Original Article

Nonsteroidal anti-inflammatory drug-induced visible and invisible small intestinal injury

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Permeation of the small intestinal mucosa is a key mechanism in the induction of enteropathy. We investigated the effect of rebamipide in healthy subjects with diclofenac-induced small intestinal damage and permeability. In this crossover study, each treatment period was 1 week with a 4-week washout period. Diclofenac (75 mg/day) and omeprazole (20 mg/day) plus rebamipide (300 mg/day) or placebo were administered. Capsule endoscopy and a sugar permeability test were performed on days 1 and 7 in each period. Ten healthy subjects were enrolled. Small intestinal injuries were observed on day 7 in 6 of 10 subjects in both groups. Urinary excretion of administered lactulose increased from 0.30% to 0.50% of the initial dose during the first treatment period in the placebo group, and from 0.13% to 0.33% in the rebamipide group. Despite recovery from small-intestinal mucosal damage, the increased permeability in both groups resulted in sustained high levels of lactulose (0.50% to 1.06% in the placebo group and 0.33% to 1.12% in the rebamipide group) through the 4-week washout period. Diclofenac administration induced enteropathy and hyperpermeability of the small intestine. The sustained hyperpermeability during the washout period may indicate the presence of invisible fragility.

***Key Words*: permeability, diclofenac, small intestinal damage, rebamipide, healthy subjects**

Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with an increased risk of upper gastrointestinal (GI) complications, which occur in approximately 15–30% of patients.[1] However, obscure GI bleeding (OGIB) remains problematic. Capsule endoscopy (CE), which was developed in the year 2000, has been shown to detect overt causes of OGIB in some cases.[2] Graham *et al.*[3] reported that chronic use of NSAIDs in patients with rheumatoid arthritis (RA) and osteoarthritis (OA) was associated with small-intestinal complications in 71% of cases. However, the mechanism of NSAID-induced small intestinal damage is not clear. Bjarnason *et al.*[4] proposed a possible mechanism to explain this process. Intraluminal factors, including bacteria, play an important role in triggering intestinal damage once the mucosal barrier has been disrupted by prostaglandin (PG) inhibition. Many NSAIDs directly cause mitochondrial disorders, which are attributable to uncoupling of oxidative phosphorylation induced by opening of the mega channel called mitochondrial permeability transition pore on the mitochondrial membrane by NSAIDs.[5] Therefore, permeability in the intestinal membrane increases. Recruitment of neutrophils and their myeloperoxidase activity ultimately induce inflammation and ulceration.[6,7]

Medding *et al.*[8] reported that GI damage can be detected by ingestion of sugars. When excreted in the urine, these sugars could be assigned to 3 categories. The first type, sucrose, is broken down after leaving the stomach. In the second category, lactulose and mannitol pass through the stomach and most of the small bowel before undergoing bacterial degradation. The third type of sugar, sucralose, remains intact during passage through the gut. Therefore, analysis of the types of sugars excreted in the urine can be used to assess the distribution of GI damage.[8] Moreover, Smecual *et al.*[9] reported that an increased ratio of lactulose and mannitol ratio induced hyperpermeation and small intestinal damage in patients taking NSAIDs. The relationship between this lactulose and mannitol ratio and small intestinal injury was also investigated, although no statistically significant association was reported.[10]

In addition to CE, the combination of upper and lower endoscopy is currently being used to image the GI tract. However, these modalities are complex, invasive, and expensive. Therefore, other safe, easy, simple, and cost-effective GI screening procedures are needed. A sugar test may be useful as a screening method for patients with NSAID-induced small intestinal injury.

