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ORIGINAL ARTICLE

Rebamipide suppresses diclofenac-induced intestinal permeability *via* mitochondrial protection in mice

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Abstract

AIM: To investigate the protective effect and mechanism of rebamipide on small intestinal permeability induced by diclofenac in mice.

METHODS: Diclofenac (2.5 mg/kg) was administered once daily for 3 d orally. A control group received the vehicle by gavage. Rebamipide (100 mg/kg, 200 mg/kg, 400 mg/kg) was administered intragastrically once a day for 3 d 4 h after diclofenac administration. Intestinal permeability was evaluated by Evans blue and the FITC-dextran method. The ultrastructure of the mucosal barrier was evaluated by transmission electron microscopy (TEM). Mitochondrial function including mitochondrial swelling, mitochondrial membrane potential, mitochondrial nicotinamide adenine dinucleotide-reduced (NADH) levels, succinate dehydrogenase (SDH) and ATPase activities were measured. Small intestinal mucosa was collected for assessment of malondialdehyde (MDA) content and myeloperoxidase (MPO) activity.

RESULTS: Compared with the control group, intestinal permeability was significantly increased in the diclofenac group, which was accompanied by broken tight junctions, and significant increases in MDA content and MPO activity. Rebamipide significantly reduced intestinal permeability, improved inter-cellular tight junctions, and was associated with decreases in intestinal MDA content and MPO activity. At the mitochondrial level, rebamipide increased SDH and ATPase activities, NADH level and decreased mitochondrial swelling.

CONCLUSION: Increased intestinal permeability induced by diclofenac can be attenuated by rebamipide, which partially contributed to the protection of mitochondrial function.

Key words: Intestinal mucosal permeability; Mitochondria; Non-steroid anti-inflammatory drugs; Oxidative damage; Rebamipide; Tight junction

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INTRODUCTION

It is well-known that traditional non-steroid anti-inflammatory drugs (NSAIDs) induce mucosal injuries in the lower gastrointestinal (GI) resulting in serious damage[1-5]. However, the mechanism of NSAIDs-related small intestinal mucosal injury is not yet clear. Bjarnason *et al*[6] suggested a hypothesis for the mechanism involved in the pathogenesis of NSAIDs-related enteropathy which was thought to be a multi-stage process, and included mitochondrial damage, increased intestinal permeability, decreased blood flow, and a reduction in prostaglandins. Several basic studies have supported the idea of increased permeability of the small intestinal mucosa as a central mechanism. Intestinal permeability regulates penetration of substances such as macromolecules, bile acids, bacteria and other intra-lumen toxins through the intestinal epithelial barrier. This may lead to low-grade intestinal inflammation by exposing the mucosa to luminal factors and might be the driving force in converting inflammation into ulcer[7]. Therefore, increased intestinal permeability was usually seen as the early stage of intestinal mucosal damage induced by NSAIDs. Many gastrointestinal inflammatory diseases, such as inflammatory bowel disease, can be detected through intestinal permeability tests, which can be used as an early predictor of relapse[8-11]. However, there are no effective drugs for the treatment of increased intestinal permeability induced by NSAIDs. Although proton pump inhibitors and prostaglandin analogues have a preventive effect in patients with traditional NSAIDs-induced upper GI adverse events, investigations into the preventive effects of drugs on NSAIDs-induced lower GI mucosal injury are inadequate.

Rebamipide is a mucosal protective agent which exerts a protective effect on NSAIDs-induced gastric injury through its antioxidant properties[12]. It was demonstrated that *Helicobacter pylori*-related macromolecular transepithelial transportation was reduced by the administration of rebamipide[13-14], which was attributed to barrier integrity reinforcement. Kishimoto *et al*[15] reported that rebamipide prevented dextran sulfate sodium-induced ulcerative colitis in rats, which was related to mucosal barrier repair. In addition, rebamipide was shown to have a healing effect in a patient with corticosteroid-resistant ulcerative colitis[16]. However, few studies have evaluated the mechanism of rebamipide on intestinal permeability in NSAIDs enteropathy.

The aim of this research was to investigate the preventive effect and mechanism of rebamipide on intestinal permeability in a diclofenac-induced enteropathy model.

MATERIALS AND METHODS

Animals and reagents

Kunming mice, 6-8 wk old weighing 20 ± 2 g were provided by the Laboratory Animal Center of Anhui Medical University. The mice were housed in animal facilities with 50% humidity and a 12:12-h light-dark cycle and fed a standard pellet diet and tap water ad libitum. All experiments were performed in accordance with the institutional and national guidelines for the care and use of laboratory animals and were approved by the Ethics Committee of Anhui Medical University. Rebamipide was purchased from Zhejiang Otsuka Pharmaceutical Corporation, Zhejiang, China. Diclofenac sodium salt, Evans blue, rhodamine 123 and fluorescein isothiocyanate dextran (FITC-D) was purchased from Sigma Co. Acetylcysteine was obtained from Beijing Solarbio Science and Technology Co., Ltd, Beijing, China. Malondialdehyde (MDA), myeloperoxidase (MPO), ATPase and succinate dehydrogenase (SDH) detection kits were bought from Nanjing Jiancheng Institute of Biotechnology, Nanjing, China.

