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Therapeutic effects of muscovite to non-steroidal anti-inflammatory drugs-induced small intestinal disease

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ABSTRACT

Objective/Background: The ability of non-steroidal anti-inflammatory drugs (NSAIDs) to injure the small intestine has been well established in humans and animals. Muscovite is one kind of natural clay consisting of an insoluble double silicate of aluminum and magnesium. It has been developed and marketed in China for the treatment of gastric diseases. The present study was designed to examine the effects of intragastric treatment of muscovite on the intestinal damage induced by administration of diclofenac in rat.

Methods: Male SD rats were treated with muscovite for 9 days, with concomitant treatment with anti-inflammatory doses of diclofenac on the final 5 days. The anatomical lesion, villous height, the thickness and the section area of small intestine were quantitatively analyzed. The change of ultrastructural organization was observed. Endotoxin level in blood was measured by photometry. Epidermal growth factor was observed by immunohistochemistry.

Results: Muscovite decreased the macroscopic and histologic damage induced by diclofenac in the rat small intestine. In the muscovite group, villous height (139.8 ± 13.2 μm) was higher than which of the model group (86.6 ± 17.1 μm) ($P < 0.05$). The index of the thickness and the section area was higher than model group. LPS level in the portal blood of muscovite (0.84 ± 1.17 EU/ml) was lower than model group (4.52 ± 0.98 EU/ml) ($P < 0.05$). The EFG of muscovite group was higher significantly compared with the model group ($P < 0.05$).

Conclusion: Muscovite can protect the small intestine from the damage induced by diclofenac in the conscious rat. Muscovite can repair NSAID-induced intestinal damage at least in part because of significant lesion in mechanical barrier function and reduction in epidermal growth factor.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for patients with febrile diseases, inflammatory diseases, arthritis, lower back pain, and collagen diseases and to prevent and treat cerebrovascular diseases and ischemic heart diseases. However, it is well known that conventional NSAID use causes serious upper gastrointestinal complications, such as bleeding and perforation. These adverse events have been shown to occur in approximately 1–1.5% of patients within the first 12 months of treatment with NSAIDs (Silverstein et al., 1995, 2000; Schnitzer et al., 2004). It has also been shown that such NSAID-associated serious gastrointestinal complications occur not only in the upper but also in the lower gastrointestinal tract. Recently, the serious problem of NSAID-induced small intestinal damage has become a topic of great interest to gastroenterologists, since capsule endoscopy and double-balloon enteroscopy are available for the detection of small intestinal lesions (Kameda et al., 2008; Watanabe et al., 2008; Sugimori et al., 2008). Graham et al. (2005) observed endoscopically evident small-intestinal mucosal breaks in 71% of patients with rheumatoid arthritis, osteoarthritis or non-specific arthritis who were chronic NSAID users, compared with in 10% of non-NSAID users.

NSAID-induced small-intestinal disease has been shown to be multifactorial, involving a combination of biochemical events that increase epithelial permeability and inflammation. When NSAIDs inhibit COX, they not only exhibit the expected anti-inhibitory effects but also could cause side effects such as gastrointestinal injury (Higuchi et al., 2009). It was recently reported that the long-term use of COX-2 selective inhibitors was linked to an increased risk of cardiovascular events, and thus physicians have reverted to the prescription of conventional NSAIDs (Baron et al., 2006). Therefore, the most logical approach is to consider treatment against the

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gastrointestinal risk posed by conventional NSAIDs. Muscovite is one kind of natural clay consisting of an insoluble double silicate of aluminum and magnesium. It has been developed and marketed in China for the treatment of gastric diseases (Qian et al., 2004; Si et al., 2005). One report has shown that muscovite has a protective effect on gastric mucosa by improving excretion of mucus and synthesis of PGE2 in gastric mucosa (Qian et al., 2004). And another report has shown that rectal administration of muscovite can ameliorate colonic inflammation of TNBS-induced colitis (Chen et al., 2009).

Based on these observations we propose that muscovite might also be used for the treatment of NSAID-induced small-intestinal disease. In the present study, we designed to examine the effects of intragastric treatment of muscovite on the intestinal damage induced by acute administration of diclofenac in the conscious rat.

2. Materials and methods

2.1. Experimental animal

Male Sprague-Dawley rats (aged 8 weeks, weight 200 ± 20 g), purchased from Zhejiang Chinese Medicine University randomly divides into the control group, the model group, Muscovite group. They were housed in a restricted access room with controlled temperature (23 °C) and a light/dark (12 h:12 h) cycle, and allocated in wire mesh cages with a maximum of 4 subjects per cage. Food and water were provided ad libitum. The study received the approval of the local Animal Ethic Committee of Zhejiang Chinese Medicine University.

