Leptin acts as a growth factor for colorectal tumours at stages subsequent to tumour initiation in murine colon carcinogenesis

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ORIGINAL ARTICLE

QUANTITATIVE ANALYSIS OF LOW-DOSE ASPIRIN-ASSOCIATED SMALL BOWEL INJURY USING A CAPSULE ENDOSCOPY SCORING INDEX

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Aim: The major limitation of capsule endoscopy (CE) has been the lack of a standardized and validated severity scale for mucosal injury. The aim of the present study was to verify the usefulness of quantifying small bowel mucosal changes associated with giving low-dose aspirin (LDA) using a CE scoring index.

Methods: The CE score for small bowel mucosal injury was investigated to evaluate the severity of mucosal injury. Healthy volunteers and patients suspected of having small bowel disease were recruited for this study. The short-term LDA group (V + S-LDA group) consisted of volunteers who took low-dose aspirin for 14 days; this group was then compared with healthy volunteers who did not receive LDA treatment (V-Control group). The long-term LDA group (L-LDA group) consisted of patients with at least a 3-month history of daily LDA use; this group was compared with non-users of LDA (P-Control group).

Results: The CE score was significantly higher in the V + S-LDA group than in the V-Control group. In the V-Control group, almost all the subjects were categorized as exhibiting a 'normal' change. 'Mild' changes were observed significantly more frequently in the V + S-LDA group than in the V-Control group. The CE score was significantly higher in the L-LDA group than in the P-Control group. 'Mild' or 'moderate or severe' changes were observed significantly more frequently in the L-LDA group than in the P-Control group.

Conclusion: The CE scoring system was useful for evaluating LDA-associated small bowel mucosal disease activity and for objectively scoring the small bowel inflammatory disease state.

Key words: capsule endoscopy, capsule endoscopy score, low-dose aspirin, mucosal injury, small bowel.

INTRODUCTION

With the advent of capsule endoscopy (CE), small bowel mucosal lesions can now be directly visualized.[1] Despite the great utility of CE,[2,3] several limitations to evaluating small bowel mucosal inflammation using this technology exist. First, the definitions of CE findings have not been standardized, especially the differentiation of ulcers from mucosal breaks, erosions, aphthae and other terms that indicate similar findings. Second, the discrimination of normal small bowel and disease states is unclear. The ability of CE to visualize the mucosa makes it possible to observe subtle redness and erosions in the small bowel, but the clinical significance of these inflammatory mucosal changes remains controversial. Finally, the precise size, number and distribution of inflammatory mucosal lesions can be difficult to judge using CE because CE images are generally close-up views and repeated observations are often impossible. Consequently, inflammatory small bowel mucosal changes are evaluated in a manner similar to that used for evaluating gastric mucosal injuries. Thus, the major limitation of CE has been the lack of a standardized and validated severity scale for mucosal injury.

To date, CE has revealed numerous inflammatory lesions and has shed light on small bowel mucosal injury induced by non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin.[4–11] Despite these investigations, a standardized and validated severity scale of NSAIDs/aspirin-associated mucosal injury has not yet been established. Recently, Gralnek *et al.*[12] reported a new scoring index (CE score) to assess mucosal inflammatory diseases detected in the small bowel during CE examinations. This score can quantify mucosal changes associated with any inflammatory process. There has been, however, no study analyzing NSAIDs/aspirin-associated mucosal injuries using this score.

The aim of this CE study was to verify the usefulness of quantifying small bowel mucosal changes associated with giving low-dose aspirin (LDA) using a CE scoring index.

METHODS

We retrospectively reviewed the CE findings for short-term (2 weeks) and long-term (>3 months) LDA users who underwent CE between September 2007 and July 2009 at a single institution (Yokohama City University Hospital). This study

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was conducted in accordance with the Declaration of Helsinki. Permission was granted by the Ethics Committee of Yokohama City University Hospital, Yokohama, Japan.

Patients

Healthy volunteers and patients suspected of having small bowel disease were recruited for this study.

Volunteer study

All volunteers had a normal physical examination and laboratory results. None of the volunteers had taken NSAIDs within the last 3 months or had a history of NSAID hypersensitivity, anti-ulcer medication use (i.e. histamine H2 receptor antagonists, proton pump inhibitors, or misoprostol), chronic alcohol consumption (>20 g alcohol/day), abdominal surgery, or any serious central nervous system, psychiatric, cardiovascular, respiratory, or gastrointestinal disease. The volunteers were divided into two groups as follows: volunteer + short-term LDA group (V + S-LDA group, $n = 13$), healthy volunteers who took LDA (enteric coated aspirin, 100 mg/day) for 14 days; and volunteer control group (V-Control group, $n = 21$), healthy volunteers who did not undergo LDA treatment. The V + S-LDA group was compared with the V-Control group.

Patient study

All the patients underwent total colonoscopy, gastroscopy, and computed tomography (CT) examinations prior to undergoing CE. Written informed consent to undergo the CE procedure was obtained from all the patients. No contraindications, such as suspected small bowel obstruction, known small bowel stricture, swallowing disorder, pacemaker implantation, or pregnancy, existed. None of the patients had a history of abdominal surgery or radiation therapy for the abdomen. None of the patients had taken NSAIDs, misoprostol, sulfasalazine, or antibiotics within the 6 months prior to the study, and none of the patients had a history of gastrointestinal disease or bowel resection. The patients were divided into two groups as follows: long-term LDA group (L-LDA group, $n = 38$), patients who had at least a 3-month history of daily LDA use for the prevention of recurrent myocardial infarction, valvular heart disease, transient ischemic attack, or angina pectoris; and patient control group (P-Control group, $n = 63$), patients with no history of LDA use who were suspected of having small bowel disease and underwent CE. The L-LDA group was compared with the P-Control group.

Capsule endoscopy procedure

All the videos were reviewed using the PillCam SB capsule endoscopy system (Given Imaging Ltd, Yoqneam, Israel). Capsule endoscopy was carried out after a 12-h fasting period. Fluid and light meals were allowed 2 and 4 h after capsule swallowing. The subjects were free to leave the hospital, with instructions to return within the 8-h study period to have the data recorder removed. The recorded digital information was downloaded from the recorder into the computer and the images were analyzed using the proprietary RAPID software.

Data analysis

Two independent investigators (H.E and K.H.) who were blinded as to whether the subjects had received aspirin, separately reviewed each of the capsule endoscopy examinations.

We investigated the CE score[12] for small bowel mucosal inflammatory changes to evaluate the severity of mucosal injury. This scoring index is based on three capsule endoscopic variables: villous appearance, ulceration and stenosis (Table 1). Mucosal inflammatory changes in villous appearance and ulceration were assessed in tertiles, dividing the small bowel transit time into three equal time allotments. The stenosis evaluation was done for one entire study. The total score was the sum of the highest tertile score plus the stenosis score. The results were classified into three categories according to the final numerical score: normal or clinically insignificant change (<135), mild change (between 135 and 790), and moderate or severe change (≥790). This scoring index is included in the new version of the capsule endoscopy software (RAPID5 Access; Given Imaging Ltd).