One of the few therapeutic options available for the prevention and/or treatment of NSAID-induced enteropathy is the use of metronidazole and sulfasalazine.[4,11] In addition, administration of PG analogs prevented NSAID- and aspirin-induced enteropathy.[12,13] Recently, Hawkey *et al.*[14] demonstrated that inhibition of prostaglandin E2 (PGE2) synthesis contributed to NSAID-induced gastroduodenal injury. These studies demonstrate the importance of PGs in the management of chemically induced small intestinal injury. Rebamipide, an endothelial PG inducer, prevented NSAID-induced small intestinal complications in healthy subjects.[14–16] Matysiak-Budnik *et al.*[17] reported that rebamipide increased the integrity of the barrier in an *in vitro* study. Joh *et al.*[18] reported that rebamipide reversed indomethacin-induced changes in epithelial permeability. However, whether rebamipide has the same protective effect against sugar permeability in the small intestinal mucosa remains unclear.

In the present study, we investigated the relationship between urinary sugar excretion and small intestinal injury induced by NSAID use and the preventive effects of rebamipide.

Methods

Study setting. This study was approved by the ethical committee of the Aichi Medical University. Written informed consent was obtained from all participants. This trial was registered on UMIN-CTR, UMIN000003258.

Subjects. Ten healthy subjects (age, 20–60 years), without evidence of either mucosal bleeding or ulcers in the small intestine

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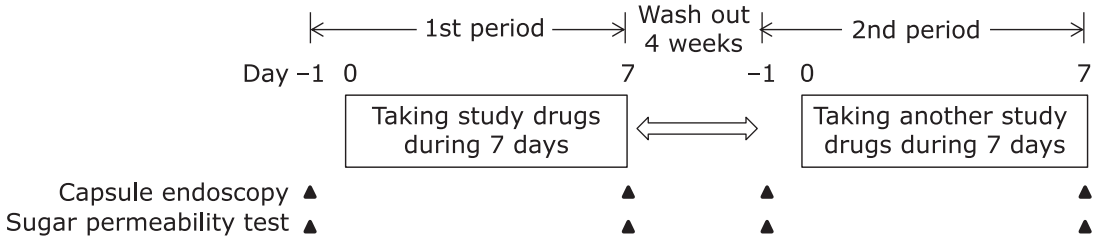

Fig. 1. Study protocol —Crossover design—.

Table 1. The mean of % initial dose of sugars permeation in urine (0 to 5 h)

	1st period				2nd period			
	Jejunum		Ileum		Jejunum		Ileum	
	P	R	P	R	P	R	P	R
1		Present*		(–)	Present		(–)	
2	Present		(–)			(–)		(–)
3		Present		(–)	(–)		(–)	
4	Present		(–)			(–)		(–)
5		(–)		(–)	(–)		(–)	
6	(–)		(–)			Present		(–)
7		Present		Present	(–)		Present	
8	Present		Present*			(–)		(–)
9		Present		Present	Present		(–)	
10	Present*		Present			Present		Present

*patients with bleeding; (–), patients without erosion; P, placebo; R, rebamipide; Present, patients with erosion.

at baseline, were eligible for randomization. Subjects with active gastrointestinal disease, use of anti-ulcer drugs within 2 weeks before start of the study, or prior gastric or intestinal surgery were excluded.

Study design. This was a prospective, randomized, double-blinded, placebo-controlled, cross-over study. The study protocol is shown in Fig. 1. The placebo group was defined as placebo + diclofenac 75 mg + omeprazole 20 mg every day for 7 days. The rebamipide group was defined as rebamipide 300 mg + diclofenac 75 mg + omeprazole 20 mg, also every day for 7 days. The subjects were assigned to either the placebo or rebamipide groups. A 4-week washout period was set. CE (PillCam 2, Given Imaging, Yoqneam, Israel) of the small intestine was performed 4 times, before and after each study period. Sugar permeability tests were performed before and after each study period.