Experimental protocol

Mice were randomly divided into the following five groups; control, diclofenac, rebamipide 100 mg/kg, 200 mg/kg, and 400 mg/kg[15]. Mice were administered diclofenac (2.5 mg/kg)[17] dissolved in 0.2% methylcellulose daily by oral gavage for 3 d, except the control group which received the vehicle. Rebamipide suspended in 0.2% methylcellulose dissolved in saline was dosed intragastrically once a day for 3 d 4 h after diclofenac administration. In the control and diclofenac groups, saline was given orally instead of rebamipide. At the end of the experiment, mice were sacrificed by decapitation, and the small intestine was quickly removed.

Intestinal permeability

Small intestinal permeability was evaluated as previously described[18]. Briefly, mice were anesthetized with ether, the abdomen was opened and a 3 cm proximal portion of the ileum from the ileocecal junction was ligated by silk suture with care to prevent injury to the superior mesenteric vessels. The ileal luminal contents were washed out gently with 4-5 mL of phosphate buffered saline (PBS). The ileocecal end was ligated to prepare the ileal loop (3 cm), and then 0.2 mL of 1.5% (w/v) Evans blue in PBS was injected into the loop. Mice were warmed with an incandescent lamp and left undisturbed for 60 min without any signs of pain. Sixty min later, mice were sacrificed by decapitation. The ileal loop was rapidly dissected out, opened, rinsed with 6 mmol/L acetylcysteine, dried on filter paper at 37 ℃ for 24 h, and then weighed and incubated with 3 mL of formamide at 50 ℃ for 24 h. The amount of dye eluted was estimated using a spectrophotometer at a wavelength of 612 nm[19]. The amount of Evans blue permeating into the intestinal wall was calculated based on the standard curve of Evans blue in formamide.

Intestinal permeability to FITC-D with a molecular mass of 4000 Da was determined using a method previously described by Chen *et al*[20] with a minor modification. Mice were anesthetized and a 5 cm segment of the ileal loop was prepared by ligating the ileum at the 3 cm and 8 cm proximal portions from the ileocecal junction

with care to prevent injury to the superior mesenteric vessels. Then 0.2 mL of PBS (pH 7.4) containing 25 mg/kg FITC-D was injected into the loop. After 30 min, a blood sample (100 μL) was taken by puncture of the portal vein under ether anesthesia and immediately diluted with 1.9 mL of 50 mmol/L Tris (pH 10.3) containing 150 mmol/L sodium chloride. The diluted plasma was centrifuged at 3000 g for 7 min and plasma FITC-D concentrations were determined by a fluorescence spectrophotometer at an excitation wavelength of 485 nm and an emission wavelength of 515 nm[21-22].

Assessment of transmission electron microscopy

For transmission electron microscopy (TEM) assessment, an ileal specimen of about 1 cm in length from the ileocecal junction to the proximal portion was excised with a sharp scalpel and fixed in 2.5% glutaraldehyde for 4 h at 4ºC, followed by fixation in osmic acid and embedding in Epon. Ultrathin sections were examined by a Hitachi TEM to detect ultrastructural injuries.

Intestinal MDA content and MPO activity

The dissected intestine was removed without any fat and mesenteries attached, and subsequently homogenized in physiologic saline for the detection of MDA content and MPO activity using commercial kits following the instruction procedures.

Preparation of liver mitochondrial and determination of mitochondrial functions

Mouse liver mitochondria were isolated as described by Johnson and Lardy[23], as it was exceedingly difficult to obtain a high yield of mitochondria from intestinal tissue[24]. Briefly, the liver was rapidly removed and placed in medium containing 250 mmol/L sucrose, 10 mmol/L Tris and 1 mmol/L EGTA (pH 7.8) at 4 ℃. The tissue was scissor minced and homogenized on ice. The homogenates was centrifuged at 600 *g* for 10 min and the resulting supernatant was centrifuged at 15 000 *g* for 5 min. The resulting mitochondrial pellet was then washed with the same medium without EGTA, and then centrifuged at 15 000 *g* for 5 min. The final mitochondrial suspension contained 5 mg/mL protein determined by Lowry's method.

Determination of mitochondrial membrane potential

Mitochondrial membrane potential (MMP) was evaluated from the uptake of rhodamine 123, which accumulates electrophoretically into energized mitochondrial in response to their negative-inside membrane potential[25]. Briefly, 1800 μL of the phosphate buffer (pH 7.2) containing 250 mmol/L sucrose, 5 mmol/L KH_2PO_4, 3 mmol/L succinate and 0.3 μmol/L rhodamine 123 was added to the cuvette, and the fluorescence was monitored by fluorescence spectrometry with excitation and emission wavelengths of 503 nm and 527 nm, respectively. After 30 s, the mitochondrial suspension (final concentration of 0.5 mg/mL protein) was added, and the fluorescence intensity was recorded continuously at 25 ℃ for 5 min. MMP was expressed by the relative value compared to the baseline intensity.

Measurement of mitochondrial swelling

Mitochondrial swelling was assessed by measuring the changes in absorbance of the suspension at 520 nm (Δ) by spectrophotometry according to Halestrap *et al*[26]. The standard incubation medium for the swelling assay contained 250 mmol/L sucrose, 0.3 mmol/L $CaCl_2$ and 10 mmol/L Tris (pH 7.4). Mitochondria (0.5 mg protein) were suspended in 3.6 mL of phosphate buffer. A quantity of 1.8 mL of this suspension was added to both sample and reference cuvettes and 6 mmol/L succinate was added to the sample cuvette only, and the $A_{520\ nm}$ scanning was started and recorded continuously at 25 ℃ for 10 min. Swelling of mitochondrial was evaluated according to decreased values in absorption at 520 nm.

