PROTECTIVE EFFECTS OF BERBERINE ON INTESTINAL ISCHEMIA AND REPERFUSION INJURY IN RATS

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Abstract: This study aims to investigate the potential protective effects of berberine on ischemia and reperfusion (IR) injury in rats. Thirty male rats were randomly divided into three experimental groups as follows: the sham group, the IR group and the berberine+IR group. Intestinal ischemia-reperfusion was performed by occlusion of the superior mesenteric artery for 30 min, followed by 2-h reperfusion. The berberine+IR group of rats were administered 200 mg/kg of berberine once a day for 7 days before laparotomy. Compared with the IR group, rats pretreated with berberine prior to IR had significantly reduced intestinal ischemia/reperfusion injury and a significant reduction in Chiu’s score (p<0.05). The level of malondialdehyde and myeloperoxidase in the berberine+IR group was significantly decreased compared with the IR group (p<0.001). Superoxide dismutase activity in the berberine+IR group was significantly higher than in the IR group (p<0.001). Compared with the IR group, diamine oxidase was markedly decreased in the berberine+IR group (p<0.01). The level of secretory immunoglobulin A in the berberine+IR group was significantly increased when compared to the IR group (p<0.001). Our results suggest that berberine can protect from intestinal IR injury and that it can be beneficial in treating conditions associated with intestinal IR injury.

Keywords: protective effects; berberine; intestinal; ischemia reperfusion injury; rats

INTRODUCTION

Many studies have suggested that intestinal ischemia-reperfusion may promote gastrointestinal tract barrier failure and bacterial translocation, contributing to many clinical conditions including neonatal necrotizing enterocolitis [1], hemorrhagic shock [2, 3] and intestinal transplant rejection [4]. The injury resulting from ischemia-reperfusion is primarily attributable to reactive oxygen metabolites associated with activation of oxidant-producing mucosal enzymes, release of lipid chemoattractants from injured cellular membranes and subsequent infiltration of
neutrophils [5,6]. However, the mechanisms of IR injury are so complex that chemotherapy for IR is far from satisfactory.

Berberine, a well-known traditional Chinese herbal medicine, is an isoquinoline alkaloid that has been widely used in China and other countries [7]. Berberine occurs as an active ingredient in the root, rhizome and stem bark of many medicinal plants [8]. However, berberine is now manufactured by chemical synthesis. The chloride and sulfate salts of berberine are used for clinical purposes [9]. Berberine is very slightly soluble in water, slightly soluble in ethanol and sparingly soluble in methanol; however, the salt forms are relatively more soluble [10]. Berberine exhibits multispectrum pharmacological actions ranging from antioxidative action to modulation of neurotransmitters, enzymes and immunomodulation [11-15]. Moreover, several clinical and preclinical studies have demonstrated the ameliorative effect of berberine against some cancers, Alzheimer’s disease (AD), cardiovascular diseases and diabetes [16-21]. Wang et al. [16] found that berberine suppressed cancer growth by significantly inhibited Hedgehog signaling pathway activity. As an anti-inflammatory agent, berberine treatment could be an effective therapy in restoring Al maltol-induced behavioral derangements in the rabbit model of AD [17]. Due to its strong antioxidative and anti-inflammatory activity, berberine significantly improves post-myocardial ischemia and reperfusion cardiac function recovery and reduces infarct size against myocardial IR injury [18].

In the present study, we investigated the potential effect of berberine on IR injury in rat intestine and we assume that berberine could alleviate intestinal ischemia-reperfusion injury due to its strong antioxidative and anti-inflammatory activity.

