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Protective effects of butyrate on intestinal ischemia–reperfusion injury in rats

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ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form
22 March 2015
Accepted 9 April 2015
Available online 16 April 2015

Keywords:
Reperfusion injury
Tight junction proteins
Butyrate

ABSTRACT

Background: Butyrate is normally fermented from undigested fiber by intestinal microflora. The goal of the present study was to determine the effects of butyrate and its underlying mechanisms on intestinal injury in a rat model of ischemia and reperfusion (I/R).

Methods: Male Sprague–Dawley rats were subjected to warm ischemia for 45 min by clamping the superior mesenteric artery after treatment with butyrate, followed by 6 and 72 h of reperfusion. Pathologic histology analysis, enzyme-linked immunosorbent assay, immunofluorescence, and Western blot were performed.

Results: Butyrate preconditioning markedly improved intestinal injury. The inflammatory factor levels and leukocyte infiltration were attenuated by butyrate. Butyrate also maintained the intestinal barrier structures, increased the expression of tight junction proteins, and decreased endotoxin translocation.

Conclusions: We conclude that butyrate administration attenuates intestinal I/R injury, which is associated with preservation of intestinal tight junction barrier function and suppression of inflammatory cell infiltration in the intestinal mucosa. This suggests butyrate as a potential strategy to prevent intestinal I/R injury.

1. Introduction

Intestinal ischemia and reperfusion (I/R) injury is commonly encountered in several clinical conditions, including trauma, shock, abdominal aortic surgery, and small bowel transplantation [1]. Intestinal I/R not only results in injury to the intestine but may also lead to sepsis, systemic inflammatory response syndrome, and even multiple organ dysfunction syndrome, owing to damage of the mucosal structure and barrier function [2]. Hence, minimizing I/R injury is of great clinical significance.

The pathophysiology of I/R injury includes both direct cellular damage due to ischemic insult and delayed dysfunction and damage resulting from inflammatory cascade [3]. Intestinal barrier injury causes translocation of endotoxin from the intestinal lumen, further aggravating I/R-induced intestinal injury by oxidative stress, free radical formation, and the release of inflammatory mediators [4]. Once reperfusion occurs, complex and multifactorial inflammatory cascade is initiated and difficult to interrupt. Thus, interfering in this pathogenetic process at multiple links, particularly in the early phases, is a

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0022-4804/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.04.031

potential strategy for the treatment of intestinal I/R injury.

There is growing interest in butyrate, a four-carbon short-chain fatty acid (SCFA) produced by the gut microbiota fermentation of nondigestible polysaccharides, as a potential therapeutic agent for cancers via histone deacetylase inhibition [5]. Beyond its established role in anticancer treatments, SCFA has recently been shown to have antioxidant, anti-inflammatory, and immunosuppressive effects [6]. Moreover, butyrate is the principal energy source for the proliferation and differentiation of colonic epithelial cells and is important for intestinal physical function [7]. For example, in physiological concentrations, SCFAs, especially butyrate, can establish and maintain the intestinal mucosal barrier, as shown in previous *in vitro* studies [8,9].

Our previous *in vivo* study showed that butyrate attenuates hepatic I/R injury [10–12]. However, its effectiveness in intestinal I/R injury remains to be determined. The present study was designed to investigate the role of butyrate in intestinal I/R injury with a focus on its potential for the modulation of inflammatory reaction and regulation of intestinal permeability.

2. Materials and methods

2.1. Animals

All experimental procedures were performed in compliance with the guidelines of the Institutional Animal Care and Use Committee in laboratory experiments. Male Sprague–Dawley rats (200–250 g) were randomized into the sham group, vehicle group, and butyrate group. For *in vivo* treatment, the rats were injected intravenously with 300-mg/kg sodium butyrate (Sigma, St. Louis, MO), as previously described [10] in the butyrate group or vehicle (normal saline solution) in vehicle group at 30 min before ischemia, followed by another injection at 12 h after reperfusion.

2.2. Intestinal I/R injury

The intestinal I/R injury model was performed as previously described [13]. In brief, the superior mesenteric artery was occluded for 45 min using a microvascular clamp, followed by reperfusion for 6 and 72 h. Animals in the sham group underwent the same procedure without vascular occlusion. Throughout the surgery, the body temperature was maintained at 36°C–37°C with a homeothermic blanket. Rats were sacrificed at the indicated time after reperfusion, and serum and small intestine samples were collected.

2.3. Histologic analysis

Jejunum tissues were fixed by 4% buffered paraformaldehyde and embedded in paraffin. Sections (4 μm) were stained with hematoxylin-eosin and quantitatively assessed for tissue damage by a pathologist blind to the experiment according to previously published criteria [14].

2.4. Myeloperoxidase activity

Tissue-associated myeloperoxidase (MPO) activity, an indicator of neutrophil infiltration in the small intestine, was determined as previously described [11].

2.5. Enzyme-linked immunosorbent assay

Tumor necrosis factor-alpha and endotoxin levels in serum were measured using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN).

2.6. Immunofluorescence

Anti-CD68 antibody (AbD Serotec, Raleigh, NC) was used for immunofluorescent staining. Secondary antibody was Fluorescein Isothiocyanate (FITC)-conjugated IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA), and the nuclei were labeled with 4′,6-diamidino-2-phenylindole (Invitrogen, Camarillo, CA).

2.7. Western blot

Western blot analysis was performed as previously described [11]. Polyclonal rabbit antibody to claudin-1, ZO-1, occludin (Abcam, Cambridge, Scotland) and monoclonal rabbit antibody to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Abcam) were used as primary antibodies; the secondary antibody was horseradish peroxidase (HRP)–conjugated goat anti-rabbit IgG antibody (Santa Cruz Biotechnology). Chemiluminescence detection was performed using a Super ECL Plus kit (GE Healthcare, Buckinghamshire, UK).

2.8. Transmission electron microscopy

Ultrathin sections of ileal segments were prepared using standard techniques and examined using a JEOL JEM 1200-EX transmission electron microscope (Hitachi, Tokyo, Japan).

2.9. Statistical analysis

Group sizes are indicated in the figure legends. Data are presented as means ± standard deviation, unless otherwise noted. Statistical analysis was performed by either an analysis of variance or Kaplan–Meier test. A difference of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Butyrate attenuates small intestine histologic damage after I/R

Histopathologic findings showed normal intestinal mucosa in the sham group, whereas the vehicle group presented with significant mucosal damage characterized by massive destruction of villi and inflammatory cell infiltration. However, butyrate treatment significantly attenuated the mucosal damage, with intestines showing slight hyperemia and edema, less inflammatory cell infiltration in the mucosa and