Determination of mitochondrial nicotinamide adenine dinucleotide-reduced level

The mitochondrial pyridine nucleotide, nicotinamide adenine dinucleotide-reduced (NADH), was monitored by measuring its autofluorescence with excitation and emission wavelengths of 360 nm and 450 nm, respectively, using a fluorescence spectrometer according to Minezaki *et al*[27]. Mitochondria (2 mg protein) were added to 1.8 mL of phosphate buffer containing 6 mmol/L succinate and the autofluorescence of NADH was determined.

Determination of mitochondrial SDH and ATPase activity

The activities of mitochondrial SDH and ATPase were detected using kits as described in the instruction manuals. The quantity of Pi production represented the activity of ATPase and was measured by the active unit mmoL Pi h mg protein. SDH activity was expressed as units/mg.protein.

Statistical analysis

All results are expressed as mean ± SEM. Statistical comparisons were made using the one-way analysis of variance (ANOVA) test. The level of significance was set to a *P*-value of 0.05. All tests were two-sided.

RESULTS

Effect of rebamipide on diclofenac-induced small intestinal permeability in mice

Non-absorbed macromolecules, such as EB and FITC-D, are often used as probes in intestinal permeability tests. The amount of Evans Blue which had permeated into the intestinal wall and the plasma FITC-D concentrations in the diclofenac group were significantly higher than those in the control group ($P < 0.01$, Figure 1). These results indicated that diclofenac damaged the small intestinal mucosal barrier, which resulted in an increase in intestinal permeability. Rebamipide significantly reduced Evans Blue and FITC-D permeation.

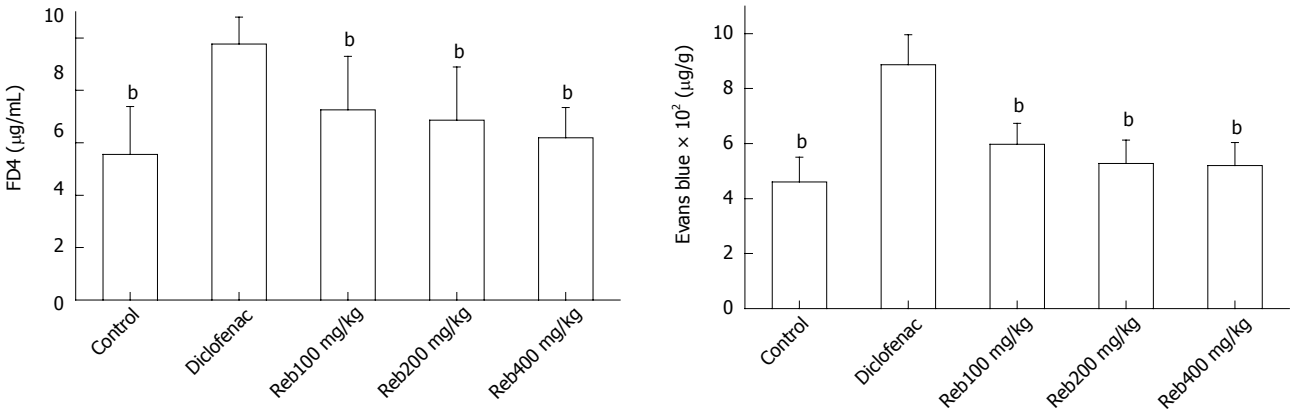

Figure 1 Effects of rebamipide on diclofenac-induced small intestinal permeability in mice. Values are mean ± SEM of data obtained from 8 mice in each group. $^{b}P < 0.01$ compared with the diclofenac group.

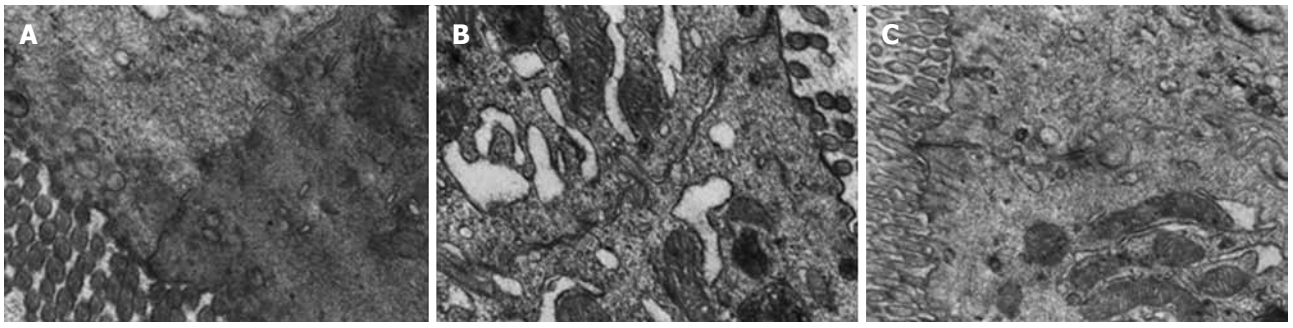

Figure 2 Transmission electron microscopic appearances of diclofenac-induced small intestinal injuries in mice (original magnification × 20 000). A: Control group; B: Diclofenac group; C: Rebamipide group (400 mg/kg). In the diclofenac group, partial deformation of intestinal epithelial cells, intestinal microvillus reduction, disarrangement of the epithelial surface and broader junctional complexes, tight junction opening were seen. Rebamipide group showed regular and intensive microvillus, and ameliorated tight junction when compared with the diclofenac group.