In addition, we assessed the classification of the CE findings, including red spots/petechiae, denuded area (loss of villi), mucosal breaks, scars and strictures. The term 'mucosal breaks' has been used in many previous studies[5–9] and has been defined as mucosal erosions and/or ulcers, both representing discrete lesions with central pallor, surrounding hyperemia, and a loss of villi.

The rate of the successful arrival of the capsule in the cecum was assessed using the capsule endoscopic images. The gastric transit time was defined as the time taken from the first gastric image to the first duodenal image. The small bowel transit time was defined as the elapsed time between the first duodenal image and the first cecal image.

Statistical analysis

Results are presented as the mean or median (± standard deviation or range) for quantitative data and the frequency (percentage) for categorical data. Data were analyzed using a chi-squared test or a Fisher's exact test. For univariate com-

Table 1. Parameters and weightings for the capsule endoscopy scoring index[12]

Parameters	Number	Longitudinal extent	Descriptors
Villous appearance	Normal	Short segment	Single
	Edematous	Long segment	Patchy
		Whole tertile	Diffuse
Ulcer	None	Short segment	<1/4
	Single	Long segment	1/4–1/2
	Few	Whole tertile	>1/2
	Multiple		
Stenosis	None	Ulcerated	Traversed
	Single	Non-ulcerated	Traversed
	Multiple		

parisons between the groups, a *t*-test or Mann–Whitney *U*-test was used, as appropriate. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

All the examinations were carried out without complications. None of the subjects developed any symptoms during the examination. The demographic data, indications for CE, and CE transit time are presented in Tables 2 and 3. There was a significant difference in the mean age between the P-Control group (mean, 55.6 years; range 18–90 years) and the L-LDA group (mean, 70.1 years; range 19–85 years; $P < 0.001$).

Table 2. Demographic data and capsule endoscopy transit time of the volunteers

	V-Control	V + S-LDA
No. volunteers	21	13
Sex (M/F)	19/2	12/1
Mean age, years	31.8 (range 21–39)	32.2 (range 25–39)
Median duration of aspirin	–	2 weeks
Median GTT, min	33.0 (range 9–55)	29.0 (range 8–241)
Median SBTT, min	203.0 (range 135–439)	232.0 (range 125–309)
Cecal completion rate, %	100	100

GTT, gastric transit time; LDA, low-dose aspirin; SBTT, small bowel transit time; V-Control group, healthy volunteers who did not receive LDA treatment; V + S-LDA group, volunteers who took low-dose aspirin for 14 days.

Table 3. Demographic data, indications for capsule endoscopy, and capsule endoscopy transit time of the patients

	P-Control	L-LDA
No. patients	63	38
Sex (M/F)	47/16	23/15
Mean age, years	55.6 (range 18–90)	70.1* (range 19–85)
Median duration of aspirin	–	21.0 months
Indications for capsule endoscopy		
Obscure gastrointestinal bleeding	49 (77.8%)	35 (92.1%)
Abdominal pain	3 (4.8%)	2 (5.3%)
Suspected small bowel tumor	5 (7.9%)	0 (0%)
Others	6 (9.5%)	1 (2.6%)
Median GTT, min	24.0 (range 1–218)	22.5 (range 3–114)
Median SBTT, min	247.0 (range 21–449)	278.0 (range 80–453)
Cecal completion rate, %	76.2	81.6

*$P < 0.01$ compared with the Control group.

GTT, gastric transit time; LDA, low-dose aspirin; L-LDA group, long-term LDA group; P-Control group, non-users of LDA; SBTT, small bowel transit time.

CE findings for each group

The CE findings are shown in Tables 4 and 5. Red spots/petechiae were significantly more common in the V + S-LDA group than in the V-Control group ($P = 0.025$). The percentage of subjects with mucosal breaks was higher in the V + S-LDA group than in the V-Control group, but the difference did not reach statistical significance ($P = 0.059$) (Table 4). The proportion of patients with denuded areas was significantly different between the P-Control group and the L-LDA group ($P = 0.012$) (Table 5). Furthermore, mucosal breaks were significantly more common in the L-LDA group than in the P-Control group ($P = 0.003$) (Table 5). Representative images of mucosal breaks in the V + S-LDA group and in the L-LDA group are shown in Figure 1.

CE score for small bowel mucosal inflammatory change associated with LDA

The CE scores for small bowel mucosal inflammatory change are shown in Figure 2. The median score of the small bowel mucosal inflammatory change was significantly higher in the V + S-LDA group (112.0; range, 0–393) than in the V-Control group (8.0; range, 0–135; $P = 0.001$) (Fig. 2). In the V-Control group, all the volunteers except one subject were categorized as normal or as having clinically insignificant changes (score < 135) (Fig. 3). However, in the V + S-LDA group, five

Table 4. Comparison of capsule endoscopy findings between the two volunteer groups

	V-Control ($n = 21$)	V + S-LDA ($n = 13$)	*P*-value
	n (%)		
Red spots/petechiae	4 (19.0)	8 (61.5)	0.025
Denuded areas	2 (9.5)	5 (38.5)	0.079
Mucosal breaks	1 (4.8)	4 (30.8)	0.059
Scars	0 (0)	0 (0)	>0.999
Strictures	0 (0)	0 (0)	>0.999

LDA, low-dose aspirin; V-Control group, healthy volunteers who did not receive LDA treatment; V + S-LDA group, volunteers who took low-dose aspirin for 14 days.

Table 5. Comparison of capsule endoscopy findings between the two patient groups

	P-Control ($n = 63$)	L-LDA ($n = 38$)	*P*-value
	n (%)		
Red spots/petechiae	30 (47.6)	25 (65.8)	0.076
Denuded areas	12 (19.0)	16 (42.1)	0.012
Mucosal breaks	24 (38.1)	26 (68.4)	0.003
Scars	2 (3.2)	2 (5.3)	0.630
Strictures	0 (0)	2 (5.3)	0.139

LDA, low-dose aspirin; L-LDA group, long-term LDA group; P-Control group, non-users of LDA.

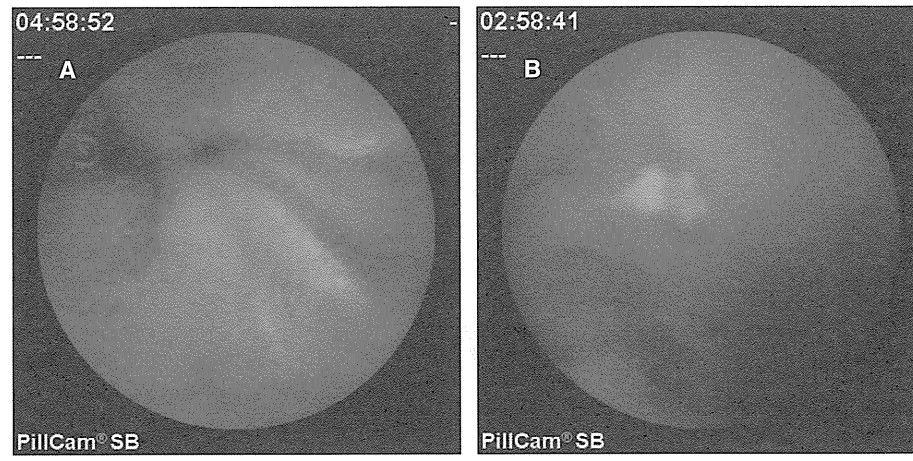

Fig. 1. Capsule endoscopic appearance of a small bowel mucosal break with surrounding erythema. (a) A jejunal small mucosal break in the S-LDA group; (b) an irregular ileal mucosal break in the L-LDA group.

