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Induction of autophagy reduces ischemia/reperfusion injury in steatotic rat livers

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ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form
23 March 2017
Accepted 11 April 2017
Available online xxx

Keywords:
Autophagy
Hepatic steatosis
Lithium chloride
Liver transplant
Ischemia/reperfusion injury

ABSTRACT

Background: Steatotic livers are particularly vulnerable to ischemia/reperfusion injury (IRI). One of the reasons is an underlying impairment of autophagy. Autophagy is regulated by glycogen synthase kinase 3b (GSK3b) and extracellular signal-regulated kinases (ERK1/2) pathways. Both of them are target proteins of a cell-protective drug, lithium chloride. Lithium chloride treatment reduces IRI in many organs including liver. Therefore, we aimed to investigate the effect of lithium chloride treatment on autophagy induction in steatotic rat livers. We also wanted to evaluate the related cell-protective effects on the enhanced hepatic IRI.

Materials and methods: After inducing hepatic steatosis, rats were injected with lithium chloride or normal saline for 3 d before being subjected to 70% selective warm ischemia for 60 min. After reperfusion, rats were observed for 30 min, 6, 24, and 48 h.

Results: Lithium chloride appeared to protect hepatocytes from IRI via its ability to induce autophagy by modulation of both GSK3b and ERK1/2 pathways. Hepatic damage was significantly decreased in the treatment group as indicated by a reduced inflammatory response, less apoptosis, less necrosis, and lower liver enzyme levels.

Conclusions: Simultaneous modulation of GSK3b and ERK1/2 pathways might be an interesting strategy to reduce IRI in steatotic livers with an impairment of autophagy.

Introduction

The lack of adequate organs for transplantation to meet the existing demand has resulted in a major organ shortage crisis. One of the strategies to solve this problem is to expand the donor organ pool by accepting marginal organs such as steatotic livers.[1]

Hepatic steatosis is associated with an increased risk for postoperative morbidity and mortality after major liver surgery. Evidence is accumulating that steatotic livers are particularly vulnerable to ischemia/reperfusion injury (IRI). A growing number of studies suggest that compared with normal livers, steatotic livers show a reduced autophagy level.

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2017.04.012

The reduction of autophagy contributes to the aggravation of hepatic damage after IRI.[2,3]

Autophagy is a cellular response to various types of cell damage, also including ischemia. Cells are protected from death via maintaining intracellular energy levels by disassembling nonessential cellular components and by degrading impaired organelles such as mitochondria.[4,5] It increases the chance of cells to survive under extenuating circumstances such as starvation, hypoxia, and IRI.[5]

IRI is a multifactorial process that affects liver function after major hepatic surgery such as extended hepatectomy with Pringle manoeuver or liver transplantation.[6] During IRI, the activation of glycogen synthase kinase 3b (GSK3b)[7] and extracellular signal-regulated kinases (ERK1/2)[8] pathways are two of the major events modulating autophagy independently. On one hand, GSK3b pathway modulates autophagy indirectly by downregulating the activity of mTOR, which is well known as an inhibitor of autophagy.[7] On the other hand, phosphorylation of ERK1/2 leads to an activation of the beclin 1 pathway, which is directly involved in the autophagy progress.[9]

On the basis of these studies, a drug which induces autophagy via a modulation of both GSK3b and ERK1/2 pathways may represent a novel strategy for protecting steatotic livers from hepatic IRI.

Lithium chloride as a neuroprotective drug has been used in bipolar disorder treatment for over 100 y.[10] It is involved in a wide range of cellular functions, including cell cycle, death, and carcinogenesis, by inhibiting GSK3b pathway directly and indirectly.[11,12] In addition, lithium also acts on stress and survival pathways such as the ERK1/2 pathway[13] to protect neurons against neurodegenerative diseases.[14,15] In previous studies, treatment with lithium chloride regulated autophagy positively.[12,16,17]

Growing evidence suggests that lithium chloride treatment may reduce IRI in many organs such as brain, kidney, heart, and liver.[18-24] However, the potential of this compound to reduce IRI in steatotic liver with the known underlying impairment of autophagy is still unclear.

In this study, we want to investigate the hypothesis that enhancing autophagy with lithium chloride treatment could protect steatotic liver from ischemia/reperfusion injury.

Materials and methods

Experimental design

Q4 Rats were given a methionine/choline deficient with high fat-content (MCD + HF, Ssniff Spezialdiaeten GmbH, Germany; key components listed in Supplementary table) diet for 2 wk to induce moderate hepatic steatosis.[25,26] During the last 3 d of feeding the diet, rats in the treatment group were pretreated with lithium chloride. Animals in the control group were subjected to treatment with an equal volume of normal saline instead of lithium chloride. Thereafter, they were subjected to 70% selective warm ischemia for 60 min. Rats were sacrificed 0.5, 6, 24, and 48 h after reperfusion (n = 6/group). The following parameters were investigated: light chain 3 II/I (LC3) and p62 levels to assess autophagy; hepatic high-mobility group box (HMGB1) translocation and expression, neutrophil infiltration, as well as serum interleukin 6 and interleukin 10 levels to assess inflammation; cleavage of caspase 3 for apoptosis; phosphorylation level of GSK3b, ERK1/2, and JNK; and mechanistic target of rapamycin (mTOR) to investigate the underlying mechanism. Furthermore, we investigated the extent of hepatic necrosis and serum liver transaminase levels to quantify hepatic damage.

Animals

Male inbred Lewis rats (Charles River Laboratories, Germany) with a body weight from 250-320g at the start of the experiment were employed in the present study. Animals were housed under standard care conditions (humidity: 45%-70%, temperature: 21 ± 03°C, 12-hour light/dark cycle). Tap water and the respective diet were offered to the rats ad libitum. All procedures were carried out according to the German Animal Welfare Legislation (Thuringia State office for consumer protection, Germany, protocol number: 02-038/14).

Drug administration

0.9% saline solution was used to dissolve lithium chloride (Sigma-Aldrich). The concentration of lithium chloride solution was 2 mmol/mL. The solution was diluted immediately before injection. In the treatment group, rats were subjected to lithium chloride treatment (2 mmol/kg subcutaneously once daily) at 72, 48, and 24 h before operation, which was continued for 24 or 48 h according to the respective observation time.[20,24,27] Rats in the control group received an equal volume of saline solution.

Partial hepatic warm ischemia/reperfusion

Rats were placed in an anesthesia induction chamber using a concentration of 3% isoflurane (Nicholas Piramal (I) Limited, United Kingdom) in a vaporizer (Sigma Delta, Netherland) and oxygen flow of 0.5 L/min. After placing a transversal incision on the abdomen, the liver was exposed and the interlobular ligaments were dissected. The left portion of the hepatoduodenal ligament (including portal vein, bile duct, and hepatic artery to median and left lateral lobes) was clamped with a microvascular clamp for 60 min to induce 70% selective warm ischemia.[28] Animals were sacrificed 0.5, 6, 24, and 48 h after reperfusion. Left lateral lobe and right superior lobe were collected for histological analysis. Right median lobe and serum were collected for further protein analysis, snap frozen, and stored in liquid nitrogen until use.

Serum aminotransferases analysis

Serum alanine and aspartate aminotransferases were analyzed by using the AEROSET System (Abbott Laboratories, Germany) according to the instructions of the manufacturers. The levels were compared to the results of serum alanine and aspartate aminotransferases levels in nonsteatotic livers, taken from our previously published study[19] (presented in Fig. 8B). Q5