Sugar Permeability tests. All participants ingested 450 ml of water containing 4 sugars as follows: 100 g sucrose (Wako Pure Chem. Ind., Ltd., Osaka, Japan), 5 g lactulose (MONILAC, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), 2 g mannitol (Wako Pure Chem. Ind., Ltd., Osaka, Japan), and sucralose (San-Ei Gen F.F.I., Inc., Toyonaka, Japan) administered as a 2 g capsule to each patient.[6] Subjects presented to the laboratory after an overnight fast and were instructed to ingest the sugar probes. All urine passed over the ensuing 5 h was collected into a pre-weighed container with 5 ml of 10% thymol in isopropanol. The urine was vigorously mixed, the total volume was recorded, and aliquots were rapidly frozen for subsequent transport and analysis. Urinary sucrose, lactulose, and mannitol concentrations were determined by an enzymatic technique. Urinary sucralose was measured by high-performance liquid chromatography-tandem mass spectrometry. The amount of sugar permeation in the urine was expressed as the mean of the total amount from 0 to 5 h.

Endpoints. The study endpoints were to evaluate the effect of rebamipide on small intestinal damage and excretion of sugars in the urine.

Evaluation. Permeability was expressed as a percent of the initial dose based on the following formula: excretion of sugar in urine (mg/ml) × urine volume (ml)/quantity of sugar loading (mg) × 100. Changes in sugar excretion amounts were calculated as differences between the amount at baseline and that on day 7. Small intestinal injury was defined as the presence or absence of erosion, ulcers, and bleeding in each subject on day 7. Erosion was defined as a lesion with slough surrounded by erythema. Ulcer was defined as loss of the villous architecture with a clear breach of the epithelium. Bleeding was defined as presence of blood with or without a detectable lesion. Small intestinal injuries were classified according to their location in the jejunum or ileum. Jejunum injuries were defined as those detected in less than half of the transit time of CE through the entire small intestine, and ileum injuries as those detected later. The preventive effect of rebamipide on small intestinal injury was assessed by CE and compared with the effect of a placebo on day 7. Changes in the excretion of sugars were compared between the rebamipide and placebo groups. Mean urinary sugar excretion calculated as the percent of the initial dose of sugar administered was determined during the first and second periods.

Statistical analysis. Differences in small intestinal injury between groups were evaluated using the chi-squared or Fisher's exact tests. Changes in sugar permeation in each group were evaluated by Wilcoxon rank-sum test. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using JMP ver. 8.0.2 software (SAS Institute, Cary, NC).

Results

Ten healthy subjects, 7 men and 3 women with a median age of 34 (range, 25–53) were enrolled in the study.

Visible changes (CE) in the small bowel. Small intestinal injury with erosion was observed at baseline in 3 of 10 subjects in the rebamipide group, and in 2 of 3 subjects in the second period.