MATERIALS AND METHODS

Experimental animals and groups
The animal experiment was approved by the Laboratory Animal Ethics Committee of Wenzhou Medical University. The ID Number is WYDW2014-0130. Thirty male Sprague-Dawley (SD) rats (250-300 g) used in this study were provided by the Laboratory Animal Center of Wenzhou Medical University. They were randomly divided into three experimental groups as follows: A – sham group (subjected to laparotomy, but no IR injury); B – IR group (animals in this group were subjected to 30-min occlusion of the superior mesenteric artery, followed by 2-h reperfusion); C – Berberine+IR group (animals in this group were subjected to 30-min occlusion of the superior mesenteric artery, followed by 2-h reperfusion; the rats were treated with berberine by gavage for 7 days before the rat developed IR). Physiological saline was given to the animals in the sham and IR groups; the berberine+IR group rats received 200 mg/kg of berberine administered by gavage once a day for 7 days before laparotomy.

Surgical procedure
After overnight fasting, the animals were anesthetized with intraperitoneal chloral hydrate; the abdomen was opened using a midline incision. IR was induced by
occlusion of the superior mesenteric artery (SMA) for 30 min and reperfusion for 2 h. The sham-group operation was performed by isolation of the SMA without clamping. In the IR group, the SMA was occluded with a micro clamp. During IR, the area of the operation was covered with a warm moist dressing to prevent hypothermia. At the end of the ischemic period, the clamp was removed and the intestine reperfused for 2 h. Immediately following reperfusion, the rats were resuscitated with a 3-ml intraperitoneal injection of warm (37°C) 0.9% NaCl solution and the abdominal cavity was closed in two layers with a running suture of 3/0 Dexon. All animals were killed following the reperfusion and their blood and intestinal tissue samples were collected.

**Histology**

Small intestine samples 1-2 cm long were taken 10 cm from the Treitz ligament. Biopsies were washed with cold saline and immediately fixed in 4% paraformaldehyde. The samples were then embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E) for routine histopathological observations. All of the tissue sections were observed under an optic microscope by a pathologist who was unaware of the details of the study design. The degree of intestinal tissue injury was evaluated and expressed using a grading scale from 0 to 5 as described previously [22].

**Measurement of malondialdehyde (MDA), superoxide dismutase (SOD) and myeloperoxidase (MPO) in intestinal tissues**

Intestinal tissues were homogenized on ice with normal saline and centrifuged for 15 min at 4000 g. Supernatants were transferred into fresh tubes for to evaluate the MDA and SOD activity by spectrophotometry. MPO activity was determined in each of the harvested ?? with an enzyme-linked immunosorbent assay.

**Measurement of secretory immunoglobulin A (SIgA) levels in intestinal mucosa**

Each intestinal segment was rinsed thoroughly with physiological saline and opened longitudinally on the antimesenteric border to expose the intestinal mucosa. The mucosa was scraped from the underlying tissue with a glass slide, then it was weighed and made into tissue homogenerate (10%). After centrifugation of the tissue homogenate at a low temperature, we obtained the suspension for the following step. Intestinal mucus levels of SIgA were determined with an enzyme-linked immunosorbent assay according to the manufacturer’s instructions.

**Measurement of diamine oxidase (DAO) in blood serum**

Three milliliters of blood were taken from the inferior vena cava of each animal at the end of the experiment, kept at room temperature for 15 min and centrifuged at 1000 g for 15 min to obtain the suspension. Serum levels of DAO were determined with an enzyme-linked immunosorbent assay.

**Statistical analysis**

All the results were expressed as means±SD. One-way ANOVA was used to analyze the differences between datasets. The ordinal values of the Chiu scores were analyzed by the Kruskal-Wallis nonparametric test. Statistical procedures were performed using GraphPad Prism 5.0 (GraphPad, San Diego, CA). A value of P <0.05 was considered statistically significant.
RESULTS