Effects of rebamipide on diclofenac-induced ultrastructure of the intestinal barrier in mice

TEM observations showed that the intestinal mucosa in the diclofenac group (Figure 2B) demonstrated visible injury and a portion of the intestinal epithelial cells were deformed, in addition, a significant reduction in intestinal microvilli, disarrangement of the epithelial surface, broader junctional complexes, and open tight junctions were observed when compared with the control group (Figure 2A). In contrast, the rebamipide group displayed nearly normal intestinal epithelial cells, regular and numerous microvilli, and clearly heightened tightness in the tight junctions (Figure 2C).

Effects of rebamipide on small intestinal MDA content and MPO activity

Compared with the control group, the small intestinal MDA content and MPO activity were significantly increased in the diclofenac group (1.65 ± 0.32 *vs* 0.97 ± 0.28 nmol/mg protein, and 0.236 ± 0.027 *vs* 0.159 ± 0.025 U/g, respectively, both $P < 0.01$), indicating that diclofenac caused oxidative damage and inflammation in small intestinal mucosa. Rebamipide significantly reduced the MDA content and MPO activity demonstrating that the antioxidative and anti-inflammatory effects of rebamipide may be related to the reduction in small intestinal injury (Figure 3).

Effects of rebamipide on diclofenac-induced impairment of liver mitochondrial functions, mitochondrial membrane potential

In the control group, the fluorescence intensity was rapidly reduced within 30 s after the mixture of mitochondria with rhodamine 123, it then began to rise and reached a steady-state within 90 s (Figure 4). Compared with the control group, the reduction in fluorescence intensity in the diclofenac group decreased slightly, indicating that the uptake of rhodamine 123 in mitochondria was smaller in the diclofenac group than in the control group. The reduction in mitochondrial rhodamine 123 uptake was reversed in the rebamipide group indicating that rebamipide significantly improved the impairment of MMP.

Mitochondrial swelling

It has been shown that pH change and high calcium can cause mitochondrial swelling, which is detected by a reduction in mitochondrial absorbance at certain wavelengths. After adding the reaction buffer (at pH 7.2) or 0.3 mmol/L of $CaC1_2$, the mitochondrial absorbance at

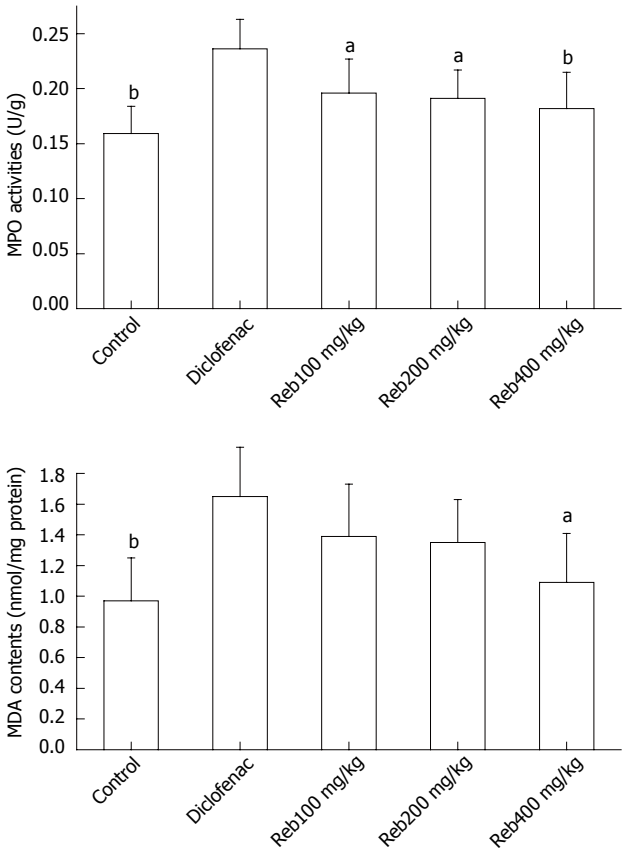

Figure 3 Effects of rebamipide on small intestinal malondialdehyde content and myeloperoxidase activity. Values are mean ± SEM of data obtained from 8 mice in each group. $^{a}P < 0.05$, $^{b}P < 0.01$ compared with the diclofenac group. MDA: Malondialdehyde; MPO: Myeloperoxidase.

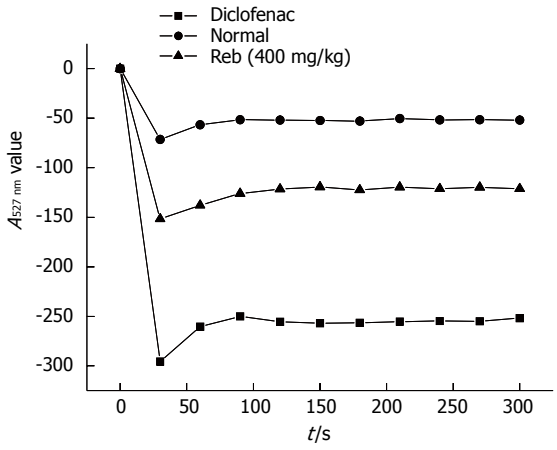

Figure 4 Effects of rebamipide on diclofenac-induced liver mitochondrial membrane potential. In the control group, the fluorescence intensity was reduced within 30 s, and then began to rise and reached a steady state within 90 s. The fluorescence intensity reduction in the diclofenac group was smaller than that in the control group, indicating that the liver mitochondrial membrane potential was decreased by diclofenac administration. The reduction in the fluorescence intensity in the rebamipide group was greater than that in the diclofenac group indicating that rebamipide improved the impairment in mitochondrial function induced by diclofenac.