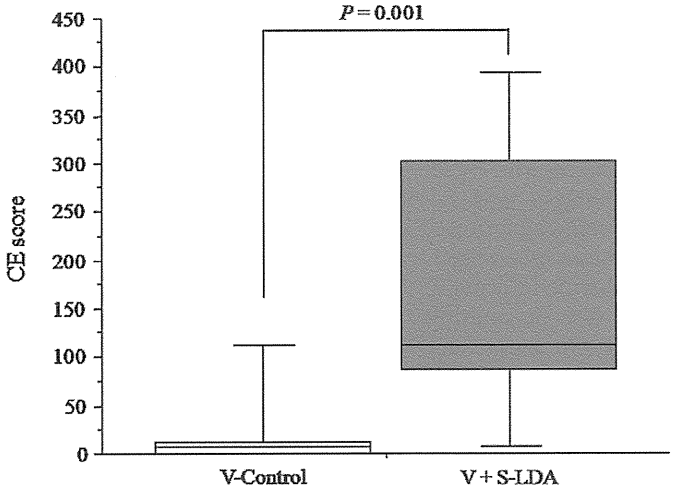

Fig. 2. Capsule endoscopy (CE) scores for small bowel mucosal inflammatory changes in the V-Control group and the V + S-LDA group. Box plots show the interquartile range (box), median (thick line) and range (thin lines) of the capsule endoscopy scores. LDA, low-dose aspirin; V-Control group, healthy volunteers who did not receive LDA treatment; V + S-LDA group, volunteers who took low-dose aspirin for 14 days.

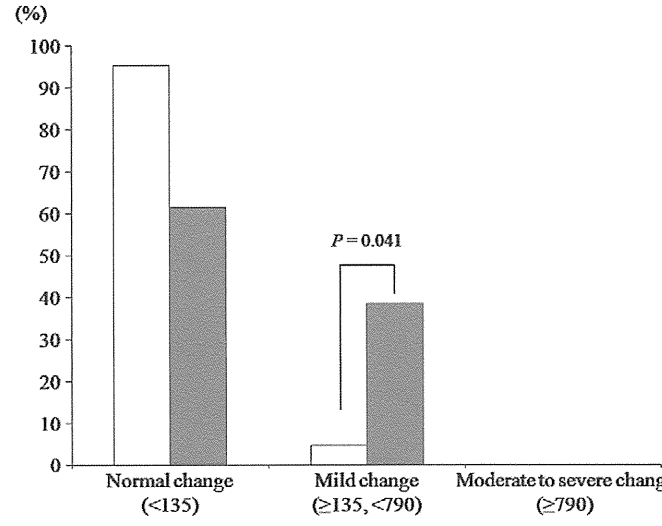

Fig. 3. Comparison of the severity of small bowel mucosal inflammatory changes between the V-Control group and the V + S-LDA group. The severity was classified into three categories according to the capsule endoscopy score: normal (<135), mild change (between 135 and 790), and moderate or severe change (≥790). LDA, low-dose aspirin; □, V-Control group, healthy volunteers who did not receive LDA treatment; ■, V + S-LDA group, volunteers who took low-dose aspirin for 14 days.

of the 13 volunteers (38.5%) exhibited a mild change (135 ≤ score < 790) in the small bowel mucosa, whereas all the others were normal (Fig. 3). A significant difference in the proportion of subjects with mild changes was observed between the two groups (4.8% *vs* 38.5%, $P = 0.041$) (Fig. 3). None of the subjects developed moderate or severe changes (score ≥ 790) in any of the groups in this study.

The median score of the small bowel mucosal inflammatory change was significantly higher in the L-LDA group (247.0; range, 0–4356) than in the P-Control group (12.0; range, 0–675; $P < 0.001$) (Fig. 4). Mild changes were significantly more common in the L-LDA group than in the P-Control group ($P = 0.002$) (Fig. 5). Furthermore, in the L-LDA group, five of the 38 patients (13.1%) developed moderate or severe change (Fig. 5). The proportion of subjects with moderate or severe change was significantly higher in the L-LDA group than in the P-Control group ($P = 0.003$) (Fig. 5).

In addition, we compared the CE scores for LDA-associated mucosal inflammatory changes according to tertiles (Fig. 6). In the V + S-LDA group, the small bowel mucosal injuries were multifocal and were evenly distributed throughout the small bowel. However, the mucosal injuries in the L-LDA group tended to increase in severity in the distal part of the small bowel (Fig. 6).

Most patients were followed for at least 3 months; however, none of the patients in the L-LDA group were newly diagnosed as having Crohn's disease, Behçet's disease or intestinal tuberculosis.

DISCUSSION

The present CE study examined the usefulness of quantifying small bowel mucosal changes associated with giving LDA,

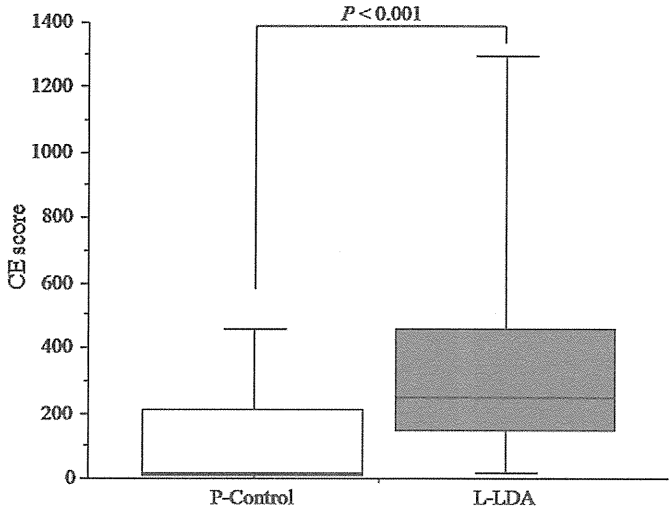

Fig. 4. Capsule endoscopy (CE) scores for small bowel mucosal inflammatory changes in the P-Control group and in the L-LDA group. Box plots show the interquartile range (box), median (thick line) and range (thin lines) of the capsule endoscopy scores. LDA, low-dose aspirin; L-LDA group, long-term LDA group; P-Control group, non-users of LDA.

