
Regular Articles

Berberine Reduces Uremia-associated Intestinal Mucosal Barrier Damage

Chao Yu, a Shanjun Tan, b Chunyu Zhou, a Cuilin Zhu, c Xin Kang, a Shuai Liu, a Shuang Zhao, a Shulin Fan, a Zhen Yu, d Ai Peng, *, a and Zhen Wang, *, a

a Department of Nephrology & Rheumatology, Shanghai Tenth People’s Hospital, Tongji University School of Medicine; Shanghai 200072, China; b Department of General Surgery, Zhongshan Hospital, Fudan University; Shanghai 200032, China; c Department of Internal Medicine, Shanghai Tenth People’s Hospital, Tongji University School of Medicine; Shanghai 200072, China; and d Department of General Surgery, Shanghai Tenth People’s Hospital, Tongji University School of Medicine; Shanghai 200072, China.

*To whom correspondence should be addressed. e-mail: pengaishsy@163.com; wangzhenshsy@163.com
ABSTRACT

Berberine is one of the main active constituents of *Rhizoma coptidis*, a traditional Chinese medicine, and has long been used for the treatment of gastrointestinal disorders. The present study was designed to investigate the effects of berberine on the intestinal mucosal barrier damage in a rat uremia model induced by the 5/6 kidney resection. Beginning at postoperative week 4, the uremia rats were treated with daily 150 mg/kg berberine by oral gavage for 6 weeks. To assess the intestinal mucosal barrier changes, blood samples were collected for measuring the serum D-lactate level, and terminal ileum tissue samples were used for analyses of intestinal permeability, myeloperoxidase activity, histopathology, malondialdehyde (MDA) level, and superoxide dismutase (SOD) activity. Berberine treatment resulted in significant decreases in the serum D-lactate level, intestinal permeability, intestinal myeloperoxidase activity, and intestinal mucosal and submucosal edema and inflammation, and the Chiu’s scores assessed for intestinal mucosal injury. The intestinal MDA level was reduced and the intestinal SOD activity was increased following berberine treatment. In conclusion, berberine reduces intestinal mucosal barrier damage induced by uremia, which is most likely due to its anti-oxidative activity. It may be developed as a potential treatment for preserving intestinal mucosal barrier function in patients with uremia.

**Key words:** berberine; intestinal mucosal barrier; uremia; oxidative stress; animal experiment
INTRODUCTION

Berberine, one of the main active constituents of *Rhizoma coptidis*, a traditional Chinese medicine, has long been used to treat various gastrointestinal disorders.\(^1\) The clinical use of berberine has been increasing worldwide especially in China, Japan, and other Asian countries, thanks to its multiple pharmacological activities, such as anti-oxidative, anti-bacterial, and cholesterol-lowering effects.\(^2\)\(^-\)\(^4\) More recent studies have demonstrated that berberine ameliorates the intestinal epithelial tight junction damage and decreases intestinal epithelial permeability, preserving intestinal mucosal barrier functions in various *in vitro* and *in vivo* models.\(^5\)\(^-\)\(^7\) We have also recently reported that berberine reduced the intestinal mucosal barrier damage following peritoneal air exposure in open abdominal surgery.\(^8\) Therefore, we speculated that berberine might be applied to protect intestinal mucosal barrier function in some chronic diseases.

Uremia is a common chronic disease, often presenting intestinal mucosal barrier dysfunction.\(^9\) The intestinal tract includes the largest community of bacteria in the body, and the number of microorganisms is much greater than that of the host cells.\(^10\) Under normal conditions, these microorganisms are restricted within the intestinal tract thanks to the integrity of intestinal mucosal barrier. However, under pathological conditions, the damage of intestinal mucosal barrier will lead to the translocation of intestinal bacteria and endotoxin, further resulting in local and systemic inflammatory responses.\(^11\)\(^-\)\(^13\) The loss of intestinal mucosal barrier function is one of the most important causes of the complications in the various diseases.\(^14\)\(^-\)\(^16\) Therefore, there is
an urgent need for developing novel intervention against intestinal mucosal barrier damage following uremia.

