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Small Bowel Tissue Concentration of Rebamipide: Study of Two Dosages in Healthy Subjects

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Summary **Non-steroidal anti-inflammatory drug (NSAID)-related small intestinal complications exist, since developed new diagnostic modalities, such as balloon and capsule endoscopies. Some experiments have shown rebamipide to protect from NSAID-induced small intestinal complications. The purpose of this study is to investigate whether the effective concentrations of rebamipide (COR) are present in the small intestine after taking an ordinary clinical dose and double dose of this drug. Twelve healthy male subjects were enrolled. After taking 100 or 200 mg of rebamipide, balloon enteroscopy was performed at 1 and 3 h, and biopsy samples were obtained from the jejunum and the stomach. Venous blood samples were taken simultaneously. Samples were analyzed by high-performance liquid chromatography. The mean COR in the jejunum was higher than 100 μM at 1 h and higher than 10 μM at 3 h in both the 100 and 200 mg groups. Mean COR in the stomach was less than 100 μM at 1 h in the 100 mg group; however it was higher than 100 μM in the 200 mg group. In conclusion, the COR level in the jejunum was sufficient to protect for NSAID-induced gastrointestinal complications.**

Key Words: **rebamipide, local concentration, small intestine, balloon enteroscopy**

Introduction

The use of non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin is common in the treatment of fever and pain. Furthermore, low-dose aspirin is generally used as a prophylaxis in cardiovascular and cerebrovascular events. However, these agents are well-known to cause the adverse event of mucosal damage in the gastrointestinal (GI) tract [1–3]. In recent years, NSAID-related complications have been reported in the stomach and small intestine. Bjarnason *et al.* [4] reported that NSAIDs caused inflammation of the small intestine in 70% of patients receiving long-term administration of these drugs. This inflammation can cause bleeding, protein loss, perforation, and strictures in the small intestine [5–7].

The pathogenesis of small-intestinal damage due to NSAIDs has been partially elucidated. Increased intestinal permeability is thought to be the essential mechanism underlying the translation of biochemical/cellular events of NSAIDs to a tissue reaction; furthermore, translocated bacteria and their degradation products lead to neutrophil infiltration in the small intestine [8]. On the other hand, biochemical actions of NSAIDs, such as cyclooxygenase (COX)-1 inhibition, which cause prostaglandin deficiency [9], damage the small intestine. NSAID-induced gastric mucosal injury can be prevented by the use of proton pump inhibitor [10], misoprostol [11], and rebamipide [12–14], however a drug for the prevention of intestinal complications related to these agents has not been developed until now.

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Rebamipide {2-(4-chlorobenzylamino)-3-[2(1H)-quino-linon-4-yl] propionic acid, Otsuka Pharmaceutical Co., Tokyo, Japan} is a anti-gastric ulcer and gastritis agent. This agent is known to have preventive effects against various acute experimental gastric mucosal lesions and to accelerate the healing of gastric ulcer. Increased mucous secretion [15], enhanced generation of endogenous pro-staglandins [16], suppression of neutrophil function [17], inhibition of inflammatory cytokines [18], and scavenging of oxygen free radicals [19] have been known to be impor-tant effects of rebamipide in the stomach. Likewise, in the mucosa of the small intestine, rebamipide's protective effects have been reported. Banan et al. [20] demonstrated that rebamipide prevented oxidation of actin and lead to the protection of actin cytoskeleton using in vitro human intestinal cell monolayers. Accordingly, they suggested that this agent prevented intestinal hyperpermeability and stabilized human intestinal barrier function. Mizoguchi et al. [21] reported protective effects of rebamipide against indomethacin-induced intestinal damage in rats, and sug-gested that the mechanism underlying its preventive action was due to the scavenging of oxygen free radicals. These pharmacological actions have been confirmed by in vitro experimental studies to require concentration of rebamipide (COR) of more than 1 μM to 100 μM [15–21].

Drug delivery in the human small intestine has not been examined until now because of the difficulty of obtaining small intestinal specimen. Using balloon enteroscopy, it is possible to successfully obtain biopsy samples of the jejunum after taking rebamipide. This is the first report on the delivery of this drug to the human small intestine.

The purpose of this study was to investigate whether an effective COR was achieved in the small intestine after taking an ordinary clinical dose and double dose of this drug by using healthy subjects.

Materials and Methods

Twelve healthy male subjects participated in this study. Written informed consent was obtained from all participants. Their mean age was 22.9 years (range 21–27 years). Each had a normal medical history. The protocol was approved by the Ethics Committee of Shinshu University School of Medicine.

Study design

Study design is shown in Fig. 1. Once entering the study, subjects were randomized through the distribution of sealed envelopes assigning them to either the 100 mg group (n = 6) or the 200 mg group (n = 6). The subjects in both groups were administered with a glass of water (50 ml). Subjects were given a bolus dose of midazolam 0.1 mg/kg intra-venously for sedation. A single balloon enteroscopy (SBE) (Olympus Co., Tokyo, Japan) was performed at 1 and 3 h after administration of rebamipide. Each time, two biopsy samples were gathered from the mucosa at the jejunum (at about 100 cm anal site from the portion of Treitz's ligament), and two biopsy samples were obtained at the large curvature of the antrum using conventional biopsy forceps. When enteroscopy was performed, venous blood samples were simultaneously taken from the subjects in both groups.