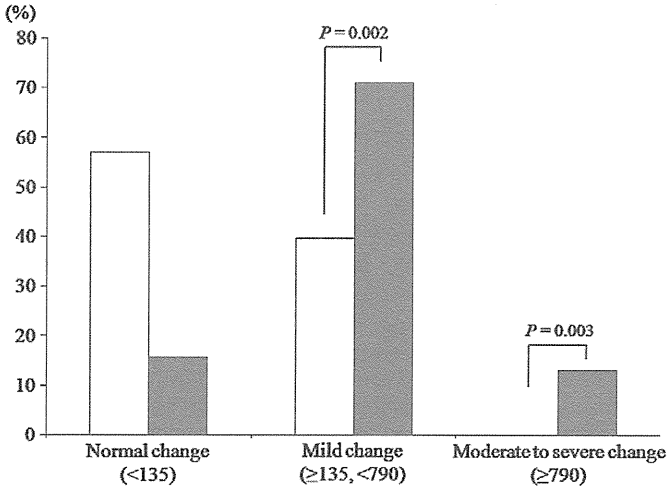

Fig. 5. Comparison of the severity of small bowel mucosal inflammatory changes between the P-Control group and the L-LDA group. The severity was classified into three categories according to the capsule endoscopy score: normal (<135), mild change (between 135 and 790), and moderate or severe change (≥790). LDA, low-dose aspirin; ■, L-LDA group, long-term LDA group; □, P-Control group, non-users of LDA.

using a CE scoring index. The CE scores of subjects taking LDA were significantly higher than those of control individuals. Furthermore, classification into three categories based on the CE score enabled us to compare the severity scale of mucosal injury between the two groups.

Previous studies evaluating NSAIDs/aspirin-associated mucosal injury have shown various ways to interpret and compare CE data. Goldstein *et al.*,[7] who compared the effects of naproxen versus celecoxib on the small bowel, simply counted the number of mucosal breaks per tertile to measure adverse drug effects. A number of investigators have attempted to create a scoring index intended specifically for use with CE. Graham *et al.*[4] assessed small bowel mucosal injury in chronic NSAIDs users. In their study, CE lesions were scored as normal, red spots, small erosions, large erosions or ulcers. Maiden and colleagues[5] graded diclofenac-induced lesions as category 1 (reddened folds), category 2 (denuded area), category 3 (petechiae/red spot), category 4 (mucosal break), or category 5 (presence of blood without visualized lesion). Despite accumulating published reports, the terminology describing CE findings and the severity scale for NSAIDs/aspirin-associated mucosal inflammatory changes have not been standardized. Therefore, for example, similar lesions are referred to as ulcers, erosions, or aphthae in different studies. In contrast, minor differences in lesion size may be exaggerated to significant qualitative difference (i.e. ulcers and erosions). Regarding this point, it is noteworthy that the CE score abandoned the differentiation of ulcers from other terms, such as erosions or aphthae, which are essentially the same lesions in nature. Because of the minimal standard terminology used in this scoring system, a quantitative evaluation of LDA-associated small bowel mucosal injury was achieved.

In the present study, we first analyzed the small bowel mucosal injuries by classifying the CE findings (Tables 4,5). This analysis was useful for the qualitative evaluation of LDA-induced lesions. However, this classification has several limitations when used to compare severity. Tables 4 and 5 do not provide information on the number of lesions per subject (one, few or multiple) or the size of the lesions (small mucosal break or large mucosal break). Thus, it is difficult to compare the severity of mucosal inflammatory changes without taking such matters into consideration. Furthermore, the mere evaluation of the proportion of patients with mucosal breaks is not sufficient to compare mild mucosal inflammation, such as mucosal injuries induced by the short-term administration of LDA. Indeed, our results showed no significant difference in the proportion of patients with mucosal breaks between the V-Control group and the V + S-LDA group, whereas the CE score clearly showed a significant difference between the two groups. In addition, this score has thresholds for distinguishing levels of mucosal disease severity. Therefore, this index could be used to discriminate between normal small bowel and disease states. These results confirmed the usefulness of quantifying small bowel mucosal changes associated with LDA using a CE scoring index.

To examine possible new directions for the use of CE scores, we analyzed the CE scores for LDA-associated mucosal inflammatory changes according to tertiles. This analysis revealed that the mucosal injuries in the L-LDA group tended to increase in severity in the distal part of the small bowel. The present result is consistent with a previous report in which chronic LDA-associated ulcers were observed mainly in the distal part of the small bowel.[11] Thus, CE scores are helpful for clarifying the distribution of LDA-associated inflammatory mucosal lesions.

LDA-associated enteropathy has been revealed with the advent of capsule endoscopy and double-balloon enteroscopy. We recently reported a pilot trial using CE to examine the effect of LDA on the small bowel in healthy volunteers.[9] Our current results are consistent with a previous study in which short-term treatment with LDA induced mild small

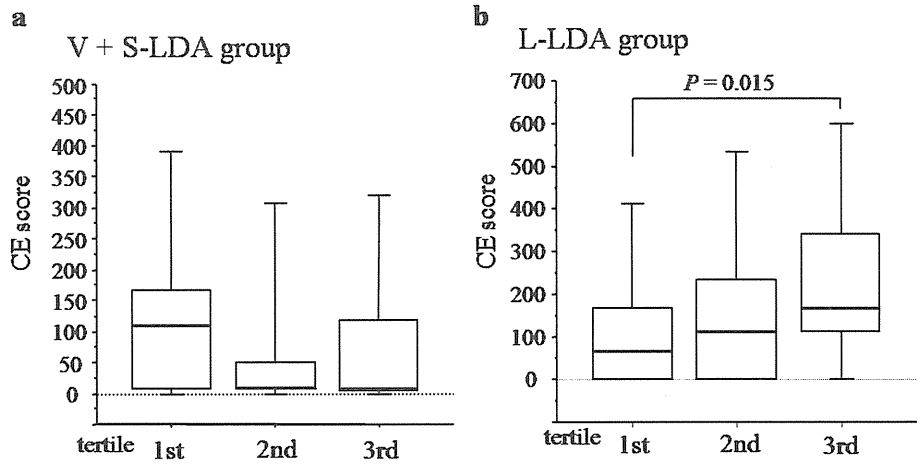

Fig. 6. Comparison of the severity of low-dose aspirin-associated mucosal inflammatory changes according to tertiles. Box plots show the interquartile range (box), median (thick line) and range (thin lines) of the capsule endoscopy (CE) scores. L-LDA group, long-term LDA group; V + S-LDA group, volunteers who took low-dose aspirin for 14 days.

bowel mucosal change. However, serious inflammatory changes were often observed in the small bowel mucosa of long-term LDA users. Further studies are needed to investigate the risk factors that may aggravate small bowel injury.

In conclusion, a CE scoring system is useful for evaluating LDA-associated small bowel mucosal disease activity using CE and for the objective scoring of small bowel inflammatory disease states. This scoring index could also be potentially used to measure and document mucosal healing in response to therapy. Thus, a scoring index might be helpful for the management of patients suffering from NSAIDs/LDA-associated small bowel mucosal disease.

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ORIGINAL ARTICLE—ALIMENTARY TRACT

Small bowel injury induced by selective cyclooxygenase-2 inhibitors: a prospective, double-blind, randomized clinical trial comparing celecoxib and meloxicam

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Abstract
Background Selective cyclooxygenase (COX)-2 inhibitors are less harmful to the small bowel mucosa than non-selective anti-inflammatory drugs. We aimed to compare the severity of small bowel mucosal injury in healthy volunteers induced by two selective COX-2 inhibitors, celecoxib and meloxicam, in a randomized, double-blind trial, using capsule endoscopy (CE).
Methods Twenty-nine healthy subjects were randomized to take either celecoxib (200 mg twice daily) or meloxicam (10 mg once daily) for 2 weeks. The incidence and the number of small bowel mucosal injuries (bleeding, ulcers, and erosions) observed by CE were compared between the two groups.
Results The overall incidence of small bowel mucosal injury was not different between the celecoxib group (6 of 14 subjects, 42.9%) and the meloxicam group (4 of 15 subjects, 26.7%, $P = 0.45$). In subjects with positive CE findings, the number of ulcers was greater in the meloxicam group than in the celecoxib group ($P = 0.02$), while such a trend was not found with regard to erosions ($P = 0.52$). The distribution of mucosal lesions within the small bowel was similar in the two groups.
Conclusions Selective COX-2 inhibitors are not completely safe for the small bowel. The mucosal lesions may be less severe with celecoxib than with meloxicam.

