Berberine Ameliorates Intestinal Mucosal Barrier Damage Induced by Peritoneal Air Exposure

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Received September 7, 2014; accepted October 3, 2014; advance publication released online October 22, 2014

Berberine, an isoquinoline alkaloid derived from many medicinal plants, has been extensively used to treat various gastrointestinal diseases. In the present study, we investigated whether berberine could ameliorate intestinal mucosal barrier damage induced by peritoneal air exposure for 3 h. Peritoneal air-exposure rats received 100, 150, and 200 mg/kg berberine orally via gavage four times before and after surgery. Blood and terminal ileum samples were collected 24 h after surgery. The serum D-lactate levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit. Intestinal permeability was determined by measuring the intestinal clearance of fluorescein isothiocyanate (FITC)–dextran (FD4). Intestinal inflammation was assessed by measuring myeloperoxidase activity. Intestinal histopathology was also assessed. The results revealed that berberine decreased the serum D-lactate level, intestinal FD4 clearance, and myeloperoxidase activity. Edema and inflammation were reduced by berberine in the intestinal mucosa and submucosa, and the Chiu’s scores, indices of intestinal mucosal injury, also decreased in the berberine-treated group. In addition, berberine exerted these protective effects in a dose-dependent manner, with a significant difference from the control group at doses of 150 and 200 mg/kg. The results suggest that berberine could ameliorate intestinal mucosal barrier damage induced by peritoneal air exposure, which is linked to its anti-inflammatory activity. Berberine may be a promising treatment for intestinal mucosal barrier damage in open abdominal surgery.

Key words berberine; open abdominal surgery; intestinal mucosal barrier; peritoneal cavity; animal experiment

Berberine (BBR) is an isoquinoline alkaloid derived from many medicinal plants, such as Cortex phellodendri (Huangbai), Hydrastis canadensis (goldenseal), and Rhizoma coptidis (Huanglian). It has been well accepted as an oral drug to treat various gastrointestinal diseases for centuries in Chinese traditional medicine. Nowadays, BBR has been worldwide used as a popular traditional herbal medicine, especially in China and other Asian countries. During the last few decades, many studies have shown that BBR exhibits multiple pharmacological activities including anti-oxidant, anti-microbial, anti-tumor, and cholesterol-lowering effects. Our previous studies also found that BBR could reinforce the intestinal epithelial tight junction and reduce epithelial intestinal permeability in vitro and in vivo, which is important to maintain the intestinal mucosal barrier function.

Peritoneal air exposure is a common clinical phenomenon in open abdominal surgery, but could induce injury to various tissues/organs. The intestinal tract is one of the target organs during various stress. Our recent studies found that peritoneal air exposure could induce damage to the intestinal mucosal barrier, which is proportional to the time length of peritoneal air exposure. It is well known that damage to the intestinal mucosa barrier will result in intestinal bacterial and endotoxin translocation and further contribute to local and systemic inflammation. The injury and inflammation in the intestinal tract is often considered as the driving force of many complications after open abdominal surgery. Therefore, methods to explore effective treatment for intestinal mucosal barrier damage are popular pursuits in modern surgery.

In the present study, therefore, we designed to investigate whether BBR could ameliorate intestinal mucosal barrier damage induced by peritoneal air exposure under experimental conditions mimicking clinical operation room atmosphere.

MATERIALS AND METHODS

Animals Healthy adult male Sprague-Dawley rats (weighing 210 to 230 g) were obtained from Jinling Hospital, Nanjing, China. The rats were housed in our laboratory in a temperature- and humidity-controlled environment; they had free access to standard rat chow and tap water. The lights were maintained on a 12:12 h light:dark cycle. This animal use and care protocol and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Jinling Hospital. The experiments were also performed according to the National Institutes of Health Guidelines on the use of laboratory animals.

Drugs BBR chloride was purchased from Sigma-Aldrich, St. Louis, MO, U.S.A. All chemicals and reagents were procured from local suppliers and were of analytical grade.

Animal Grouping and Administration After an adaptation period for one week, 30 rats were randomly divided into 5 groups (n=6 each): a control group (CG), a exposure group with peritoneal air exposure for 3 h (EG), and three BBR-treated EG groups at a dose of 100, 150, and 200 mg/kg, respectively (BG, including BG100, BG150, and BG200).

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The peritoneal air exposure was induced as described in our previous study. Briefly, after full anesthesia with 2% pentobarbital sodium (3.5 mL/kg), rats in the EG and the BG groups were laparotomized through a 3 cm midline abdominal incision, and then the wound edge was retracted to allow for maximal peritoneal air exposure for 3 h. For the CG group, the animals underwent the same anesthesia but without any operative procedures. The surgical procedures were performed in an aseptic environment with controlled temperature and humidity.

Rats in three BG groups were separately administrated BBR orally at a dose of 100, 150, and 200 mg/kg through gavage once daily at 3 d, 2 d, and 1 d before surgery, and at 6 h after surgery. Each dose was dissolved in saline and diluted once daily at 3 d, 2 d, and 1 d before surgery, and at 6 h after surgery.

**Histopathology**

The ileum tissue was fixed in 4% buffered formaldehyde, and embedded in paraffin. Slices of 4-μm thick were prepared, stained with hematoxylin and eosin (H&E), and then examined by a pathologist blinded to this study design using light microscopy. The degree of intestinal mucosa injury was assessed by using Chiu’s scoring system as described previously. The intestinal mucosal changes after peritoneal air exposure graded as follows: Grade 0, Normal mucosal villi; Grade 1, Development of sub-epithelial Gruenhagen’s space, usually seen at the apex of the villus; often with capillary congestion; Grade 2, Extension of the sub-epithelial Gruenhagen’s space with moderate lifting of epithelial layer from the lamina propria; Grade 3, Massive epithelial lifting down the sides of villi, with a few tips being denuded; Grade 4, Denuded villi with lamina propria and dilated capillaries exposed; Increased cellularity of lamina propria; and Grade 5, Digestion and disintegration of lamina propria; hemorrhage and ulceration. The average scores of the exposure groups were compared with that of the controls.

