet the specific energy needs of the cell and is coordinated through the activity of several transcription factors to the nuclear level, and the peroxisome proliferator-activated receptor-γ coactivator 1 alpha (PGC-1α) is one of the most important [47]. This protein promotes the transcription of mitochondrial transcription factor A (TFAM) that controls mitochondrial DNA replication [16,33]. Literature data already showed that during the first 3 days after BDL, the transcription of TFAM protein levels were decreased when compared to controls, indicating that mitochondrial biogenesis is impaired early in the development of cholestasis [51]. Therefore, mitochondrial damage is not accompanied by a proper mitochondrial biogenesis to recover mitochondrial function.

Non-functional mitochondria are important sources of reactive oxygen species (ROS) that promote the recruitment and activation of inflammatory cells, including Kupffer cells, responsible for the production of pro-fibrogenic cytokines such as TGF-β, IL-1, TNF-α e IL-6 [29], which induce parenchyma cells apoptosis [39]. In addition, TGF-β directly acts on hepatic stellate cells by activating them and promoting extracellular matrix production [1] that, when deposited in the perisinusoidal space, hinders the movement of nutrients and oxygen to the hepatocytes, leading to apoptosis of these cells ([19]; [11,42]).

Currently, the only effective treatment available for cirrhosis is liver transplantation. However, the shortage of organs, among other factors, limits the treatment and leads to the imminent need for developing new anti-fibrotic therapies [25,43]. Our group has recently demonstrated that transplantation of bone marrow mononuclear cells (BMMNC) on rats with liver fibrosis induced by BDL restores the normal balance between the components of extracellular matrix (such as collagen and laminin), leading to fibrosis resolution and normalizing the hepatic function [10–12]. On this way, the currently work aimed to better understand the mechanisms by which BMMNC transplantation has a positive effect on cholestasis investigating its role on liver mitochondrial bioenergetics.

Materials and methods

Hepatic fibrosis induction and experimental groups

To induce hepatic fibrosis, three-month old male Wistar rats (250–270 g) were anesthetized with halothane and the bile duct ligation (BDL) followed by resection was carried out. BMMNC were isolated from the tibias and femurs of two-month old healthy male Wistar rats, sacrificed in a CO_2 camber, as described by Carvalho et al. [10]. Briefly, the medullar cavities were exposed and washed with cold DMEM (Dulbecco's Modified Eagle's Medium, Sigma-Aldrich) pH 7.2. The bone marrow cells were submitted to a Ficoll–Hypaque (Sigma-Aldrich) density gradient and centrifugation at 2000 rpm. The mononuclear cells interface was collected and transplanted via jugular vein in rats with 14 days fibrosis.

The animals were divided into four groups ($n=8$): normal animals, animals with hepatic fibrosis after 14 and 21 days of BDL (F14d and F21d groups, respectively), and animals with hepatic fibrosis after 14 days of BDL which received 10^7 BMMNC via jugular vein and were sacrificed after 7 days of transplantation (F14d+7d BMMNC). All animals used in this study were submitted to protocols approved by the Animal Experiments Committee in accordance with the standard guidelines on animal experimentation, received water and standard food ad libitum and were sacrificed in CO_2 camber.

High-resolution Respirometry

We used the high-resolution Respirometry protocol according to Cortez et al. [14]. Immediately after sacrifice, liver fragments were mechanically permeabilized by using sharpened forceps. After that, the tissue were transferred into vessels with cooled (in ice) Mitochondrial Respiration Medium, MIR05 (in mM: EGTA 0.5, $MgCl_2$ 3.0, K-MES 60, taurine 20, K2HPO4 10, HEPES 20, Sucrose 110 and BSA 1 g/L, pH 7.1 adjusted at 25 °C) and incubated at mild stirring for 2 min. After, the tissue was weighted and 5 mg was used per chamber. The respiratory rates of liver were measured with the Oroboros 2k-Oxygraph (Oroboros Instruments, Innsbruck, Austria) in 2 ml of MIR05 at 37 °C with continuous stirring. Before adding the tissue into the chamber, wet weight measurements were taken and a sample of 5 mg was used per chamber. Oxygen limitation was avoided by maintaining oxygen levels above 400 mM O_2 in the chamber. Datlab software (Oroboros Instruments, Innsbruck, Austria) was used for data acquisition and analysis. Oxygen consumption rates were expressed as pmol of O_2● s-1●mg wet weight-1. Studies were performed with two independent sets of substrates. In the carbohydrate protocol, substrate combinations were used for electron flow through CI and CII (in mM): glutamate, 10; malate, 2; and succinate, 10. In the fatty acid, protocol respiration was measured with (in mM): palmitoyl-l-carnitine, 0.02 and malate, 2. Respiratory parameters were defined as follows: maximally ADP (5 mM)–stimulated