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Keywords Selective cyclooxygenase-2 inhibitor · Small bowel mucosal injury · Capsule endoscopy

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) frequently show gastrointestinal (GI) toxicity. For instance, gastroduodenal ulcers occur in 20–30% of chronic NSAID users [1–3]. It has also been shown that colonoscopy detects ulcers in the lower GI tract in 3% of chronic NSAID users [4, 5]. Although it had become evident in the 1980s that NSAIDs also damaged the small bowel, in practice, the mucosal injury could not be visualized until capsule endoscopy (CE) and double-balloon endoscopy (DBE) became widely used. While a postmortem examination identified small bowel ulcerations in 21 (8.4%) of 249 NSAID users [3], it has subsequently become evident in CE and DBE studies that NSAIDs cause small bowel mucosal injury more frequently, with a prevalence of up to 70% [6–8].

Recent clinical studies have shown that the incidence of upper GI injury was lower in subjects treated with selective cyclooxygenase (COX)-2 inhibitors than in those treated with non-selective NSAIDs [9–12]. Furthermore, celecoxib, one of the selective COX-2 inhibitors, has been shown to cause small bowel mucosal injury and lower GI events less frequently than non-selective NSAIDs [12–14]. Meloxicam, an agent synthesized as a traditional NSAID, also has a selective inhibitory action against COX-2 [15, 16]. In vitro studies showed that meloxicam had less potent inhibitory action on the synthesis of prostaglandin E, 6-keto-prostaglandin $F_{1\alpha}$, and thromboxane B_2 in human gastric mucosa when compared to indomethacin [17]. Ex vivo analysis of monocytes obtained from meloxicam-pretreated humans revealed that the drug had a five- to tenfold higher inhibitory

effect on COX-2 than on COX-1 [18–20]. In clinical trials, meloxicam was associated with a lower incidence of upper GI toxic events when compared to other traditional NSAIDs [21–23]. However, small bowel mucosal injury caused by meloxicam has not been examined to date.

In order to examine whether selective COX-2 inhibitors are protective against small bowel injury in humans, and to investigate possible differences between the small bowel toxicity of two selective COX-2 inhibitors, celecoxib and meloxicam, we performed a prospective, double-blind, randomized, controlled study.

Methods

Study design

This study was a prospective, double-blind, randomized trial. Prior to randomization, all subjects underwent laboratory tests (complete blood cell count, serum chemistry, and detection of *Helicobacter pylori* antibody), an electrocardiogram (ECG), and a baseline CE. Any subjects who had abnormal laboratory test results or an abnormal ECG were excluded from the study. Subjects who had small bowel erosions or ulcers at baseline CE were also excluded. All remaining subjects were then randomized, by a computer-generated randomization system, to receive either celecoxib (200 mg twice daily) or meloxicam (10 mg once daily) for 2 weeks. The dose of each drug was determined on the basis of the dose approved by the Japanese Ministry of Health and Welfare and applied to other clinical trials [24, 25]. In both groups, omeprazole (20 mg once daily) was given in consideration of possible gastric mucosal injury. Celecoxib and meloxicam were prepared in dummy capsules and the subjects were instructed to take a capsule twice per day for 2 weeks. The use of other NSAIDs, aspirin, or anti-ulcer drugs was strictly prohibited during the study period. After 2 weeks of medication, the subjects completed a questionnaire about GI symptoms, underwent repeated laboratory tests, and received a second CE.

The study protocol was approved by the institutional review board of the International University of Health and Welfare Fukuoka Sanno Hospital (FS-2-0903-049), and the study was conducted in accordance with the Helsinki Declaration. This trial has been registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) as number UMIN000003871. All subjects provided their written informed consent before entry into the study.

Subjects

Healthy volunteers with normal physical examinations and normal laboratory test results were eligible for the present investigation. Exclusion criteria were as follows: (1) a history of peptic ulcers, (2) a history of recent (within a month) use of NSAIDs or aspirin, (3) a history of aspirin-induced asthma, (4) allergy to sulfonamide, (5) recent treatment with anti-ulcer drugs, (6) stenosis of the GI tract, (7) a history of adhesion ileus, (8) pregnant or nursing females, and (9) the presence of other disorders regarded as causing the subject's participation in the present study to be inappropriate.

Capsule endoscopy

The baseline and the second CEs were performed using a PillCam SB (Given Imaging, Yokneam, Israel). After an overnight fast for 12 h, each subject was prepared with sensor arrays and a data recorder, and instructed to swallow the capsule with a small amount of water. CE images were recorded for the subsequent 8 h. All the digital video image streams were downloaded to the Given Imaging Reporting and Processing of Images and Data (RAPID) system.

Two observers (M.E. and Y.M.) independently assessed the CE images. Positive CE findings were classified as mucosal bleeding or mucosal injuries. Mucosal injuries were further divided into ulcers and erosions on the basis of the classification reported by Fujimori et al. [26] and Niwa et al. [27] with slight modifications. Mucosal bleeding was defined as the presence of luminal blood in the small intestine. A large mucosal defect with obvious whitish mucous was defined as an ulcer (Fig. 1a), while a small mucosal break surrounded by redness was regarded as an erosion (Fig. 1b). The small intestine was divided equally into the jejunum and the ileum by the small bowel transit time. If the CE findings were different between the two observers, they then discussed the case until a consensus opinion was reached.

Endpoints

The primary endpoint was the incidence of positive CE findings of any type at the second CE.

The secondary endpoints were the incidence of CE findings in the jejunum and in the ileum, the numbers of each CE finding in subjects with positive CE results, GI symptoms, and the presence or absence of anemia. GI symptoms were assessed at the end of the medication period by using a GI symptom rating scale (GSRS) [28]. Anemia was defined as a decrease in the hemoglobin level by more than 2.0 g/dl from the baseline value.

