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Butyrate protects liver against ischemia reperfusion injury by inhibiting nuclear factor kappa B activation in Kupffer cells

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ABSTRACT

Background: The inflammatory response after hepatic ischemia reperfusion (I/R) contributes to liver dysfunction and failure after transplantation. Butyrate is a four-carbon fatty acid, normally produced by bacterial fermentation of fiber in mammalian intestines, with anti-inflammatory activities. The purpose of the present study was to investigate the protective effect of butyrate preconditioning, if any, against hepatic I/R injury in rats and the underlying mechanisms involved.

Methods: Male Sprague-Dawley rats were subjected to a partial (70%) hepatic ischemia for 60 min after pretreatment with either vehicle or butyrate, followed by 3, 6, and 24 h of reperfusion. Hepatic injury was evaluated by biochemical and histopathologic examinations. Neutrophil infiltration was measured by myeloperoxidase (MPO) activity. The expression of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) was measured by enzyme-linked immunosorbent assay (Elisa) and Real-time reverse-transcriptase polymerase chain reaction (RT-PCR). The expression of nuclear factor kappa B (NF-κB) p65 was determined by immunohistochemistry and Western blot analysis.

Results: Butyrate treatment markedly improved hepatic function and histology, as indicated by reduced transaminase levels and ameliorated tissue pathologic changes. The expression of tumor necrosis factor-alpha, interleukin-6, and myeloperoxidase activity was attenuated by butyrate. Butyrate also reduced I/R-induced nuclear translocation of NF-κB p65 in Kupffer cells.

Conclusion: Our results suggest that butyrate alleviates I/R-induced liver injury, possibly by suppressing inflammatory factors production and preventing NF-κB activation in Kupffer cells.

1. Introduction

Liver transplantation is the most effective means for the treatment of various types of end-stage liver disease. However, its utilization is seriously limited by a critical shortage of donors. Donation after cardiac death (DCD) is the most likely source of donation. However, primary graft nonfunction and biliary complications remain significant risks for recipients of DCD livers, compared with brain death and living organ donations [1]. The main reason for this is the prolonged ischemic insult as a result of liver retrieval, with preservation and engraftment leading to more serious reperfusion injury [1,2]. Hence, reducing ischemia reperfusion (I/R) injury and improving DCD transplantation outcomes is of

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great clinical significance. The molecular mechanisms underlying I/R, however, have not been fully clarified, despite a recent resurgence of interest in this area.

The I/R insult is a multifaceted process that combines two distinctive phases with unique mechanisms of tissue damage [3]. The initial phase, within the first 2 h of reperfusion, is characterized by the activation of immune cells and formation of reactive oxygen species, followed by a late phase, from 6 to 24 h after reperfusion, marked by neutrophils accumulation and hepatocellular injury. Kupffer cells (KCs), the largest population of resident macrophages, are the major innate immune cells in the liver. Overactive KCs have been identified as the primary cell type in the initiation and perpetuation of I/R injury. They produce proinflammatory cytokines and chemokines to recruit and activate circulating neutrophils, which all contribute to the inflammation-associated damage. Therefore, the catastrophic cascade of inflammatory events, triggered by KCs and neutrophils, play pivotal roles in I/R pathogenesis, suggesting therapeutic interventions that inhibit the inflammatory pathway may attenuate I/R-induced liver injury.

The inflammatory response is tightly regulated by the transcription factor nuclear factor-κB (NF-κB). NF-κB is a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NFKB1/p105, NFKB1/p50, REL, and NFKB2/p52 and the heterodimeric p65-p50 complex appears to be the most abundant one. In an inactive form, NF-κB dimers are sequestered in the cytoplasm complexed with members of the Inhibitor of κB (IκB) family. When stimulated, NF-κB complex is then freed to enter the nucleus where it can turn on the expression of downstream genes. However, there are conflicting experimental results regarding the inhibition of NF-κB signaling mainly because it also serves as a key regulator of both the regenerative and antiapoptotic responses. The dual and opposing roles of NF-κB signaling in I/R may be explained by their specific function with different cells, for example, NF-κB activation in KCs promotes inflammatory gene expression, whereas activation in hepatocytes promotes cell survival [4]. Because of this, selective inhibition of NF-κB signaling in KCs has potential therapeutic applications in hepatic I/R injury.

Butyrate, a four-carbon short-chain fatty acid, normally produced by the colonic bacterial anaerobic fermentation of undigested carbohydrates and fiber polysaccharides, has received considerable attention as a potential therapeutic agent for cancers because of its histone deacetylase (HDAC) inhibition [5]. In addition to their anticancer activity, recent data have demonstrated that short-chain fatty acids have potent anti-inflammatory or immunomodulatory effects, at noncytotoxic dosing levels [6]. Kim *et al.* [7] have demonstrated that HDAC inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat ischemic model of stroke. However, it remains unclear whether pretreatment with butyrate can protect the liver against I/R injury. Hence, in the present study, we investigated the effects of butyrate administration on I/R-induced liver injury, by measuring liver function and histopathologic changes. We also analyzed the related mechanisms of its anti-inflammatory effect and NF-κB activity during liver I/R injury.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g) were purchased from the Department of Laboratory Animal Science at Fudan University and housed in a laminar flow, specific pathogen-free atmosphere. Animal protocols were approved by the Fudan University Animal Care Committee, and the experiments were performed in adherence to the guidelines provided by the National Institutes of Health for the use of animals in laboratory experiments.

2.2. Warm hepatic I/R model

Partial (70%) warm ischemia was performed as previously described [8]. In brief, a microvascular clamp was used to occlude the portal triad (hepatic artery, portal vein, and bile duct) to the left and median liver lobes for 60 min to introduce partial warm ischemia. Sham controls underwent the same procedure without vascular occlusion. Reperfusion was initiated by removal of the clamp. The rectal temperature was maintained at 37°C throughout the surgery by a warming pad. For the pretreatment experiments, some rats were injected intravenously with 300 mg/kg sodium butyrate (Sigma, Saint Louis), as previously described [7], or vehicle (normal saline solution) at 30 min before ischemia.

2.3. Liver damage assessment

To assess hepatic function and cellular injury following liver ischemia, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured in blood samples obtained at predetermined time points (3, 6, and 24 h) after reperfusion with a standard automatic analyzer (type 7150; Hitachi, Tokyo, Japan).

2.4. Histopathology

Liver tissues were fixed by immersion in 4% buffered paraformaldehyde and embedded in paraffin. Sections (4 μm) were stained with hematoxylin-eosin and assessed for inflammation and tissue damage.

2.5. Liver myeloperoxidase activity

Tissue-associated myeloperoxidase (MPO) activity, an indicator of neutrophil infiltration, was determined as previously described [9].

2.6. Real-time reverse-transcriptase polymerase chain reaction

Total RNA was extracted from the liver using TRIzol reagent (Life Technologies, Carlbad) according to the manufacturer's instructions. The messenger RNA (mRNA) for tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was quantified in duplicate by SYBR green two-step, real-time