In the present study, we therefore used some typical biochemical parameters to investigate effects of berberine on intestinal mucosal barrier damage in a rat uremia model induced by the 5/6 kidney resection. We also determined the underlying mechanisms by analyzing the status of intestinal oxidative stress after berberine treatment in comparison with control animals. It was hoped that our results from the present study would help develop berberine as an effective treatment for intestinal mucosal barrier dysfunction in patients with uremia.
MATERIALS AND METHODS

**Animals**  Male Sprague-Dawley (SD) rats, weighing 170 -200 g, were provided by the Tenth People’s Hospital of Shanghai, Shanghai, China, and housed under an environment with controlled humidity and temperature in our animal experimental laboratory. The light was kept on a 12 h/12 h light-dark cycle. The animals had free access to standard rat chow and tap water. The animal use and care and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Tenth People’s Hospital of Shanghai. The animal experiments were also conducted in accordance with the National Institutes of Health Guidelines on the use of laboratory animals.

**Drugs**  Berberine hydrochloride (purity greater than 98%) was obtained from Shanghai Tauto Biotech Co. Ltd., Shanghai, China. The chemicals and reagents used in the present study were of analytical grade and procured from the local suppliers.

**Animal Grouping and Treatments**  After a 1-week acclimation, the SD rats were randomly divided into 3 groups (10/group): control group, uremic group, and berberine-treated uremic group. The rat uremia model was established by a 5/6 kidney resection as described previously.\textsuperscript{17} Briefly, rats in the uremic and the berberine-treated uremic groups were anesthetized by the subcutaneous injection of 3.5 mL/kg 2% pentobarbital sodium, and underwent laparotomy with a 2-cm dorsal incision, after which the 2/3 left kidney was removed, and 7 days later, the whole right kidney was removed under anesthesia. The control animals underwent sham-operation but without any kidney resection.
At 4 weeks after the operation, rats in the berberine-treated uremic group were administrated berberine at a daily dose of 150 mg/kg by oral gavage for 6 weeks. The berberine solution was prepared with saline at a final concentration of 10 mg/mL. This dosage used in the present study was chosen according to the results in the previous studies and our preliminary experiment.\textsuperscript{8, 18, 19} Rats in the control and the uremic groups were given equal volume of saline by oral gavage. At 10 weeks after operation (6 weeks after treatment), the animals were sacrificed and the blood and terminal ileum samples were collected. The selection of terminal ileum as the investigated site was on the basis of the results reported previously.\textsuperscript{20-22} These studies have demonstrated that the terminal ileum is the most sensitive part of the entire intestinal tract for observation of the intestinal mucosal barrier damage under various pathological conditions.\textsuperscript{23}

\textbf{Serum D-lactate Measurement} The blood samples were collected from the inferior vena cava and were centrifuged at 1,500 rpm at 4 °C for 15 min. The serum samples were collected for the analysis of the D-lactate levels using the ELISA kit for rats (R&D Systems, USA) following the manufacturer’s instruction.

\textbf{Intestinal Permeability Measurement} The intestinal permeability was assessed by measuring the intestinal clearance of fluorescein-isothiocyanate dextran (FD4) as described in our previous studies.\textsuperscript{8, 12, 24} Briefly, a 8-cm segment of terminal ileum was collected, and the intestinal mucosa was everted; one end of the segment was closed to make an intestinal sac, to which 1.0 mL Krebs-Henseleit bicarbonate buffer was injected from the other end. The filled intestinal sac was then incubated in a 0.5
mg/mL FD4-contained bathing solution at 37 °C. The solution was aerated through gentle bubbling with a mixture of 95% O₂ and 5% CO₂ for 30 min. The intestinal mucosal surface area (A) was then measured, and the fluorescence in the solution was analyzed using a fluorescence spectrophotometer. The intestinal clearance of FD4 was calculated as follows:

\[
C = \frac{[\text{FD4}]_{\text{ser}} \times 1 \text{ mL}}{[\text{FD4}]_{\text{muc}} \times A \times 30 \text{ min}}
\]

where \( A = \pi LD \); \( C \) is the mucosal to serosal clearance of FD4 (µL·min⁻¹·cm⁻²); \([\text{FD4}]_{\text{ser}} \) is the FD4 level in the serosal fluid taken from the sac at the end of experiment; \([\text{FD4}]_{\text{muc}} \) is the FD4 level in the mucosal fluid taken from the sac at the beginning of the experiment; \( L \) is the sac length; and \( D \) is the sac diameter.

**Intestinal Myeloperoxidase (MPO) Measurement**  The ileum tissue was homogenized, and centrifuged at the 4,000 rpm at 4 °C for 15 min, and then the supernatants were obtained. The MPO activity (units/g tissue) was quantitatively measured by spectrophotometry at 460 nm as described in our previous study.²⁵)

**Histopathological Analysis**  The removed terminal ileum was fixed with 10 % buffered formaldehyde solution and embedded in paraffin. Slices of 4-µm thickness were made, and stained with hematoxylin and eosin (H&E). The histopathological changes were evaluated under a light microscope, independently by two pathologists blinded to the present study design. The Chiu’s scoring system was used to describe the extent of intestinal mucosa injury.²⁶)
Intestinal Malondialdehyde (MDA) and Superoxide Dismutase (SOD)

Measurement  The ileum tissue homogenates were centrifuged at the 4,000 rpm at 4 °C for 15 min, and the supernatants were collected for the analyses of MDA and SOD. The measurement of MDA (nmol/mg tissue) and SOD (units/mg tissue) was accomplished using the commercial kits (Nanjing Jiancheng Biocompany, Nanjing, China) following the manufacturer’s instructions.

Statistical Analysis  The experimental data were presented as the mean ± S.D. The statistical analyses were accomplished with the SPSS 20.0 software (SPSS Inc., USA). The significance of differences among experimental groups was determined by one-way analysis of variance (ANOVA), followed by least significant difference test for multi-group comparisons. $p < 0.05$ was considered statistically significant.
RESULTS

Effects of Berberine on the Serum D-lactate Level  As shown in Fig.1, the serum D-lactate level in the uremic group was significantly higher than that in the control group \( (p < 0.05) \); while berberine treatment significantly decreased the serum D-lactate level in the berberine-treated uremic group when compared with that in the uremic group \( (p < 0.05) \), although the serum D-lactate level in the berberine-treated uremic group was still significantly higher than that in the control group \( (p < 0.05) \).

Fig. 1. Effects of Berberine on the Serum D-lactate Level. Values are presented as the mean ± S.D. \( *P < 0.05 \) means the significance of the uremic and the berberine-treated uremic groups compared with the control group, and \( #P < 0.05 \) means the significance of the berberine-treated uremic group compared with the uremic group, respectively.

Effects of Berberine on the Intestinal Permeability  As shown in Fig.2, the intestinal clearance of FD4 in the uremic group was significantly greater than that in the control group \( (p < 0.05) \); while berberine treatment significantly decreased the intestinal clearance of FD4 in the berberine-treated uremic group when compared with that in the uremic group \( (p < 0.05) \), reaching the similar level observed in the control group \( (p > 0.05) \).
Fig. 2. Effects of Berberine on the Intestinal Clearance of FD4. Values are presented as the mean ± S.D. *$P < 0.05$ means the significance of the uremic and the berberine-treated uremic groups compared with the control group, and **$P < 0.05$ means the significance of the berberine-treated uremic group compared with the uremic group, respectively.