Statistical analysis

The incidence of small bowel mucosal injury after 2 weeks of celecoxib has been shown to range from 6 to

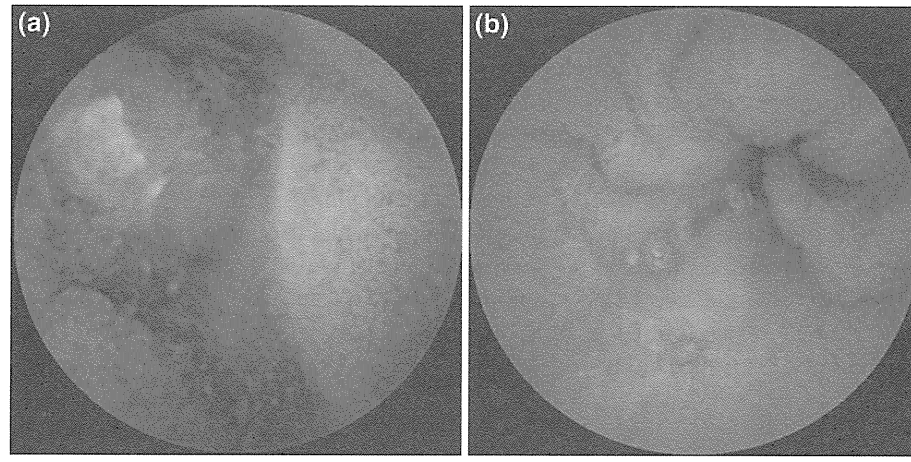

Fig. 1 Examples of mucosal injury observed by capsule endoscopy (CE) (**a** ulcer, **b** erosion)

16% [13, 14]. The incidence of small bowel mucosal injury caused by meloxicam was unknown. We thus presumed the incidence to be equivalent to that of non-selective NSAIDs (68–75%) [6, 7]. In the present study, the sample size was calculated on the assumption that the incidence of small bowel mucosal injury would be 10% for celecoxib and 60% for meloxicam. To detect this difference with a 0.05 significance level and a statistical power of 80%, it was calculated that 15 subjects per group would be required.

Parametric data were expressed as medians (ranges). The data were compared between the groups using the Mann–Whitney *U*-test. Non-parametric data were expressed as frequencies, and analyzed by Fisher's exact probability test or the χ^2 test. A *P* value of <0.05 was considered to be statistically significant for each test.

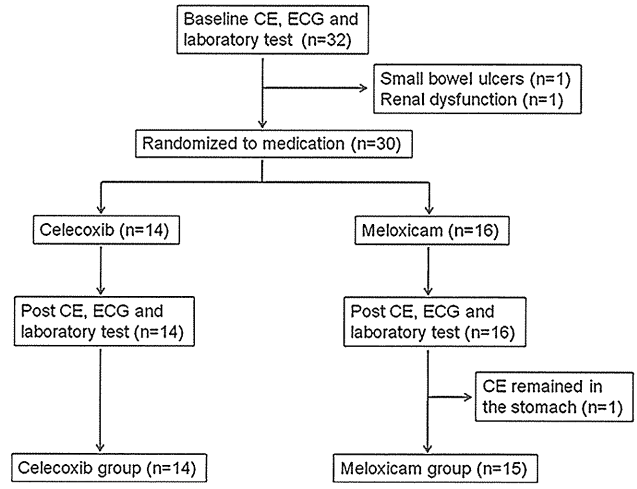

Fig. 2 Flow chart of the study subjects

Results

Subjects

The study was conducted from April to August 2010. During the study period, 32 subjects were enrolled. A flow chart of the study subjects is shown in Fig. 2. Two subjects were excluded, one because of multiple small bowel ulcers and one because of a slight increase in the serum creatinine level (1.2 mg/dl) at baseline. The remaining thirty subjects were then randomized to either the celecoxib or the meloxicam group. The second CE enabled total enteroscopy in 29 subjects, because the capsule remained in the stomach during the second CE in one subject (who had been taking meloxicam). Consequently, the celecoxib and meloxicam groups comprised 15 subjects and 14 subjects, respectively.

Table 1 shows a comparison of the demographic data in the two groups of study subjects. There were no significant differences in age, gender, or body weight between the two groups. *Helicobacter pylori* infection was detected in 3 subjects in the celecoxib group and in one subject in the meloxicam group. The prevalence of the infection was not different between the two groups. One subject in the celecoxib group continued taking concurrent medication for his essential hypertension.

Table 1 Comparison of demographic data between the celecoxib and meloxicam groups

	Celecoxib group	Meloxicam group	*P* value
Number of subjects	14	15	
Age (years)	33 (25–50)	30 (24–46)	0.60
Gender (female/male)	6/8	6/9	0.88
Body weight (kg)	66 (45–79)	59 (39–76)	0.68
Helicobacter pylori infection	3	1	0.33
Concurrent medication	1[a]	0	0.48

Parametric data are expressed as medians (ranges)

[a] The subject continued taking an angiotensin II receptor blocker

In the subjects who completed the full study protocol, we did not encounter any extra-abdominal symptoms or significant changes in laboratory data.

Capsule endoscopy findings

In each subject, the two observers reported a concordant result as to the presence or absence of positive findings at the second CE. However, there were two subjects in whom the determination of an ulcer or erosion was discordant between the two observers, thereby requiring a discussion. As a result of the discussion, a consensus was reached that there were erosions in 6 subjects in the celecoxib group, three of whom also had ulcers. In the meloxicam group, ulcers were found in 4 subjects, three of whom also had erosions. Consequently, the incidence of small bowel mucosal injuries was not significantly different between the two groups (42.9% in the celecoxib group and 26.7% in the meloxicam group, $P = 0.45$) (Fig. 3). When the total number of mucosal injuries was compared, no significant difference was found between the celecoxib group (0 [range 0–14]) and the meloxicam group (0 [range 0–18]). Similarly, neither the number of ulcers nor the number of erosions differed between the two groups.

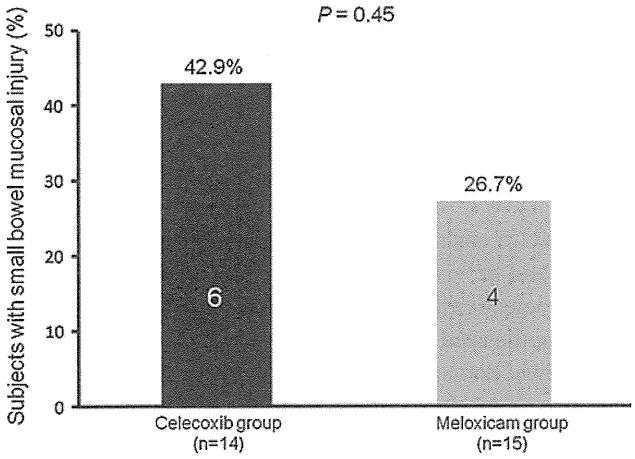

Fig. 3 Comparison of the incidence of small bowel mucosal injury between the celecoxib and meloxicam groups

We then compared the severity of mucosal injuries in the two groups in subjects with positive CE findings (Fig. 4). Six subjects in the celecoxib group and four subjects in the meloxicam group were the subjects for the comparison. The number of ulcers in subjects taking celecoxib was 1 (range 0–1), while the number was higher (3 [range 1–3]) in subjects taking meloxicam ($P = 0.02$). The number of erosions was 6 (range 1–13) in subjects taking celecoxib and 13 (range 0–16) in subjects taking meloxicam ($P = 0.52$). The total number of mucosal injuries was no different between the two groups of subjects (6 [range 1–14] in subjects with celecoxib and 16 [range 3–18] in subjects with meloxicam, $P = 0.18$).

