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5-Aminolevulinic acid combined with ferrous iron ameliorate ischemia–reperfusion injury in the mouse fatty liver model

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ABSTRACT

Background: The fatty liver could increase the risk of serious acute ischemia reperfusion (I/R) injury, and hepatic steatosis is indeed a major risk factor for hepatic failure after grafting a fatty liver.
Materials & methods: Fatty liver models of methionine- and choline-deficient high-fat mice were subjected to I/R injury with or without 5-aminolevulinic acid (5-ALA)/sodium ferrous citrate (SFC) treatment. Levels of hepatic enzymes, lipid peroxidation and apoptosis, inflammatory cytokines and heme oxygenase (HO)-1, and the carbon monoxide (CO) in the liver, and reactive oxygen species (ROS), inflammatory cytokines and members of the signaling pathway in isolated Kupffer were assessed.
Results: Alanine aminotransferase and aspartate aminotransferase levels, the number of necrotic areas, thiobarbituric acid reactive substance content, TUNEL-positive cells, infiltrated macrophages, and the inflammatory cytokine expression after I/R injury were dramatically decreased, whereas the endogenous CO concentrations and the HO-1 expression were significantly increased by 5-ALA/SFC treatment. The expression of toll-like receptors 2 and 4, NF-κB and inflammatory cytokines and ROS production in Kupffer cells were significantly decreased with 5-ALA/SFC treatment.
Conclusion: 5-ALA/SFC significantly attenuates the injury level in the fatty liver after I/R injury.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease in developed countries [1]. NAFLD is a progressive liver disease and the spectrum includes a simple fatty liver and nonalcoholic steatohepatitis, which includes inflammation and liver fibrosis or cirrhosis [2]. The contrariety between the shortage of liver donors and the number of patients waiting for liver transplantation has increased dramatically, thus we have to use the extended criteria donor (ECD) liver for transplantation. In all ECD, the fatty liver is one of the most common indications. If we use a fatty liver for liver transplantation, then the most critical risk is an impaired liver function after transplantation or implantation failure [3]. The tolerance of the fatty liver to acute injury induced by hepatic ischemia and reperfusion (I/R) is worse [4]. The mortality rate of the fatty liver was 14%, however, that of the healthy liver was only 2%, and the risk of major functional loss and dysfunction was also higher [5]. Thus the development of protective strategies to minimize the adverse effects of I/R injury in fatty livers is an urgent need. Liver I/R activates leukocytes,

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endothelial cells and Kupffer cells and causes their interaction with one another, resulting in the release of inflammatory cytokines such as tumor necrosis factor (TNF)−α [6]. As key mediators of the immune response, Kupffer cells play an important role in hepatic I/R injury [7].

Heme oxygenase (HO) is the rate-limiting degradation enzyme in the process of heme metabolism. It can catalyze heme to iron ion, carbon monoxide (CO) and biliverdin. Heme oxygenase-1 (HO-1) is an inducible enzyme of HO, and the HO-1 expression is also caused by the inflammation signal or hypoxia [8]. Several recent studies have shown that 5-aminolevulinic acid (5-ALA), an endogenous and natural amino acid, is an intermediate in heme synthesis and fundamental for aerobic energy metabolism, combined with sodium ferrous citrate (SFC) induces upregulation of the expression of HO-1 [9–11]. Treatment with 5-ALA and SFC (5-ALA/SFC) resulted in the increased HO-1 expression in the mouse kidney and subsequently degraded heme to CO, which exhibited immunomodulatory protective effects in renal I/R injury [9]. Furthermore, both HO-1 and metabolites of heme are widely acknowledged to have antioxidant and anti-inflammatory activities in the human body [12]. The HO-1/CO axis exerts both cytoprotective and therapeutic capacities for liver injury. In addition, HO-1 and CO are significantly effective in protecting hepatocytes from chronic injury by cell death via both apoptotic and necrotic pathways [13].

Reactive oxygen species (ROS), a type of by-product of cellar metabolism, are a clear target to reduce tissue injury during hepatic I/R injury [14]. Activated Kupffer cells are the key players in I/R injury due to upregulation of ROS production, the pro-inflammatory cytokines expression, and aggregation of neutrophils, which play a role in liver inflammatory injury [6]. In the activation of Kupffer cells, the Toll-like receptor 4 (TLR4)/nuclear factor kappa beta (NF-κB) signaling pathway contributes to the damage of hepatic ischemia reperfusion [15].