**Effects of Berberine on the Intestinal MPO Activity** As shown in Fig.3, the intestinal MPO activity in the uremic group was significantly higher than that in the control group ($p < 0.05$); while berberine treatment significantly decreased the intestinal MPO activity in the berberine-treated uremic group when compared with that in the uremic group ($p < 0.05$), although the intestinal MPO activity in the berberine-treated uremic group was still significantly higher than that in the control group ($p < 0.05$).
Fig. 3. Effects of Berberine on the Intestinal MPO Activity. Values are presented as the mean ± S.D. $^*P < 0.05$ means the significance of the uremic and the berberine-treated uremic groups compared with the control group, and $^\#P < 0.05$ means the significance of the berberine-treated uremic group compared with the uremic group, respectively.

**Effects of Berberine on Histopathological Changes** As shown in Fig. 4, remarkable edema and inflammation in the intestinal mucosa and submucosa were observed in the uremic group when compared with those in the control group; while these edema and inflammation were significantly decreased by berberine treatment. Furthermore, as shown in Fig. 5, the Chiu’s scores calculated for assessing intestinal mucosal injury were significantly reduced in the berberine-treated uremic group when compared with those in the uremic group ($p < 0.05$).

Fig. 4. Microscopic changes of the intestinal tissue stained with H&E (×100).
Remarkable edema and inflammation (arrow) in the intestinal mucosa and submucosa were observed in the uremic group when compared with those in the control group; while these edema and inflammation were significantly decreased by berberine treatment.

Fig. 5. Effects of Berberine on the Chiu’s Scores. ND (not detectable). Values are presented as the mean ± S.D. *P < 0.05 means the significance of the berberine-treated uremic group compared with the uremic group.

**Effects of berberine on the Intestinal MDA and SOD Levels** As shown in Fig. 6, the intestinal MDA level in the uremic group was significantly higher than that in the control group (p < 0.05); while berberine treatment significantly reduced the intestinal MDA level in the berberine-treated uremic group when compared with that in the uremic group (p < 0.05), reaching the similar level observed in the control group (p > 0.05). Furthermore, the intestinal SOD activity in the uremic group was significantly increased when compared with that in the control group (p < 0.05), which was further increased in the berberine-treated uremic group (p < 0.05).
Fig. 6. Effects of Berberine on the Intestinal MDA and SOD Levels. Values are expressed as the mean ± S.D. *$P < 0.05$ means the significance of the uremic and the berberine-treated uremic groups compared with the control group, and #$P < 0.05$ means the significance of the berberine-treated uremic group compared with the uremic group, respectively.
DISCUSSION

The results from the present study demonstrated that berberine treatment significantly reduced the serum D-lactate level, intestinal permeability, and intestinal MPO activity in the berberine-treated uremic group. The uremia-associated edema and inflammation in the intestinal mucosa and submucosa observed by light microscope were remarkably reduced by berberine treatment, and the degree of intestinal mucosal injury, as measured by the Chiu’s scores, was also reduced in the berberine-treated uremic group. Furthermore, berberine treatment significantly decreased the intestinal MDA level and increased the intestinal SOD activity, indicating that it modulated the oxidative stress responses in the treated animals.

Uremia often results in intestinal mucosal barrier dysfunction. The damage of intestinal mucosal barrier will lead to intestinal bacterial and endotoxin translocation, further resulting in local and systemic inflammatory responses. It has been suggested that novel treatments against intestinal mucosal barrier damage are needed for the clinical management of uremia in the modern nephrology. Berberine is a traditional herbal, and has been worldwide used for long time to treat various gastrointestinal disorders. Berberine’s protective effects on the intestinal epithelial tight junction and epithelial permeability have been reported in studies with in vivo and in vitro models. However, the therapeutic value of berberine have not yet been investigated in preventing uremia-associated intestinal mucosal barrier dysfunction.