Figure 5 shows a comparison of the incidence of jejunal and ileal injuries in the two groups. Ulcers were found only in the ileum, with an incidence of 21% (3 subjects) in the celecoxib group and an incidence of 27% (4 subjects) in the meloxicam group (Fig. 5a). While the incidence of erosions in the jejunum was not different between the two groups (7.1% in the celecoxib group and 6.7% in the meloxicam group, $P = 1.0$), the incidence of ileal erosions was higher in the celecoxib group (42.9%) than in the meloxicam group (20%). However, the difference did not reach statistical significance ($P = 0.25$).

Symptoms, laboratory data, and complications

One subject in the celecoxib group complained of epigastric pain. In the meloxicam group, two subjects experienced abdominal discomfort and one subject had diarrhea. As shown in Table 2, the GSRS score was 17 (range 15–25) in the celecoxib group and 18 (range 15–26) in the meloxicam group. None of the subjects manifested anemia at the end of the medication period.

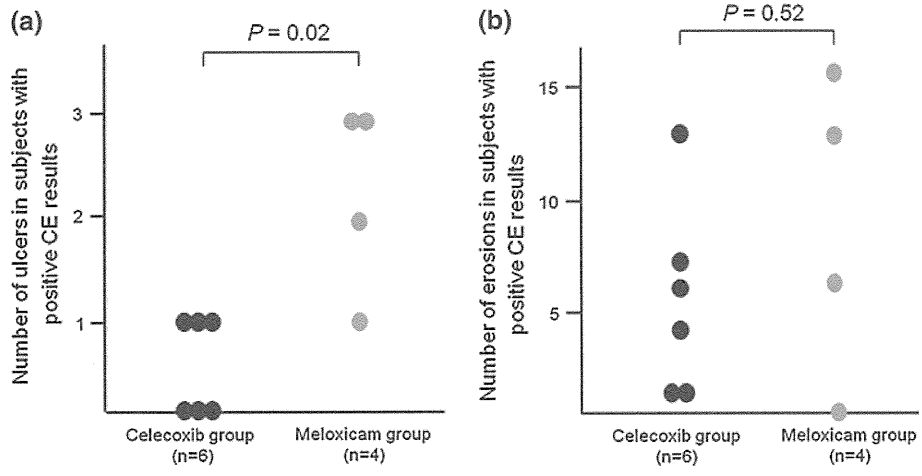

Fig. 4 Comparison of the number of lesions in subjects with positive CE results (**a** number of ulcers, **b** number of erosions)

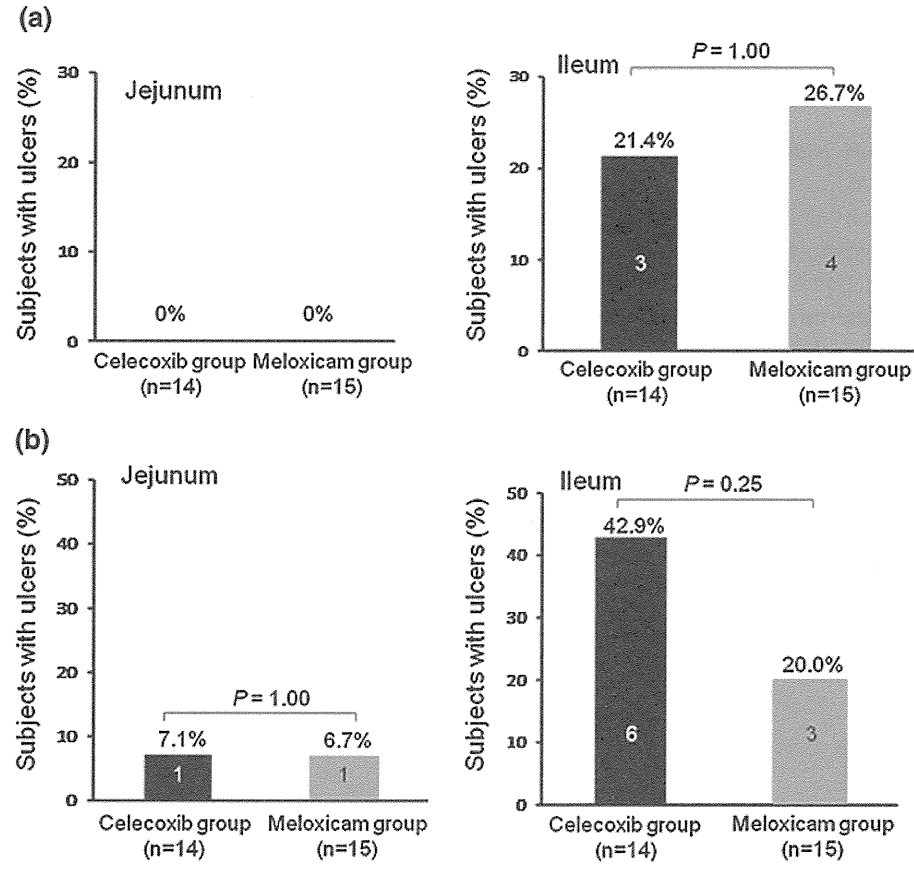

Fig. 5 Comparison of the incidence of small bowel mucosal injuries between the two groups according to their site (**a** ulcers, **b** erosions)

Table 2 Abdominal symptoms and laboratory data

	Celecoxib group ($n = 14$)	Meloxicam group ($n = 15$)	P value
Symptoms	1	3	0.60
GSRS	17 (15–25)	18 (15–26)	0.52
Anemia	0	0	

Anemia was defined as a decrease in the hemoglobin level of at least 2.0 g/dl from baseline

GSRS gastrointestinal symptom rating scale, *GSRS* data are expressed as medians (ranges)

Discussion

NSAIDs and selective COX-2 inhibitors are generally classified by their COX-2/COX-1 selectivity determined by in vitro or ex vivo experiments. In this regard, meloxicam and celecoxib are classified in the same category of NSAIDs, with selectivity ranging from 5 to 50 [29, 30]. However, the relative risk of upper GI toxicity is threefold higher with meloxicam than with celecoxib [31]. Lanas et al. [32] reported a much higher risk of upper GI bleeding in patients administered meloxicam than in those administered celecoxib in a hospital-based, case–control study. These observations suggest that the in vivo COX-2/COX-1 selectivity of each of these NSAIDs is different from their in vitro and ex vivo selectivities, and that the in vitro and ex vivo selectivities are not predictive of GI toxicity. We thus hypothesized that the incidence and the severity of small bowel damage would be different between celecoxib and meloxicam. In accordance with prior clinical trials, we carried out a double-blind prospective study with healthy subjects treated with short-term NSAIDs [13, 14]. As has been confirmed in other prospective studies treating healthy volunteers [7, 13, 14], we found small bowel mucosal lesions in 3% of our subjects prior to the administration of the test drugs.

Our results indicated that the incidence of small bowel mucosal damage induced by celecoxib (43%) was not different from that induced by meloxicam (27%), with rather a higher value for celecoxib than for meloxicam. Interestingly, the incidence of celecoxib-induced small bowel mucosal damage in our subjects was equivalent to that induced by diclofenac or naproxen in Western and Eastern subjects verified by randomized trials [7, 13, 27, 33] and it was higher than that induced by ibuprofen in Western subjects [14]. It thus seems reasonable to conclude that the selective COX-2 inhibitors available at present are not unequivocally safe for the small bowel. However, because celecoxib and meloxicam have anti-COX-1

properties, it is still possible that COX-1 inhibition contributes to the pathogenesis of the mucosal damage even in subjects treated with selective COX-2 inhibitors.