Measurement of obtained samples

After the jejunum and gastric mucus adhering to the
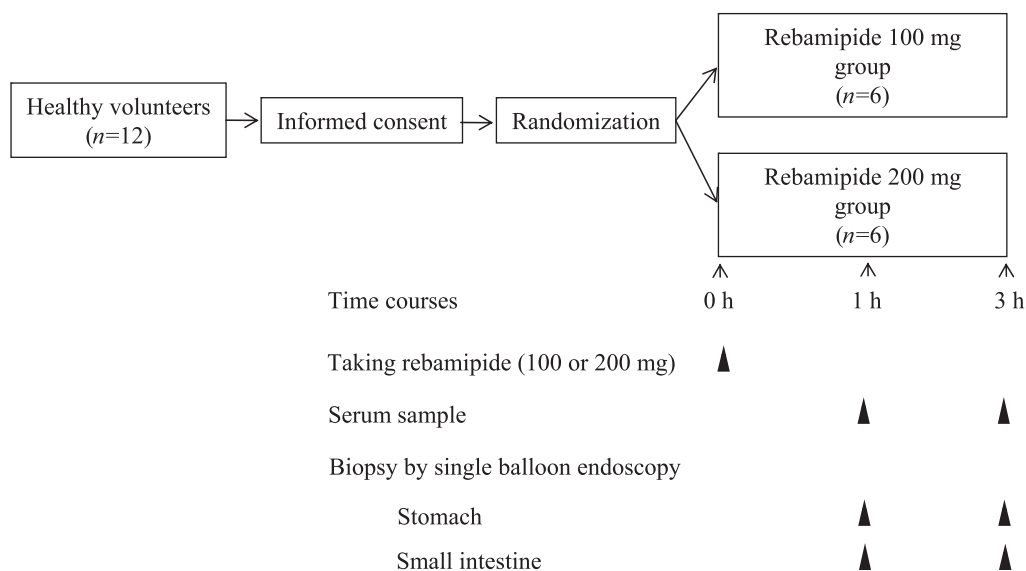

Fig. 1.   Study design.

biopsy specimens was removed using filter paper, rebamipide (attached to the surface well) of collected jejunum and gastric specimens was washed away, and these specimens were immediately placed in preserving tubes and weighed. Biopsy specimens and serum were stored frozen at −20 degrees until being assayed for COR. The COR was determined by high-performance liquid chromatography as reported previously [22–23].

Safety assessment

All symptoms, the incidence of adverse events by examination of balloon endoscopy, and other complications through this study period were observed carefully by endoscopists.

Results

COR in the jejunum

In the 100 mg group, the mean COR level was 146.3 ± 203.1 μM at 1 h after taking rebamipide, and 54.9 ± 47.8 μM at 3 h. In the 200 mg group, the mean COR level was 153.5 ± 183.1 μM at 1 h, and 36.0 ± 43.5 μM at 3 h. The mean COR level in both groups was higher than 100 μM at 1 h, and higher than 1 μM at 3 h (shown in Fig. 2).

COR in the stomach

In the 100 mg group, the mean COR level was 68.3 ± 79.0 μM at 1 h after taking rebamipide, and 22.5 ± 19.2 μM at 3 h. In the 200 mg group, the mean COR level was 173.9 ± 207.5 μM at 1 h, and 25.8 ± 28.0 μM at 3 h. The mean COR level in both group was higher than 1 μM at 1 and 3 h (shown in Fig. 2).

COR in serum

In the 100 mg group, the mean COR level was 0.69 ± 0.220 μM at 1 h after taking rebamipide, and 0.87 ± 0.21 μM at 3 h. In the 200 mg group, the mean COR level was 1.14 ± 0.51 μM at 1 h, and 1.33 ± 0.66 μM at 3 h. The mean COR level was lower than 1 μM at 1 and 3 h in the 100 mg group, and lower than 10 μM in the 200 mg group (shown in Fig. 2). The mean COR level was higher in the 200 mg group than in the 100 mg group.

Safety assessment

The incidence of adverse events and other complications caused by the study drug and examination of endoscopy were not observed throughout the study period.