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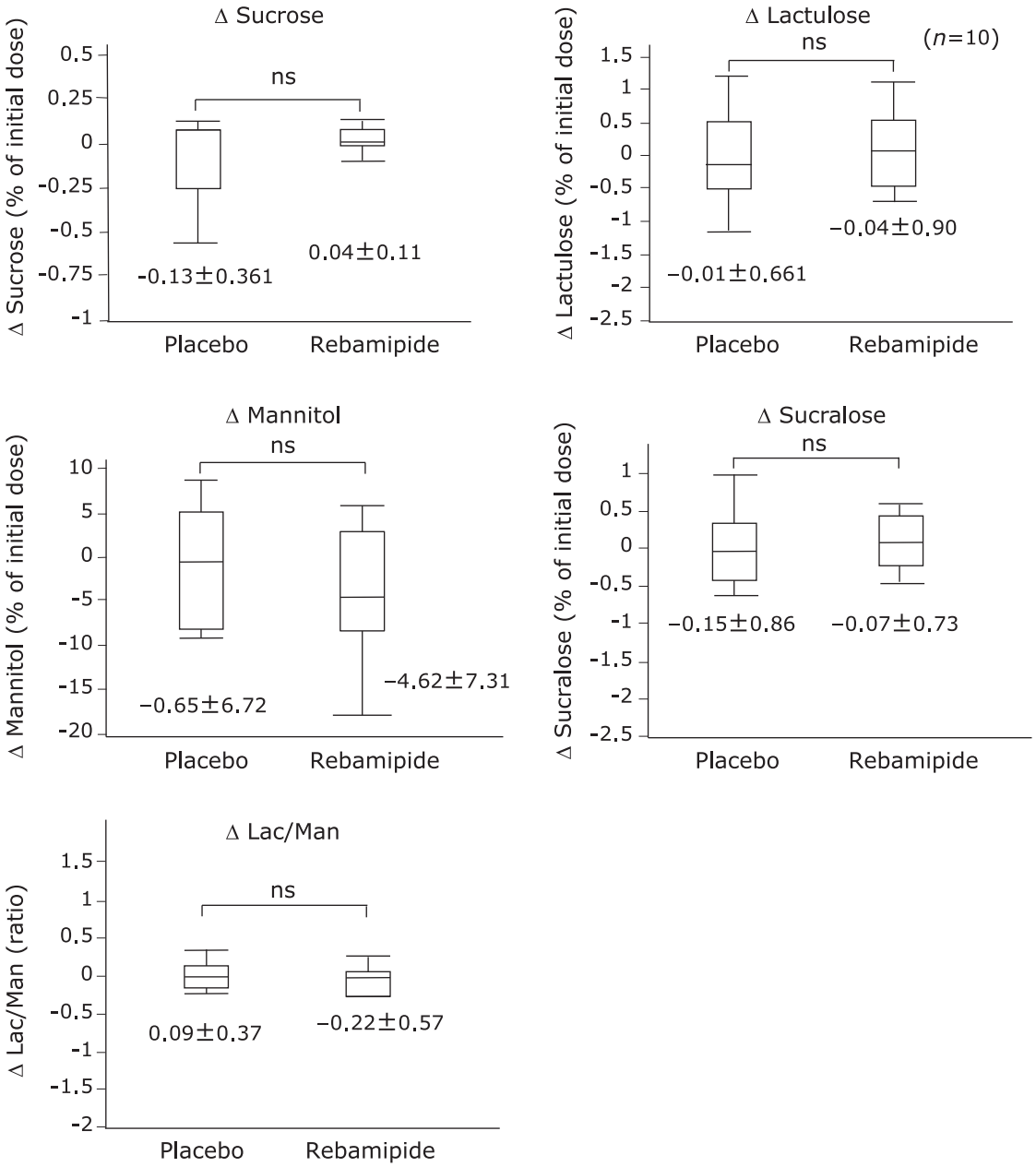

Fig. 2. Changes in sugar excretion. Permeability was described as percent of the initial dose according to the following formula: excretion of sugar in urine (mg/ml) × urine volume (ml)/quantity of sugar loading (mg) × 100. Changes in excretion of sugars were defined as differences between the baseline and day 7 levels.

Small intestinal injuries were observed on day 7 in 6 of 10 subjects in both groups (Table 1). Bleeding was observed in 2 subjects in the placebo group, and in 1 in the rebamipide group. There were no statistically significant differences between the groups. Small intestinal injuries in the jejunum and ileum were the same in the 2 groups, with 6 of 10 subjects showing jejunum damage and 4 showing ileum injury. There were no subjects with ulcers in either of the groups.

Invisible changes (permeability) in the small bowel. Differences in the urinary excretion of all the sugars tested between the rebamipide and placebo groups were not statistically significant (Fig. 2). In the placebo group, lactulose excretion increased from 0.30% to 0.50% of the initial dose during the first period, whereas lactulose/mannitol increased from 0.07% to 0.12%. In the rebamipide group, lactulose increased from 0.13% to 0.33%, sucrose from 0.12% to 0.16%, and lactulose/mannitol from 0.02% to 0.05% of the initial dose during the first period. Sucrose increased from 0.07% to 0.08% of the initial dose during the second period (Table 2).