Histopathological analysis
Intestinal injury was evaluated by Chiu’s scoring after microscopy (×100). In the sham group (Fig. 1A), the intestinal mucosa was normal. The gap of epithelial cells was in the normal range and we failed to identify any enlarged gap between epithelial cells. There were no significant pathological changes in the intestinal tissue of the sham and berberine groups. In the IR injury group (Fig.1B), severe edema of mucosal villi and infiltration of necrotic epithelial and inflammatory cells were observed, and intestinal glands showed evidence of severe injury. In addition, a large number of intestinal villi were severed, the gap of epithelial cells increased significantly and blood and lymph vessels expanded markedly, indicating severe mucosal damage. Berberine pretreatment significantly reduced the histological damage compared to the IR group. We also observed edema of mucosal villi, infiltration of necrotic epithelial and inflammatory cells (Fig. 1C). The score of intestinal mucosa pathology is given in Fig. 1D. Compared with the IR group, rats preconditioned with berberine (200 mg/kg) prior to intestinal IR had significantly reduced intestinal IR injury and significant reduction in Chiu’s scores (p <0.05; Fig. 1D).

Intestinal tissue levels of MPO, MDA and SOD
The MPO activity was used to reflect neutrophil migration into the small intestine. As shown in Fig. 2A, the activity of MPO in the berberine+IR group was lower than in the IR group (p<0.05). IR was associated with a significant increase in the lipid peroxidation product MDA, which was reduced by berberine pretreatment (Fig. 2B). SOD is an important free radical scavenging enzyme, and by measuring its activity we can quantify the body’s ability to eliminate the oxygen radical that derived from the inflammation reaction. SOD activity in the IR group was significantly lower than that in the sham group; berberine markedly restored SOD activity (p<0.001; Fig. 2C).

Serum levels of DAO
DAO is an enzyme, also named as histaminase, that catalyses the conversion of histidine to histamine, which is related to the metabolism, oxidation and inactivation of histamine. The highest content of the enzyme is observed in the digestive tract and placenta. An increased DAO level indicates intestinal epithelial injury. In the present study, the serum levels of DAO in the IR injury group were higher than those in the sham group (p<0.001). DAO was reduced in the berberine+IR group compared to the IR group (p<0.01; Fig. 3).

Intestinal mucosa levels of SIgA
SIgA acts as an important immunologic barrier that could protect gut mucosa from luminal pathogens. The SIgA level in the intestinal mucous was decreased in the IR group compared to the sham group. The secretion of SIgA in the berberine+IR group was increased significantly compared to the IR group (p<0.001; Fig.4).

DISCUSSION
IR injury is a major problem associated with high morbidity and mortality after
trauma, hemorrhagic shock, abdominal aortic aneurysm surgery and small bowel transplantation because tissue hypoxia, inflammation and cell infiltration result in the loss of the mucosal barrier [4, 23-26]. Of the internal organs, the intestine is probably the most sensitive to IR injury [4]. Intestinal IR injury can lead to, or further enhance, oxidative stress during IR, resulting in complications in postoperative myocardial functional recovery. Therefore, effective approaches to prevent IR injury are important. Owing to the relative failure of the clinical treatment of reperfusion injury and in light of previous studies, the importance of targeting reperfusion injury in cases of intestinal ischemic injury has been brought into question. Recent studies demonstrated that ukrain, an alkaloid thiophosphoric acid derivative of greater celandine (Chelidonium majus, a member of the Papaveraceae family), helps to prevent intestinal tissue breakdown during intestinal IR injury and that this effect can be achieved by antioxidant activities [27]. Gu et al. [28] suggested that IR-induced intestinal tight junction dysfunction can be improved by berberine, thereby demonstrating the therapeutic potential of berberine for intestinal IR.

Berberine has a 3000-year-long history of use as a Chinese medicine due to its potent antimicrobial, antiprotozoal, antiarrhythmic and antidiarrheal and antitrichomonal actions [29]. Clinical investigations of berberine have demonstrated a wide spectrum of pharmacological effects. Several reports highlighting significant antihypertensive, antiarrhythmic, antihyperglycemic, anticancer, antidepressant, anxiolytic, neuroprotective, antioxidant, anti-inflammatory, analgesic and hypolipidemic activities of berberine are available [30-32]. Moreover, laboratory studies have shown several molecules and signaling pathways that account for its therapeutic effects [33-36]. In the present study, we investigated the potential protective effect of berberine on IR injury in rat intestine.