520 nm declined, indicating mitochondrial swelling due to abnormal osmotic pressure. The extent of absorbance

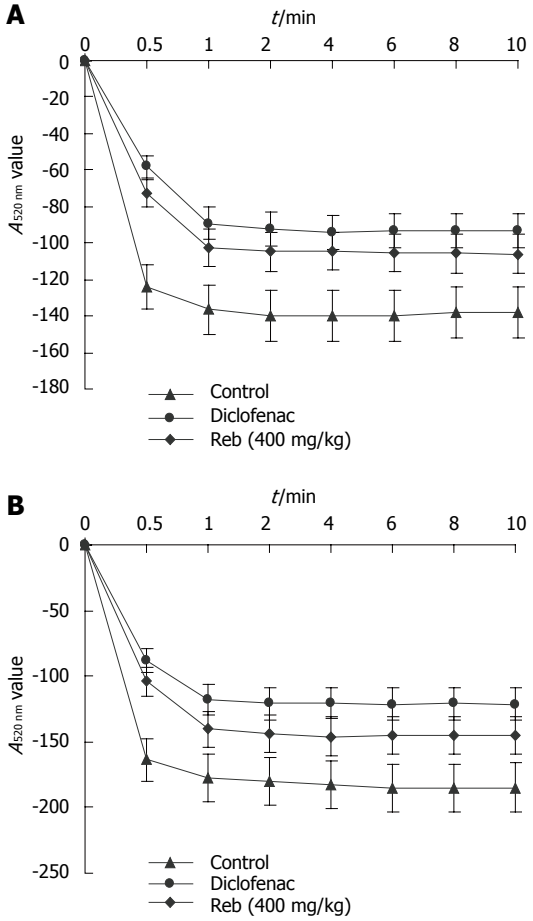

Figure 5 Effects of rebamipide on diclofenac-induced liver mitochondrial swelling in mice. A: After adding the reaction buffer, the absorbance at 520 nm in the control mitochondria declined rapidly. The decrease was smaller in the presence of diclofenac compared with that in the control, demonstrating that liver mitochondrial dysfunction was induced by diclofenac administration. This reduction in absorbance was significantly increased in the presence of rebamipide, indicating that rebamipide improved impaired mitochondrial function; B: After adding 0.3 mmol/L $CaC1_2$ reaction buffer, the absorbance at 520 nm in the control mitochondria declined rapidly, suggesting significant swelling of mitochondria. The decrease was smaller in the presence of diclofenac compared with that in the control, demonstrating that liver mitochondrial dysfunction was induced by diclofenac administration. This reduction was significantly increased in the presence of rebamipide, indicating that rebamipide improved impaired mitochondrial function.

decrease in the diclofenac group was smaller than that in the control group (Figure 5A and B), demonstrating that liver mitochondrial dysfunction was induced by diclofenac administration. Compared with the diclofenac group, the absorbance of rebamipide was significantly increased, indicating that rebamipide improved impaired liver mitochondrial functions.

Mitochondrial NADH levels, SDH and ATPase activities

Compared with the control group, significant decreases in NADH levels, ATPase and SDH activities in liver mitochondrial were seen in the diclofenac group (Figure 6). Rebamipide significantly increased these levels and activities, demonstrating that rebamipide enhanced cellular stress response capacity and maintenance of mitochon-