Discussion

Our hypothesis in this pilot study was that diclofenac use increases the permeability of the small intestinal membrane, and that this effect can be prevented by rebamipide, a PGE2 inducer. However, there were no statistically significant differences in the urinary excretion of either of the sugars tested or in the prevalence of small intestinal injury between the placebo and rebamipide groups. Although the surface area of the small intestine is very large, a dose of 300 mg/day of rebamipide, which is the dose used

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Table 2. The mean of % initial dose of sugars permeation in urine (0 to 5 h)

	1st period			2nd period		
	Baseline	Day 7	Difference	Baseline	Day 7	Difference
Placebo group (n = 5, % initial of dose)						
Sucrose	0.26 ± 0.43	0.08 ± 0.03	−0.18	0.17 ± 0.24	0.09 ± 0.04	−0.08
Lactulose	0.30 ± 0.24	0.50 ± 0.64	0.20	1.06 ± 0.86	0.88 ± 0.70	−0.18
Mannitol	10.84 ± 1.20	9.60 ± 4.60	−1.24	11.77 ± 3.34	9.54 ± 3.03	−2.23
Sucralose	1.07 ± 0.91	0.86 ± 0.29	−0.21	0.72 ± 0.39	0.55 ± 0.22	−0.17
Lac/Man	0.07 ± 0.06	0.12 ± 0.14	0.05	0.26 ± 0.23	0.23 ± 0.15	−0.03
Rebamipide group (n = 5, % initial of dose)						
Sucrose	0.12 ± 0.09	0.16 ± 0.19	0.04	0.07 ± 0.02	0.08 ± 0.06	0.01
Lactulose	0.13 ± 0.18	0.33 ± 0.64	0.20	1.12 ± 1.42	0.91 ± 0.73	−0.21
Mannitol	13.30 ± 8.80	9.32 ± 7.54	−3.08	11.73 ± 4.09	10.55 ± 2.38	−1.18
Sucralose	0.87 ± 0.85	0.46 ± 0.38	−0.41	0.90 ± 0.13	0.87 ± 0.40	−0.03
Lac/Man	0.02 ± 0.03	0.05 ± 0.07	0.03	0.24 ± 0.27	0.22 ± 0.16	−0.02

The value of sucrose, lactulose, mannitol, and sucralose were shown by % of initial dose. Lac/Man was lactulose/mannitol. The value of sucrose, lactulose, mannitol, and sucralose were described by mean ± SD.

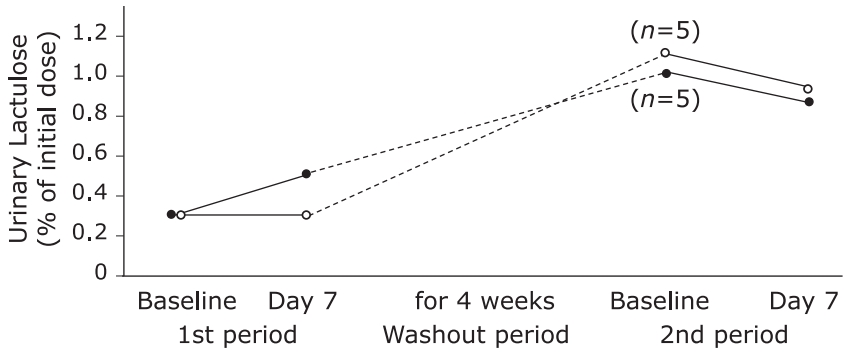

Fig. 3. Time course of lactulose excretion in urine. Closed circles represent the placebo group, and open circles represent the rebamipide group. Diclofenac use is associated with a linear increase of lactulose urinary excretion level during the washout period despite the recovery from small intestinal injury.

for stomach ulcers, was used in this study. Therefore, a higher dose of rebamipide may be necessary to treat small intestinal damage.

