Oxymatrine Attenuates Intestinal Ischemia/Reperfusion Injury in Rats

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Abstract

Purpose. Intestinal ischemia/reperfusion (I/R) is a common and serious clinical condition. The anti-inflammatory and anti-apoptotic properties of oxymatrine, the extract from a traditional Chinese herb, Sophora flavescens Ait, have been shown to protect the liver from I/R injury and attenuate colitis. The objective of this study was to investigate if oxymatrine could attenuate intestinal I/R injury induced in rats.

Methods. The experimental design consisted of three groups of 24 Wistar rats each: a sham-operation group (control group), a group subjected to intestinal I/R and then given saline (saline group), and a group subjected to intestinal I/R and then given oxymatrine (oxymatrine group). Intestinal I/R was induced by occluding the superior mesenteric artery for 45 min. Six rats from each group were killed at selected time points, and blood and intestinal samples were collected.

Results. Morphological alteration, reduction of γ-glutamyl transpeptidase (γ-GGT) activity, and increased cell apoptosis confirmed intestinal I/R injury. The oxymatrine group had a significantly lower histological score and apoptosis index than the saline group, demonstrating that the preadministration of oxymatrine attenuated gut damage. Moreover, oxymatrine inhibited the production of lipid peroxides (LPO), decreased the serum levels of tumor necrosis factor (TNF)-α, and downregulated expression of phosphorylated p38 mitogen-activated protein kinase, Fas, and FasL, which had been elevated by I/R.

Conclusions. These results provide further evidence of the anti-inflammatory and anti-apoptotic activities of oxymatrine, which may become a potent drug for protecting the intestines against I/R injury.

Key words Oxymatrine · Intestinal ischemia/reperfusion · Apoptosis · Tumor necrosis factor-α · Fas/Fas ligand · p38 mitogen-activated protein kinase

Introduction

Intestinal ischemia/reperfusion (I/R) is a common but serious event, which occurs in ischemic bowel disease, necrotizing enterocolitis, intestinal transplantation, hemodynamic shock, and sepsis. During I/R, tissues are subjected to the destructive proinflammatory cytokines and reactive oxygen species released by inflammatory cells, leading to inflammatory injury and cell apoptosis. Intestinal I/R can also cause injury to the secondary organs and even multiple organ failure (MOF). Therefore, novel agents to attenuate intestinal I/R are being sought urgently.

Oxymatrine, the extract from a traditional Chinese herb, Sophora flavescens Ait, has been used widely to treat chronic hepatitis. Its pharmacological effects include regulation of immune reaction, anti-inflammation, anti-hypersensitive reaction, and inhibition of histamine release. We reported previously that oxymatrine attenuated hepatic I/R injury in rats through its anti-apoptotic activity, which depends on downregulating Fas and FasL in livers. Oxymatrine has also been shown to ameliorate inflammation in dextran sulfate sodium-induced colitis in rats, and protect lungs from acute injury induced by oleic acid in mice. Thus, we hypothesized that oxymatrine might be effective against intestinal I/R induced injury.
Materials and Methods

Animals

Male Wistar rats, weighing 250–280 g, were supplied by The Animal Research Center of the First Clinical Medical School of Harbin Medical University, Harbin, China. The animals were kept under standard conditions and fed rodent chow and water. Animal care and all surgical procedures were approved by the Animal Ethics Committee of Harbin Medical University.

Surgical Procedures and Treatment

Animals were randomly assigned to one of three experimental groups: a sham group (n = 24), a saline group (n = 24), and an oxymatrine group (n = 24). The rats in the saline and oxymatrine groups were given an intravenous injection of 2 ml physiological saline or an equal volume of oxymatrine injectable solution (Cat. 0406111; Jiangsu Chia-tai Tianqing Pharmaceutical, China) 40 mg/kg body weight via the penis vein, 30 min before I/R, respectively. The rats in the sham group were subjected to the same surgical procedures except that the superior mesenteric artery (SMA) was not clamped. The rats were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg), and operated on through a midline laparotomy. In the preliminary experiments, we tested four doses (10, 20, 40, 80 mg/kg) of oxymatrine on intestinal I/R in rats. Oxymatrine 10 mg/kg did not produce a significant effect, but 20, 40, and 80 mg/kg attenuated intestinal injury. Oxymatrine 40 mg/kg showed a significantly stronger effect than 20 mg/kg, but there was no significant difference between 40 and 80 mg/kg; thus, we used 40 mg/kg throughout this study. After identifying the SMA, the small intestine was subjected to warm ischemia by occluding the SMA with a nontraumatic vessel clamp. Adequate occlusion was confirmed by pallor and the absence of pulsation in the mesenteric vessels of the small intestine. The clamp was removed 45 min later and reperfusion was started. The abdominal cavity was closed with sutures, and analgesia was provided by an injection of buprenorphine (0.1 mg/kg s.c.). Six rats from each group were killed 2, 3, 12, and 36 h after reperfusion, respectively. Blood samples were collected and full-thickness samples of small intestine were removed for further analysis. The blood samples were spun at 1000 × g for 10 min, and the sera were decanted and stored at −80°C. To study the survival beneficial effect of oxymatrine, 12 rats were randomly assigned into either a saline group (n = 6) or an oxymatrine group (n = 6), treated as above, and closely monitored.

Morphological Analysis

Specimens were fixed in 10% formalin and embedded in paraffin. Sections of 4 μm were prepared and stained with hematoxylin–eosin (H&E), and blindly examined under light microscopy. The semiquantitative histopathologic assessment for evaluating intestinal damage has been described previously. Briefly, a total score was derived from the sum of scores for 11 histologic criteria, including villus fusion and stunning, disruption of the brush border and surface enterocytes, reduction in the number of goblet cells, reduction in number of mitotic figures, crypt loss, and architectural disruption, disruption or distortion of crypt cells, crypt abscess formation, infiltration of polymorphonuclear cells and lymphocytes, dilatation of lymphatics and capillaries, and thickening and edema of the submucosal and muscularis external layers. Histologic examinations were done in a blinded manner. Each histologic variable was scored from 0 (normal) to 3 (maximal damage) to give a maximum possible score of 33 for each intestinal sample.

γ-Glutamyl Transpeptidase (γ-GGT) Assay

The method used to measure γ-GGT was described previously. Briefly, a segment of intestine was isolated and flushed with phosphate-buffered saline, cut into pieces, placed in Tris–Triton buffer, and stirred for 2 min. The tissues were incubated on ice for 30 min, followed by centrifugation to collect the supernatants. The supernatants were added to a reaction mixture containing glycyl-glycine, Tris–Triton buffer, and γ-glutamyl-p-nitroanilide. After incubation for 10 min at 37°C, the reaction was stopped by glacial acetic acid. After centrifugation, supernatants were read in an ELISA reader, and the results are expressed as 405 nm absorbance.

Measurement of Lipid Peroxides (LPO)

The amount of LPO in the intestines was calculated by measuring malondialdehyde (MDA), as described previously. Briefly, intestinal tissues were homogenized and incubated with the reaction solution containing sodium dodecyl sulfate, acetic acid solution, and TBA solution, at 95°C for 60 min. After cooling, H₂O and n-butanol were added to the mixture, which was shaken vigorously, followed by centrifugation at 3000 × g for 10 min. Absorbance of the organic layer was measured at 535 nm with a spectrophotometer, and 1,1,3,3-tetraethoxypropane served as a standard.