**Intestinal Myeloperoxidase (MPO) Activity Asssessment**

The ileum tissue was homogenized, centrifuged at a speed of 20000×g, at 4°C for 15 min, and then the supernatants were obtained. The protein concentration in the supernatant was quantitatively measured by spectrophotometry at 460 nm as described in our previous study. Values were expressed as units/g in the ileum tissue.

**RESULTS**

**Effects of BBR on n-Lactate Level**

The results were shown in Fig. 1. The n-LA level of the EG group was significantly increased (p<0.05) when compared with that of the CG group. However, BBR induced a progressive decrease in the n-LA level in a dose-dependent manner, differed significantly in the BG150 and the BG200 groups when compared with that in the EG group (p<0.05). In addition, while the n-LA level of the BG100 and the BG150 groups was still significantly higher than that of the CG group (p<0.05), that of the BG200 group returned to the level of the CG group.
Effects of BBR on Intestinal Permeability  The results were shown in Fig. 2. The intestinal clearance of FD4 of the EG group were significantly increased ($p<0.05$) when compared with that of the CG group. However, BBR induced a progressive decrease in the intestinal clearance of FD4 in a dose-dependent manner differed significantly in the BG150 and the BG200 groups when compared with that in the EG group ($p<0.05$). In addition, while the intestinal clearance of FD4 of the BG100 and the BG150 groups was still significantly higher than that of the CG group ($p<0.05$), that of the BG200 group returned to the level of the CG group.

Effects of BBR on Intestinal MPO Activity  The results were shown in Fig. 3. The MPO activity of the EG group was significantly increased ($p<0.05$) when compared with that of the CG group. However, BBR induced a progressive decrease in the MPO activity in a dose-dependent manner, differed significantly in the BG150 and the BG200 groups when compared with that in the EG group ($p<0.05$). In addition, while the MPO activity of the BG100 group was still significantly higher than that of the CG group ($p<0.05$), that of the BG200 and the BG150 groups returned to the level of the CG group.

Histopathology  The results were shown in Fig. 4. There was no obvious structural injury in intestinal tissue among groups. However, edema and inflammation were observed in the intestinal mucosa and submucosa in the EG group when compared with that of the CG group, while BBR reduced this edema and in three BG groups when compared with that of the EG group. In addition, as shown in Fig. 5, BBR also caused a progressive decrease in Chiu’s score, differed significantly in the BG150 and the BG200 groups, when compared with the EG group ($p<0.05$).

DISCUSSION

In the present study, we designed to investigate whether BBR could ameliorate intestinal mucosal barrier damage induced by peritoneal air exposure under experimental conditions mimicking clinical operation room atmosphere. Our results showed that BBR decreased serum D-lactate level, intestinal FD4 clearance, and intestinal MPO activity. The edema and inflammation was reduced by BBR in intestinal mucosa and submucosa, and the Chiu’s scores, indices for in-
Intestinal mucosal injury, was also decreased in the BBR treated group. In addition, BBR exerted this protective effect in a dose-dependent manner, and with significance at doses of 150 and 200 mg/kg.

Peritoneal air exposure is a common clinical phenomenon in open abdominal surgery, but could induce injury to various tissues/organs. Therefore, maintenance of the intestinal mucosal barrier function is important to avoid infection. Our recent studies found that peritoneal air exposure could induce damage to the intestinal mucosal barrier, which is proportional to the time length of peritoneal air exposure. As we known, bacterial colonization of the gut is extensive and bacteria are confined to the gastrointestinal tract by the intestinal mucosal barrier. Damage to the intestinal mucosa will result in intestinal bacteria and endotoxin translocation, and further contribute to local and systemic inflammation and other complications.

BBR is a traditional herbal used to treat many gastrointestinal diseases worldwide. Many studies have shown that BBR exhibits multiple pharmacological activities including antioxidant, anti-microbial, anti-tumor, and cholesterol-lowering effects. Therefore, we choose d-lactate as a marker for the changes in intestinal mucosal barrier function. In addition, FD4 is a relatively large molecule, which could not come across the normal intestinal mucosal barrier. When the intestinal permeability is increased under pathological conditions, FD4 can penetrate the intestinal mucosal barrier. Therefore, the FD4 clearance is also employed as a marker for intestinal mucosal barrier function. In the present study, we found that BBR caused decreases in both serum d-lactate level and intestinal clearance of FD4. In addition, although there was no significant changes in histopathology among groups, the Chiou’s scores assessed for intestinal mucosal injury were significantly decreased by BBR when compared with the peritoneal air exposure group. In addition, BBR exerted this protective effect in a dose-dependent manner, and with significance at doses of 150 and 200 mg/kg. Therefore, all of these results indicated that BBR could ameliorate intestinal mucosal barrier damage induced by peritoneal air exposure.

In conclusion, our data demonstrates that BBR could ameliorate intestinal mucosal barrier damage induced by peritoneal air exposure, which is linked to the anti-inflammatory activity. BBR may be a promising treatment for intestinal mucosal barrier damage in open abdominal surgery.

Acknowledgments The authors would like to thank Prof. Qirong Li, Research Institute of General Surgery at Jinling Hospital, for her excellent technical assistance. This study was supported by 12th five-year Major Program of Army Grants (AWS12J001); Jiangsu Province’s Special Project of Science and Technology in Medicine (BL2012006).

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