We identified MPO activity levels and quantified the tissue neutrophil contents. While extracellular MPO activity is thought to correspond to MPO-induced tissue damage, intracellular activity should correlate with tissue neutrophil numbers. According to our study, berberine can attenuate neutrophil accumulation in IR injury and further reduce excessive tissue damage by the reduction of intestinal MPO activity. There is increasing evidence showing the involvement of oxidative stress in IR injury [4,37]. In this study, we investigated the antioxidant activities of berberine. MDA has been recognized as an important lipid peroxidation indicator, since subjects affected by several diseases have increased MDA levels [38]. SOD plays an important role in defense against oxidant injury, and its activity can reflect the scavenging capacity of endogenous free radicals. There is a sharp rise in oxygen free radical generation during ischemia-reperfusion, resulting in SOD consumption during the elimination of oxygen free radicals [4]. It has been reported that oxidative stress occurs in intestinal IR injury in mice, and that it is manifested by a significant increase in serum MDA and a decrease in serum SOD [39]. In our study, reperfusion of the ischemic intestinal circulation led to a profound increase in MDA levels in intestinal tissues, which is inhibited by pretreatment with berberine. On the other hand, SOD levels in intestinal tissues were significantly increased in the berberine+IR group when compared to the IR group.
Diamine oxidase (DAO), which catalyzes oxidative deamination of histamine-like diamines, increases in the plasma during intestinal ischemia, inflammation and other stress [40]. Intestinal barrier functioning was directly assessed by examining DAO activity. DAO is a type of structural enzyme in the intestine, present in high amount; after injury of the intestinal epithelium, DAO is released into the blood [41]. In this work, the serum levels of DAO in the berberine+IR group was significantly decreased compared to the IR group. These results suggested that berberine had a beneficial effect on intestinal barrier function.

It has been reported that the immune function of the intestinal mucosa declines after ischemia-reperfusion, and that it is closely related to intestinal bacterial replacement [42]. SIgA is the principal immune defense against luminal pathogens at gut mucosal surfaces. It also has anti-inflammatory activities that may be important for the maintenance of mucosal surface integrity. In this experiment, the secretion of SIgA in the berberine+IR group was significantly increased compared to the IR group; berberine may serve to maintain intestinal barrier function and thereby decrease the systemic inflammatory response by increasing the secretion of SIgA.

In summary, the presented data indicate that berberine can protect the intestines from IR injury by reducing oxygen free radicals, inhibiting neutrophil aggregation neutrophils, modulating intestinal barrier functioning and enhancing intestinal immunity in rats. These findings suggest that berberine may be beneficial as a treatment for conditions associated with intestinal IR injury.

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Authors’ contributions: BL conceived and designed the study. TZC performed the experiments and wrote the paper.

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Figure Legends

**Fig. 1.** The evaluation of intestinal injury with Chiu’s scores by optical microscopy. Black arrow denotes areas of bleeding and necrosis. A – sham group; B – IR injury group; C – berberine pretreatment group. Intestinal histology was evaluated using Chiu’s score (D), (scale 0-5). (H&E, 100X) (Scale bar-50µm). Ten rats were present in each group. ***p<0.001 compared with the sham group; #p<0.05 compared with the IR group.

**Fig.2.** Intestinal tissue levels of MPO (A), MDA (B) and SOD (C). Ten rats were present in each group. ***p<0.001 compared with the sham group. #p<0.05 compared with the IR group. ###p<0.001 compared with the IR group.

**Fig.3.** Serum levels of DAO. Ten rats were present in each group. ***p<0.001 compared with the sham group. ##p<0.01 compared with the IR group.

**Fig.4.** Intestinal mucosa levels of SIgA. Ten rats were present in each group. ***p<0.001 compared with the sham group. ###p<0.001 compared with the IR group.
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