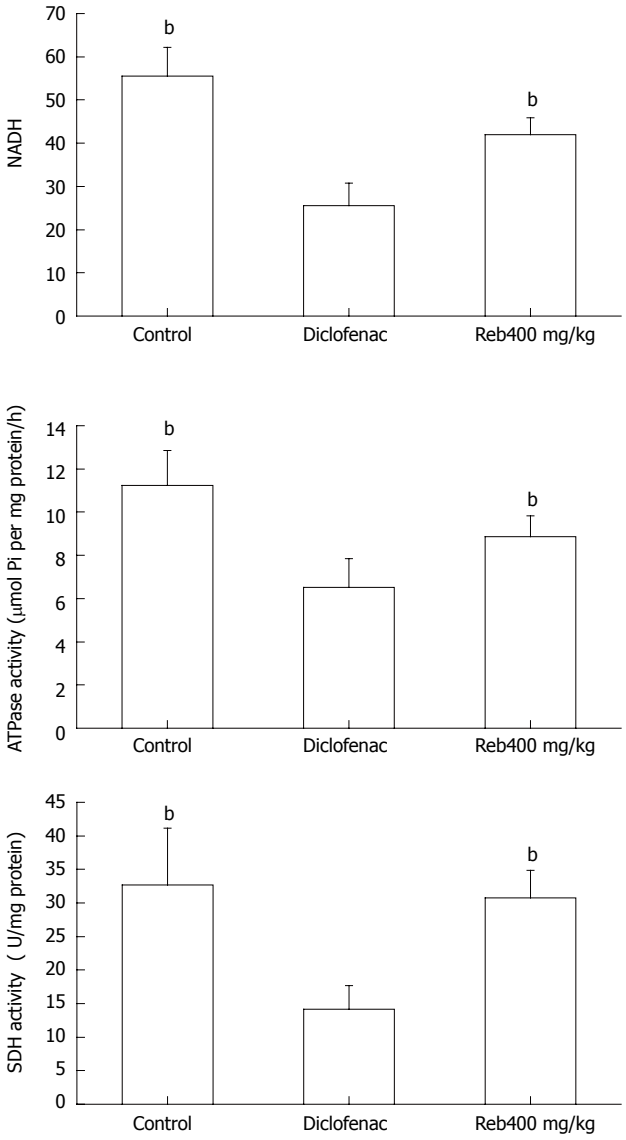

Figure 6 Effects of rebamipide on diclofenac-induced decreases in liver mitochondrial nicotinamide adenine dinucleotide-reduced levels, succinate dehydrogenase and ATPase activities in mice. Values are mean ± SEM of data obtained from 8 mice in each group. $^{b}P < 0.01$ compared with the diclofenac group. SDH: Succinate dehydrogenase; NADH: Nicotinamide adenine dinucleotide-reduced.

drial energy metabolism.

DISCUSSION

Recently, several investigators have shown that the administration of NSAIDs induces small intestinal damage. Bjarnason *et al*[28] demonstrated that in 97 patients who took NSAIDs for more than 2 mo these agents caused enteropathy in 66% of these patients. Sigthorsson *et al*[29-30] demonstrated that intestinal adverse events, defined as hospitalization for intestinal perforation or hemorrhage, occurred in 72% of 286 patients who took 12 different NSAIDs. These trials showed that chronic NSAIDs use not only causes upper GI injury, but also lower GI injury. To date, there are no effective drugs to prevent NSAID-induced lower GI complications.

Bjarnason *et al*[31] advocated that an increase in the permeability of small intestinal mucosa was an important factor in the mechanism of NSAID-induced small intestinal injury. Increased intestinal permeability leads to large molecules contained in food substances, bile acids, pancreatic juice, bacteria and other toxins within the lumen passing through the intestinal epithelial barrier, causing intestinal inflammation and injury. Therefore, the change in small intestinal permeability could directly reflect small intestinal mucosal integrity and barrier function, and help to identify early damage to intestinal mucosa.

In the present study, we investigated intestinal permeability induced by diclofenac and the protective effects of rebamipide. Because non-absorbed macromolecules, such as EB and FITC-D, are often used as probes in intestinal permeability tests, we investigated the amount of Evans Blue permeating into the intestinal wall (460.6 ± 89.7 *vs* 887.1 ± 108.3) and the significant elevation in plasma FITC-D concentrations (5.56 ± 1.82 *vs* 9.77 ± 1.03) (Figure 1). By using TEM technology, visible injury, deformed intestinal epithelial cells, a significant reduction in intestinal microvilli, disarrangement of the epithelial surface, broader junctional complexes, and opened tight junctions were observed in the diclofenac group (Figure 2B). Administration of rebamipide significantly improved small intestinal mucosal permeability and the ultrastructure changes showed more regular and numerous microvilli, and ameliorated tight junctions (Figure 1 and Figure 2C).

On the other hand, our results demonstrated that small intestinal injury induced by diclofenac was associated with increased permeability, ulcers and edema macroscopically, and small intestinal villi damage, partial epithelial denudation, and infiltration of inflammatory cells microscopically. Rebamipide reduced these small intestinal injuries both macroscopically and microscopically. Administration of rebamipide prevented small intestinal injury and histopathologic changes induced by diclofenac (data not shown).

Matysiak *et al*[13], using HT29-19A intestinal epithelial cells, reported that rebamipide may exert its protective effect on gastric mucosa by reinforcing the epithelial barrier. Banan *et al*[32], using human intestinal (Caco-2) cell monolayers, reported that rebamipide prevented oxidation of actin and led to the protection of actin cytoskeleton and intestinal barrier integrity against oxidant insult. These two reports show that rebamipide acts in the management of tight junctions in small intestinal mucosa. It is suggested that rebamipide improved intestinal mucosa integrity by reducing intestinal permeability.

Rebamipide removes oxygen free radicals in epithelial cells. It has been reported to promote healing and prevent relapse of gastric ulcers, which is attributed to inflammatory cell migration into the gastric mucosa[33]. Kim *et al*[34] reported that rebamipide significantly inhibited upper GI mucosal damage induced by NSAIDs in a randomized controlled trial carried out in healthy volun-

teers. In addition, rebamipide was protective in NSAID-induced lower GI injuries. Niwa *et al*[35] reported that taking rebamipide plus diclofenac significantly prevented small intestinal injury compared with placebo plus diclofenac. However, the protective mechanism of rebamipide on intestinal mucosal injury remains unclear.