Discussion

The mean COR levels in the jejunum were higher than 100 μM at 1 h and 1 μM at 3 h after taking rebamipide. These results have shown the possibility of demonstrating actions which rebamipide has, such as enhancing generation of endogenous prostaglandins [16], inhibition of inflammatory cytokines [18], scavenging of oxygen free radicals [19], preventing intestinal hyperpermeability, and stabilized human intestinal barrier function [20]. On the other hand, the COR which was administered by higher dosage of rebamipide compared with indicating dosage for gastric ulcer and gastritis were also measured in this study. However, the mean COR levels in the jejunum were not elevated despite administration of 200 mg of rebamipide. Nakamura *et al.* [24] investigated the localization of the binding sites of rebamipide, 3H-rebamipide by binding radioisotope, in rats with 100% acetic acid-induced gastric
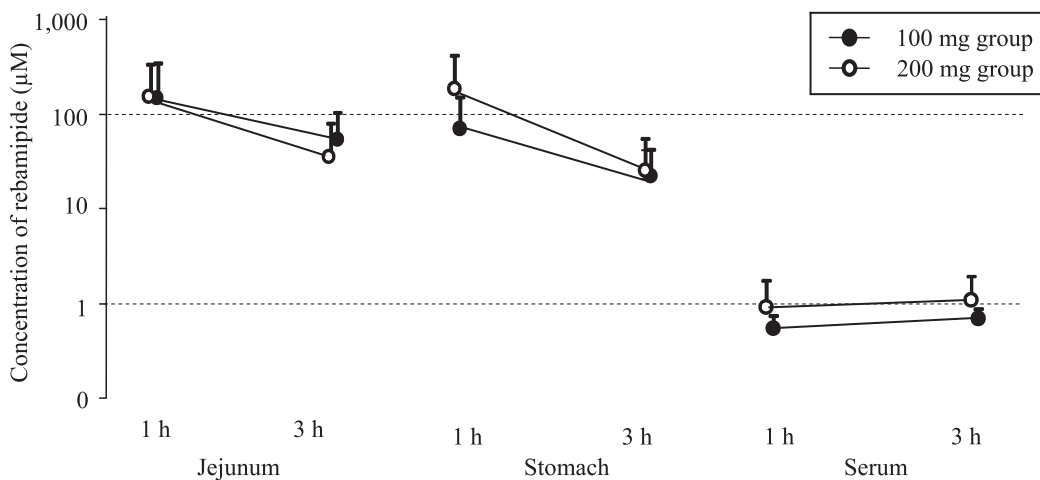

Fig. 2.   Gastric mucosa, small intestine and serum concentration of rebamipide at 1 h and 3 h after administration (mean ± SD, *n* = 6 each). Broken lines show pharmacological actions of rebamipide have been confirmed by *in vitro* experimental studies to require COR of more than 1 μM to 100 μM.

injury. In the control rats, 3H-rebamipide was found to bind to the surface epithelial cells. On the other hand, 3H-rebamipide in the acetic acid-treated group was observed in the lamina propria mucosa. Combination of autoradiography and immune-histochemistry has revealed that iNOS-immunoreactive cells had strong binding of 3H-rebamipide in the acetic acid-treated group. This result may show that rebamipide is induced to i-NOS-expressed sites, but not to sites that do not to express i-NOS. In our study, we did not use any stimulators to induce small intestinal injury, such as NSAIDs. The result of our study showed COR in the jejunum was not elevated despite using a higher dosage of rebamipide. The COR for several organs of rats were reported, localizing in the stomach and small intestine mostly was shown compared with the other organs involving serum levels [25]. The experimental data of this study was limited as different given dosages were not examined. On the other hand, the mean COR levels in the serum were very low compared with the jejunum and the stomach, and increased with the dosage (increased from 100 to 200 mg). This result suggested that the COR presented in the jejunum and the stomach resulted not from systemic circulation to the GI lumen, but from topical.

There are several limitations to this study. First, the effective concentration of rebamipide to various action mechanisms is unknown in basic research and in humans. In addition, the small intestine is an internal organ with wide surface area compared with stomach. Therefore, there is the necessity of investigating high-dose (more than 300 mg/day) rebamipide dosing, in order to realize the preventive effect of rebamipide with NSAID-induced small-intestinal injury. It is necessary to investigate the COR in sufficient dosage of rebamipide to demonstrate efficacy. Secondly, NSAID-induced enteropathy is recognized not only in the jejunum but also in the ileum. However, we could not obtain specimens from the ileum. This was from an ethical perspective in using healthy subjects. In the future, new technology such as a video capsule endoscopy (VCE) with a device for obtaining biopsy specimens or with an elaborate sensor to measure concentrations of drugs will make it possible to study drug delivery in the entire small intestine.

Since developing VCE and balloon endoscopy, so-called "obscure GI bleeding" is not obscure, and the incidence of high frequencies ratio of small-intestinal complications, related to NSAID use, is obvious. The preventive therapeutic strategy for NSAID-induced complications is required not only in the upper GI but in the small intestine and the lower GI. However, there is no drug with efficacy for small intestinal injury. Recently, Niwa *et al.* [26] reported the efficacy of rebamipide in preventing NSAID-induced small-intestinal complications. They found small intestinal mucosal injuries after taking diclofenac in 8 of 10 subjects in the placebo group, but in only 2 of 10 in the rebamipide group

($p = 0.023$). This result may suggest its being one candidate drug for NSAID-induced small intestinal complications.

In conclusion, the COR in the jejunum has fitted in the range which was sufficient to demonstrate protective actions after taking an ordinary clinical dose and double dose, however further study is required concerning COR in the ileum.

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Disclosure

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