In the present study, we investigated the protective effect of 5-ALA combined with SFC in acute liver I/R injury in a fatty liver model using methionine, choline-deficient and high fat (MCDHF) diet. Methionine and choline deficiency (MCD) diet model is a well-characterized dietary rodent model of NAFLD. MCD disturbs biosynthesis of phosphatidylcholine in the liver and secretion of lipoprotein from the liver, which causes severe fatty change in the liver. As we already reported MCDHF as a NAFLD model [16,17], this NAFLD model showed excessive ROS, inflammation, and fibrosis in the liver with the advantage of this model is the shortage of the time course to establish NAFLD. Further, we examined whether the HO-1/CO axis and TLR4/NF-κB immune signaling pathway are involved in the therapeutic effect of 5-ALA/SFC.

2. Materials and methods

2.1. Preparation of 5-ALA/SFC

5-ALA (COSMO ALA, Co., Ltd., Tokyo, Japan) and SFC (Eisai Food & Chemical, Co., Ltd., Tokyo, Japan) were dissolved in distilled water (DW). SFC was dissolved in the 5-ALA solution immediately prior to oral administration. 5-ALA (100 mg/kg) and SFC (157 mg/kg) were administered at 48 h, 24 h, and 30 min before ischemia. Other materials and methods are given in the Supplementary data.

3. Results

3.1. 5-ALA/SFC suppressed I/R injury in the fatty liver and decreased oxidation stress by I/R

To assess the preventive effect of 5-ALA/SFC for I/R injury of the fatty liver, necrotic areas in the liver specimen were quantitatively analyzed. Almost no inflammatory cells or necrotic areas in the MCDHF diet group, whereas inflammatory cell infiltration and necrotic areas formed after 1 h and 3 h reperfusion following 15 min ischemia (Fig. 1A, 1st and 2nd rows and Fig. 1B, upper panel). 5-ALA/SFC exhibited significantly reduced elevated Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by I/R injury (Fig. 1C, upper and middle panel). On the evaluation of the fatty liver oxidant stress in I/R damage, the fatty livers subjected to I/R injury showed significantly elevated thiobarbituric acid reactive substance (TBARS), while 5-ALA/SFC pretreatment drastically inhibited the increase in TBARS in the fatty livers after I/R injury (Fig. 1C, lower panel). The protective effect of 5-ALA/SFC correlated with the histological alterations and biochemical markers.

3.2. The mRNA expression of inflammatory cytokines reduced by 5-ALA/SFC in the fatty livers suffering from I/R

The injury degree of I/R in the fatty liver was associated with multiple inflammatory cytokines. A quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis demonstrated that higher levels of the expression of TNF-α, interleukin (IL)-6, osteopontin (OPN), inducible nitric oxide synthase (iNOS), and interferon (IFN)-γ mRNA in the fatty livers after 1 h (Fig. 2A) and 3 h (Fig. 2B) reperfusion following 15 min ischemia were significantly reduced by 5-ALA/SFC.

3.3. Suppression of the macrophage infiltration and apoptotic cell death in the fatty livers after I/R by 5-ALA/SFC treatment

Infiltration of macrophages in the fatty livers following I/R injury was evaluated by immunohistochemistry. Macrophages that widely distributed in the liver after 1 h and 3 h reperfusion following 15 min ischemia were significantly decreased by 5-ALA/SFC (Fig. 1A, 3rd and 4th rows, and Fig. 1B, middle panel). Furthermore, apoptosis of hepatocytes following I/R injury was also inhibited by 5-ALA/SFC dramatically (Fig. 1A, 5th and 6th rows, and Fig. 1B, lower panel).

3.4. Pretreatment of 5-ALA/SFC generated high endogenous CO content in the fatty livers of mice

5-ALA/SFC could generate endogenous CO was evaluated. As shown in Fig. 2C, the endogenous CO concentrations in the fatty liver mice treated with 5-ALA/SFC were significantly higher than those treated with DW, 5-ALA, or SFC ($p < 0.01$ for all) with time dependency (30 min and 1 h). The results showed that the endogenous CO concentration was enhanced at 30 min and decreased at 1 h after 5-ALA/SFC administration in the fatty livers (Fig. 2C, left panel).

3.5. 5-ALA/SFC pretreatment induced the HO-1 expression in the fatty livers subjected to I/R injury

As high level expression of HO-1 ameliorated I/R injury in the fatty liver [18], examination of the involvement of HO-1 in present study was examined. As shown in Fig. 2D, the mRNA level of HO-1 was dramatically upregulated in the fatty livers in the 5-ALA/SFC pretreatment group subjected to 15 min ischemia and 1 h reperfusion (Fig. 2D, right panel) or 3 h reperfusion (Fig. 2D, left panel) compared with sham fatty livers and fatty livers subjected to I/R injury. These results suggest that 5-ALA/SFC administration induced the protective effect of HO-1 on I/R injury in the fatty livers.

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