The separation between first and second periods is an issue of concern. Diclofenac-related small intestinal injury during the first period was observed in 70% of subjects, and only lactulose and lactulose/mannitol excretion in the urine were increased during the first period in both groups. We have confirmed that sugar permeation into the urine increases in patients with Crohn's disease and ulcerative colitis as measured by our sugar permeability test (inhouse data). Smecuol *et al.*[9] reported an increase in the ratio of lactulose and mannitol after the administration of indomethacin (75 mg for 2 days). Furthermore, Smecuol *et al.*[10] reported that the ratio of lactulose and mannitol ratio increased in patients receiving enteric-coated aspirin for 14 days. These previous reports are in agreement with our results.

However, diclofenac-related small intestinal injury was observed in 40% of subjects during the second period, whereas lactulose and lactulose/mannitol excretion in the urine was not increased in either of the groups during the second period. This could be attributed to the fact that the baseline level of lactulose excretion in the urine during second period was not recovered to the baseline level observed during the first period. Diclofenac use resulted in a linear increase of lactulose urinary excretion level during the washout period despite the recovery from small intestinal injury (Fig. 3). To date, no study has evaluated the permeability of the small intestine using a design similar to that of the present study, which included a 4-week washout period and re-administration of NSAIDs. Intestinal injury evaluated using CE, which assesses visible damage, indicates short-term recovery. However, the permeability of the mucosal membrane, which reflects invisible damage, might indicate a long-term recovery to baseline level than visible damage. The mechanism of increased intestinal permeability might lead to low-grade intestinal inflammation by exposing the mucosa to luminal factors (bile, bacteria, etc.), while the concomitant, predominantly systematically mediated, inhibition of cyclooxygenase appears to be the driving force in converting the inflammation to ulcers.[19] This sequence may result in a time lag between hyperpermeation and small intestinal damage.

The prevalence of total small intestinal injury in the second period was lower than that of the first period. Lipscomb *et al.*[20] reported that upper GI injury associated with NSAID use frequently resolves despite its continuous intake, which could be related to a process of adaptation. Their report addressed upper GI injury, whereas our study was concerned with lower GI damage. There are few reports on adaptation in lower GI injury.

In our study, the prevalence of total small intestinal injury in the ileum was higher than that of the jejunum. Only lactulose excretion in the urine increased in the first period. The increase of lactulose excretion might be associated with the prevalence of erosion in the jejunum, because lactulose was digested by enteric bacteria.

This study had several limitations, including the small sample size and short study period. It is not clear whether a 4-week washout period is appropriate to evaluate small intestinal invisible damage. Furthermore, the permeability of the small intestine

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should be assessed at earlier time points than at 7 days, such as after 2 or 3 days. Therefore, future studies should assess permeability at several time points, including the early phase. We observed a low incidence of small intestinal injury induced by diclofenac at 75 mg/day. Maiden *et al.*[21] reported that small intestinal changes were observed in 68% of subjects, with more than one-third having discrete mucosal breaks (erosive-ulcerative damage), and increased calprotectin levels were observed in 75% of subjects after they took diclofenac at 150 mg for 14 days. Thus, the dose of diclofenac must be increased to 150 mg. Moreover, changing the type of NSAIDs to aspirin should be considered in future studies. Endo *et al.*[22] reported that 95.5% of chronic aspirin users (>3 months) had some small bowel mucosal injury.

In conclusion, measurement of urinary lactulose excretion could help determine NSAID-induced small intestinal mucosal damage. The increase in the permeability of the small intestinal mucosa continued during NSAID withdrawal, despite recovery from small intestinal injuries. Our results suggest that the consequences of the long-term use of NSAIDs are an issue of concern.

Abbreviations

CE	capsule endoscopy
GI	gastrointestinal
NSAIDs	nonsteroidal anti-inflammatory drugs
OA	osteoarthritis
OGIB	obscure GI bleeding
PG	prostaglandin
RA	rheumatoid arthritis

Conflict of Interest

No potential conflicts of interest were disclosed.

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