In the present study we used various measurements to systematically assess the effects of berberine on the intestinal mucosal barrier function after uremia. D-lactate
is a metabolic product of bacteria, and its serum level is very low under normal conditions. If the intestinal mucosal barrier is damaged under certain pathological conditions, the intestinal permeability is increased, and the serum D-lactate will increase. Furthermore, FD4, a relatively large molecule, could not pass through the normal intestinal mucosal barrier; if the intestinal permeability is increased under pathological conditions, FD4 will pass through the intestinal mucosal barrier. Therefore, serum D-lactate level and intestinal clearance of FD4 have been considered as sensitive biomarkers to assess the intestinal mucosal barrier function. In the present study, berberine treatment significantly reduced both serum D-lactate level and intestinal clearance of FD4 in the uremic rats. The Chiu’s scores calculated for assessing the degree of intestinal mucosal injury were also significantly decreased by berberine treatment when compared with those in the uremic group. In addition, intestinal inflammation has been considered as an important indicator for the intestinal mucosal barrier function changes. An increase in intestinal inflammation has a positive correlation with the severity of intestinal mucosal barrier damage. In the present study, berberine treatment significantly reduced the intestinal mucosal and submucosal inflammation in histopathological observation, and the MPO activity, a sensitive index to evaluate the extent of inflammatory response in various inflammatory disease, was also significantly decreased by berberine treatment. Of note, in the pilot experiment prior to this study, we also checked the effect of berberine on intestinal mucosal integrity in normal rats, and the results demonstrated that there were not any changes in the intestinal permeability in normal animals that
treated with berberine for 6 weeks, and therefore the possibility has been excluded that berberine can directly affect the intestinal mucosal integrity. Taking together, these results indicated that berberine could reduce intestinal mucosal barrier damage following uremia, which may clinically relevant, i.e., berberine may be a potential treatment to preserve intestinal mucosal barrier function, and further reduce gut-derived complications.

The underlying mechanisms for the protective effects of berberine exerted on uremia-associated intestinal mucosal barrier damage remain unclear. It has been reported that berberine exerts many pharmacological activities such as anti-oxidative, anti-bacterial, and other organ function protective activity.\(^2, 4, 32\) Considering that intestinal oxidative stress injury plays an important role in the pathogenesis of several gastrointestinal diseases and that reducing intestinal oxidative stress could improve various gastrointestinal injury,\(^{33-35}\) we measured some typical biomarkers, such as MDA and SOD in the intestine, for evaluating the status of oxidative stress in the setting of uremia,\(^{36, 37}\). The results revealed that the intestinal MDA level was significantly increased in the uremic group, compared with that in the control group. As an important lipid peroxidation production in the oxidative stress process, an MDA increase may indicate the increased oxidative stress injury followed by uremia.\(^{25}\) SOD, one of the main antioxidant enzymes, could prevent the body from oxidative stress injury.\(^{38}\) In the present study, berberine treatment was shown to significantly increase the intestinal SOD activity, compared with the uremic group. In addition, in the present study, we also checked whether berberine for 6 week-treatment affected
the uremia condition by analyses of blood urea nitrogen and creatinine levels, and the results demonstrated that the uremic group presented similar blood urea nitrogen and creatinine levels when compared with the control group, showing that berberine could not ameliorate the uremia condition by itself. Taken together, we speculate that the berberine’s protective effects on the intestinal mucosal barrier function followed by uremia may be linked to the anti-oxidative activity. The underlying mechanism for this anti-oxidative enzyme increase was not investigated in the present study; but we speculate that there may be a feedback or compensation mechanism for this increase to protect the target organ from oxidative stress injury in uremia progression. Nevertheless, further study is now underway to explore the exact mechanism for the anti-oxidative effect of berberine and the involvement of other pharmacological activities such as anti-bacterial action in the uremia setting.

In summary, the results from the present study demonstrate that berberine could reduce uremia-associated intestinal mucosal barrier damage, which is most likely due to its anti-oxidative activity. These results provide a basis for future clinical investigations to develop berberine as a potential approach to preserve intestinal mucosal barrier function in patients with uremia.

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Conflict of Interest The authors declare no conflict of interest.
REFERENCES