Many researchers have demonstrated that mitochondrial injury is part of the mechanism of intestinal damage induced by NSAIDs, which also includes dissipating the mitochondrial transmembrane potential, and inducing the mitochondrial permeability transition pore, which liberates cytochrome c, resulting in the caspase cascade and cellular lipid peroxidation, inducing cellular apoptosis. The fluorescent molecular probe, rhodamine 123, can enter the mitochondrial matrix through mitochondrial membrane potential dependent-substrate, and can reflect mitochondrial membrane potential by measuring the changes in optical density[25]. Research has shown that rebamipide exerted a protective effect on mitochondrial membrane stability in gastric epithelial cells[36], however, the mechanism remains unknown. In our study, as it was exceedingly difficult to obtain a high yield of mitochondria from intestinal tissue[24], mouse liver mitochondria were isolated to examine mitochondrial function instead of small intestinal mitochondria according to the method of Somasundaram. We found that rebamipide had a protective role in mitochondrial damage induced by diclofenac, which was related to the maintenance of mitochondrial membrane potential, an improvement in mitochondrial function, enhanced enzyme activities in the mitochondrial respiratory chain and the maintenance of energy metabolism, which indicated that the protective effect of rebamipide may be related to mitochondrial protection.

In conclusion, the administration of diclofenac in mice induced small intestinal mucosal damage and was associated with small intestinal hyperpermeation. Rebamipide showed a preventive effect on hyperpermeation, which was related to mitochondrial protection.

COMMENTS

Background

It is well-known that traditional non-steroid anti-inflammatory drugs (NSAIDs) induce mucosal injury in the lower gastrointestinal resulting in serious damage. However, the mechanism of NSAIDs-related small intestinal mucosal injury has not yet been clearly defined and the available protective drugs for NSAIDs enteropathy are inadequate.

Research frontiers

Many researchers have demonstrated that mitochondrial injury is part of the mechanism of intestinal damage induced by NSAIDs, which also includes dissipating the mitochondrial transmembrane potential, and inducing the mitochondrial permeability transition pore, which liberates cytochrome c, resulting in the caspase cascade and cellular lipid peroxidation, inducing cellular apoptosis.

Innovations and breakthroughs

The administration of diclofenac in mice induced small intestinal mucosal damage and was associated with small intestinal hyperpermeation. Rebamipide showed a preventive effect on the permeability induced by diclofenac, which was related to mitochondrial protection.

Applications

These findings may provide experimental evidence for the clinical application of rebampide in the treatment of NSAIDs enteropathy.

Terminology

NSAIDs enteropathy, mitochondria, rebamipide, intestinal mucosal permeability.

Peer review

This is an interesting paper investigating the effects of NSAIDs on intestinal permeability and the protective effect of rebamipide on diclofenac-induced injury. However, for this kind of pharmacological study, a clear dose-dependency has not been found.

REFERENCES

1 **Bjarnason I**, Zanelli G, Prouse P, Smethurst P, Smith T, Levi S, Gumpel MJ, Levi AJ. Blood and protein loss via small-intestinal inflammation induced by non-steroidal anti-inflammatory drugs. *Lancet* 1987; **2**: 711-714

2 **Bjarnason I**, Hayllar J, MacPherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology* 1993; **104**: 1832-1847

3 **Allison MC**, Howatson AG, Torrance CJ, Lee FD, Russell RI. Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1992; **327**: 749-754

4 **Wallace JL**. Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology* 1997; **112**: 1000-1016

5 **Tibble JA**, Sigthorsson G, Foster R, Scott D, Fagerhol MK, Roseth A, Bjarnason I. High prevalence of NSAID enteropathy as shown by a simple faecal test. *Gut* 1999; **45**: 362-366

6 **Bjarnason I,** MacPherson A, Hollander D. Intestinal permeability: an overview. *Gastroenterol* 1995; **108**: 1566-1581

7 **Knowles RG**. Nitric oxide biochemistry. *Biochem Soc Trans* 1997; **25**: 895-901

8 **Teahon K,** Smethurst P, MacPherson AJ, Levi J, Menzies IS, Bjarnason I. Intestinal permeability in Crohn's disease and its relation to disease activity and relapse following treatment with an elemental diet. *Eur J Gastroenterol Hepatol* 1993; 5: 79-84

9 **May GR**, Sutherland LR, Meddings JB. Is small intestinal permeability really increased in relatives of patients with Crohn's disease? *Gastroenterology* 1993; **104**: 1627-1632

10 **Arnott ID**, Kingstone K, Ghosh S. Abnormal intestinal permeability predicts relapse in inactive Crohn disease. *Scand J Gastroenterol* 2000; **35**: 1163-1169

11 **Wyatt J**, Vogelsang H, Hübl W, Waldhöer T, Lochs H. Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* 1993; **341**: 1437-1439

12 **Hiratsuka T**, Futagami S, Shindo T, Hamamoto T, Ueki N, Suzuki K, Shinji Y, Kusunoki M, Shinoki K, Wada K, Miyake K, Gudis K, Tsukui T, Sakamoto C. Rebamipide reduces indomethacin-induced gastric injury in mice via down-regulation of ICAM-1 expression. *Dig Dis Sci* 2005; **50** Suppl 1: S84-S89

13 **Matysiak-Budnik T**, de Mascarel A, Abely M, Mayo K, Heyman M, Mégraud F. Positive effect of rebamipide on gastric permeability in mice after eradication of Helicobacter felis. *Scand J Gastroenterol* 2000; **35**: 470-475

14 **Matysiak-Budnik T**, Thomas-Collignon A, Mégraud F, Heyman M. Alterations of epithelial permeability by Helicobacter and IL-1beta in vitro: protective effect of rebamipide. *Dig Dis Sci* 2001; **46**: 1558-1566

15 **Kishimoto S**, Haruma K, Tari A, Sakurai K, Nakano M, Nakagawa Y. Rebamipide, an antiulcer drug, prevents DSS-induced colitis formation in rats. *Dig Dis Sci* 2000; **45**: 1608-1616