2.2. Induction of intestinal damage

Enteritis was induced in separate groups ($n = 8$ rats for each group) of unfasted rats, by means of a single intragastric administration of 7.5 mg/kg diclofenac, 2/d, the control groups were given the same dose of saline. The doses of diclofenac were selected to cause intestinal lesions in rats, converting from human dosage. The rats were killed 24 h and 5 days after the treatments and were killed under deep ether anesthesia, and the small intestines were excised for observation and treated with 2% formalin for fixation of the tissue walls.

2.3. Treatment

Muscovite: There is 3 g in a bag which is supplied from Zhejiang Chinese Medicine University. We dissolved muscovite into physiological saline to make muscovite solution (12 mg/ml). The treatment dose of muscovite for the treat group was 120 mg/kg/d. The dose of physiological saline for control group was the same to treat group.

2.4. Groups dividing

Control groups: 8 SD rats were treated with saline for 9 days, with concomitant treatment with anti-inflammatory doses of saline on the final 5 days.

Model groups: 8 SD rats were treated with saline for 9 days, with concomitant treatment with anti-inflammatory doses of diclofenac (7.5 mg/kg diclofenac, 2/d) on the final 5 days.

Muscovite groups: 8 SD rats were treated with muscovite (120 mg/kg/d) for 9 days, with concomitant treatment with anti-inflammatory doses of diclofenac (7.5 mg/kg diclofenac, 2/d) on the final 5 days.

2.5. Macroscopic evaluation

The animals were killed after the administration by under deep ether anesthesia. The abdomen was opened immediately, the stomach and small intestine was removed. The intestine was flushed with physiological saline to clear any residual food particles. The intestine was then opened along its antimesenteric border and the tissue spread out carefully.

The damage of small intestinal mucosal was calculated as anatomical lesion score, using the criteria outlined adapted from Wallace et al. (1992): ulceration: 0 score (nornal appearance); 1 score (focal hyperaemia, no ulcers); 2 scores (ulceration without hyperaemia or bowel wall thickening); 3 scores (ulceration with inflammation at one site); 4 scores (≥two sites of ulceration and inflammation); 5 scores (major sites of damage extending >1 cm along the length of the intestine). Damage extended to >2 cm along the length of the intestine, increase the score by one for each additional cm of damage. Adhesions: 0 score (no adhesions to surrounding tissue); 1 score (minor adhesions (intestine can be separated from other tissues with little effort)); 2 scores (major adhesions).

2.6. Histology

The small intestine was removed from each animal and the first 25 cm of the proximal region, starting from the pylorus and 25 cm away from ileocecal junction, were taken for histology. Histology was carried out on segments of small intestine removed from the rats of each group. Intestinal segments were immediately injected with 10% formalin and left in the same fixative solution. After 30 min, they were opened along the anti-mesenteric border, cleaned of fecal content and fixed in 10% formalin for 24 h. From each intestinal segment six sections were randomly chosen and processed into paraffin. Serial paraffin sections (4 μm) were then prepared and stained with hematoxylin–eosin and PAS for morphological examination. Histologic damage was assessed by two observers, blind to the treatment, according to the scoring system by Chiu scale (Chin et al., 1970). The scale consists of values from 0 to 5, where 0 normal mucosa; 1, development of sub-epithelial (Gruenhagen's) spaces; 2, extension of the sub-epithelial space with moderate epithelial lifting from the lamina propria; 3, extensive epithelial lifting with occasional denuded villi tips; 4, denuded villi with exposed lamina propria and dilated capillaries; and 5, disintegration of the lamina propria, hemorrhage, and ulceration. The Chiu scale were counted from each sample in 20 fields of vision randomly on each slide under a light microscope to quantitatively analyze the height of pile, the thickness and the section area of mucous membrane by Carl Zeiss Imaging Systems.

2.7. Ultrastructure observation

After fixation, specimens were rinsed for 30 min in 0.1 M cacodylate buffer and post-fixed for 1 h at 4 °C in 1% osmium tetroxide. Subsequently, tissue samples were dehydrated by graded ethanol and embedded in EM bed-812 resin. Fragments were cut into 100 Å sections with an Ultramicrotome System 2128 (Ultratome, Bromme, Germany) ultramicrotome and examined in a Hitachi H-600 (Hitachi Ltd., Tokyo, Japan) at 50 kV at a magnification of 35,000×. From each animal an average of 250 consecutive TJ were observed by an observer unaware of the relative treatment, avoiding those next to goblet cells known to have minor resistance. TJ were considered to have increased permeability where the electrondense marker penetrated into the junctional complex and electrondense material was identified on the luminal surface.