16 **Makiyama K**, Takeshima F, Kawasaki H, Zea-Iriarte WL. Anti-inflammatory effect of rebamipide enema on proctitis type ulcerative colitis: a novel therapeutic alternative. *Am J Gastroenterol* 2000; **95**: 1838-1839

17 **Tugendreich S**, Pearson CI, Sagartz J, Jarnagin K, Kolaja K. NSAID-induced acute phase response is due to increased intestinal permeability and characterized by early and consistent alterations in hepatic gene expression. *Toxicol Pathol* 2006; **34**: 168-179

18 **Lange S**, Delbro DS, Jennische E. Evans blue permeation of intestinal mucosa in the rat. *Scand J Gastroenterol* 1994; **29**: 38-46

19 **Kitajima S**, Takuma S, Morimoto M. Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. *Exp Anim* 1999; **48**: 137-143

20 **Chen LW**, Hsu CM, Cha MC, Chen JS, Chen SC. Changes in gut mucosal nitric oxide synthase (NOS) activity after thermal injury and its relation with barrier failure. *Shock* 1999; **11**: 104-110

21 **Yang ZL**, Yang LY, Huang GW, Liu HL. Tri-iodothyronine supplement protects gut barrier in septic rats. *World J Gastroenterol* 2003; **9**: 347-350

22 **Wang Q**, Fang CH, Hasselgren PO. Intestinal permeability is reduced and IL-10 levels are increased in septic IL-6 knockout mice. *Am J Physiol Regul Integr Comp Physiol* 2001; **281**: R1013-R1023

23 **Johnson D,** Lardy H. Isolation liver or kidney mitochondtia. *Meth in Enzymol* 1967; **10**: 94-97

24 **Somasundaram S**, Rafi S, Hayllar J, Sigthorsson G, Jacob M, Price AB, Macpherson A, Mahmod T, Scott D, Wrigglesworth JM, Bjarnason I. Mitochondrial damage: a possible mechanism of the "topical" phase of NSAID induced injury to the rat intestine. *Gut* 1997; **41**: 344-353

25 **Emaus RK**, Grunwald R, Lemasters JJ. Rhodamine 123 as a probe of transmembrane potential in isolated rat-liver mitochondria: spectral and metabolic properties. *Biochim Biophys Acta* 1986; **850**: 436-448

26 **Halestrap AP**, Davidson AM. Inhibition of Ca2(+)-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase. *Biochem J* 1990; **268**: 153-160

27 **Minezaki KK**, Suleiman MS, Chapman RA. Changes in mitochondrial function induced in isolated guinea-pig ventricular myocytes by calcium overload. *J Physiol* 1994; **476**: 459-471

28 **Bjarnason I**, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ. Nonsteroidal antiinflammatory drug-induced intestinal inflammation in humans. *Gastroenterology* 1987; **93**: 480-489

29 **Sigthorsson G**, Tibble J, Hayllar J, Menzies I, Macpherson A, Moots R, Scott D, Gumpel MJ, Bjarnason I. Intestinal permeability and inflammation in patients on NSAIDs. *Gut* 1998; **43**: 506-511

30 **Langman MJ**, Morgan L, Worrall A. Use of anti-inflammatory drugs by patients admitted with small or large bowel perforations and haemorrhage. *Br Med J (Clin Res Ed)* 1985; **290**: 347-349

31 **Bjarnason I**, Thjodleifsson B. Gastrointestinal toxicity of non-steroidal anti-inflammatory drugs: the effect of nimesulide compared with naproxen on the human gastrointestinal tract. *Rheumatology* (*Oxford*) 1999; **38** Suppl 1: 24-32

32 **Banan A**, Fitzpatrick L, Zhang Y, Keshavarzian A. OPC-compounds prevent oxidant-induced carbonylation and depolymerization of the F-actin cytoskeleton and intestinal barrier hyperpermeability. *Free Radic Biol Med* 2001; **30**: 287-298

33 **Murakami K**, Okajima K, Uchiba M, Harada N, Johno M, Okabe H, Takatsuki K. Rebamipide attenuates indomethacin-induced gastric mucosal lesion formation by inhibiting activation of leukocytes in rats. *Dig Dis Sci* 1997; **42**: 319-325

34 **Kim HK**, Kim JI, Kim JK, Han JY, Park SH, Choi KY, Chung IS. Preventive effects of rebamipide on NSAID-induced gastric mucosal injury and reduction of gastric mucosal blood flow in healthy volunteers. *Dig Dis Sci* 2007; **52**: 1776-1782

35 **Niwa Y**, Nakamura M, Ohmiya N, Maeda O, Ando T, Itoh A, Hirooka Y, Goto H. Efficacy of rebamipide for diclofenac-induced small-intestinal mucosal injuries in healthy subjects: a prospective, randomized, double-blinded, placebo-controlled, cross-over study. *J Gastroenterol* 2008; **43**: 270-276

36 **Nagano Y**, Matsui H, Muramatsu M, Shimokawa O, Shibahara T, Yanaka A, Nakahara A, Matsuzaki Y, Tanaka N, Nakamura Y. Rebamipide significantly inhibits indomethacin-induced mitochondrial damage, lipid peroxidation, and apoptosis in gastric epithelial RGM-1 cells. *Dig Dis Sci* 2005; **50** Suppl 1: S76-S83

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