When we compared the CE findings in subjects with positive CE results, we found a greater number of ulcers in the meloxicam-treated subjects than in the celecoxib-treated subjects. This observation suggests that meloxicam induces more severe mucosal lesions in subjects who are at a high risk of NSAID enteropathy. Possible explanations for this difference between meloxicam and celecoxib include differences in the effects of the two drugs on the enterohepatic recirculation [34], in their effects on bacterial flora and bile acid composition, and presumably, in their effects on in vivo COX-2/COX-1 selectivity. Because severe mucosal damage is likely to cause GI complications such as bleeding and perforation, celecoxib may be safer than meloxicam for the small bowel.

In both our celecoxib and meloxicam groups, most mucosal damage was found in the distal part of the small bowel. It has been confirmed that NSAIDs increase intestinal permeability through enterocytic mitochondrial damage and a decrease in prostaglandin synthesis, and, as a consequence, the intestinal mucosa becomes more susceptible to the actions of luminal agents such as bile acid, bacterial flora, and ingested foods [34–37]. Changes in the composition of bile acids and an increase in bacterial flora in the ileum may explain the more severe mucosal damage at this site [37]. A similar trend in the distribution of mucosal injuries has been confirmed in recent studies using other NSAIDs [26, 38, 39], indicating that the ileum seems to be the predominant site prone to mucosal injury in patients taking NSAIDs or COX-2 inhibitors.

The incidence of small bowel mucosal injuries in our celecoxib group was 43%, which was much higher than was predicted (10%). We predicted the incidence of small bowel mucosal injury in the celecoxib group based on the prospective studies done by Goldstein et al. [13, 14], and this discordant result may therefore have been a consequence of the differences in subjects' ethnicities and physiques between the studies done by Goldstein et al. [13, 14] and our present trial. In fact, the body weight of our subjects (median 59 kg) was much lower than that in the study by Goldstein et al. [14] (73 kg). However, it should also be noted that in an observational study done by Maiden et al. [40] in the United Kingdom, CE detected minute small bowel mucosal injuries in 50% of patients taking COX-2 inhibitors (celecoxib, etoricoxib, rofecoxib, or valdecoxib). It thus seems possible that COX-2 plays a significant role in the preservation of the mucosal integrity of the small bowel, and the inhibition of COX-2 can easily lead to mucosal breaks.

Our present study has some limitations. First, because the predicted incidence of mucosal injury in the celecoxib group was lower than the actual incidence, we should have recruited a larger number of subjects for each group to prove an insignificant difference in the incidence of mucosal injuries between the two groups. We thus cannot deny a significantly higher incidence of injuries in the celecoxib group. However, our conclusion that celecoxib possibly damages the small bowel should not be modified. Second, the small sample size suggests that there may be a type 2 error in the comparison of the severity of mucosal injuries, which means that the number of ulcers was not actually different between the two groups. Finally, subjects in the meloxicam group were administered a 10-mg dose of meloxicam, which is the standard dose in Japan but is lower than that in Western countries (15 mg).

In conclusion, our prospective study indicated that the incidence of small bowel mucosal damage was not different between subjects treated with celecoxib and those treated with meloxicam, suggesting that selective COX-2 inhibitors are not completely safe for the small bowel. Our sub-analysis of subjects with positive CE findings suggested celecoxib to be less harmful than meloxicam, indicating that factors other than in vitro COX-2/COX-1 selectivity may be associated with small bowel toxicity. The conspicuously high incidence of mucosal damage in our subjects treated with celecoxib warrants further studies to establish the role of selective COX-2 inhibitors for the prevention of small bowel injuries in patients scheduled to receive long-term NSAID treatment.

Conflict of interest The authors declare that they have no conflict of interest.

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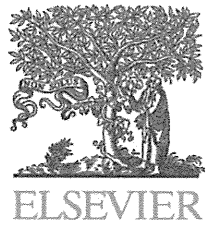

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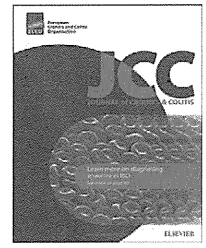

Chronic nonspecific multiple ulcer of the small intestine segregates in offspring from consanguinity

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Chronic enteropathy;
Anemia;
Hypoproteinemia;
Family history

Abstract

Background and aims: Chronic nonspecific multiple ulcer of the small intestine is a recently proposed enteropathy characterized by persistent blood and protein loss from the small-bowel. We examined possible segregation of the disease in family pedigrees.
Methods: All cases of the disease diagnosed at our institution were reviewed with respect to particular focuses on the presence of close consanguinity in the families, the enteroscopic findings and the long-term clinical course. The diagnosis was based on persistent occult gastrointestinal bleeding and hypoproteinemia for more than 5 years, and irregularly shaped shallow ulcers in the ileum.
Results: During a 45-year-period, 13 patients were diagnosed as having the disease. There were 11 females and 2 males, with ages ranging from 8 to 37 years at the time of the initial presentation and with those from 13 to 38 years at the diagnosis. Enteroscopy performed in 11 patients with a time duration ranging from 0.5 to 44 years after the diagnosis revealed active ileal ulcers in 10 patients. Parents' consanguineous marriage was verified in 6 patients, two of whom also had siblings with the enteropathy. Another patient without consanguinity had a sibling with protein-losing enteropathy.
Conclusion: Chronic nonspecific multiple ulcer of the small intestine seems to segregate in offspring from consanguineous marriage.

1. Introduction

The use of capsule endoscopy and balloon endoscopy has led to an increase in the chance of encountering small-bowel ulcers, especially in patients with obscure gastrointestinal bleeding.[1,2] While Crohn's disease, intestinal tuberculosis,

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radiation enteropathy, and nonsteroidal anti-inflammatory drug (NSAID) enteropathy are entities predisposing to chronic or recurrent small-bowel ulcers, there are cases of ulcers with obscure origin.

We recently reported on a peculiar form of enteropathy characterized by chronic blood and protein loss through persistent small-bowel ulcers.[3] Because the ulcers of the disease had nonspecific histology, we referred to the condition as "chronic nonspecific multiple ulcer of the small intestine (CNSU)".[3,4] CNSU does not seem to be a rare entity, because cases of exactly the same clinicopathologic features have subsequently been reported in the literature.[5–7] Furthermore, a similar enteropathy with different nomenclatures has been described in Caucasians and referred to as "diaphragm disease of the small bowel without apparent NSAID use"[8] or as "cryptogenic multifocal ulcerous stenosing enteritis".[9] More recently, Adler et al.[10] reported a novel enteropathy in a middle aged American male characterized by blood loss from recurrent small-bowel ulcers. Surprisingly, Adler's case had compound heterozygous mutations in the encoding regions of *cytosolic phospholipase A2α (cPLA2α)* gene. Based on the description, we hypothesized CNSU to be a hereditary condition with genetic alterations. We thus retrospectively investigated family histories of CNSU in patients with the disease identified at our institution.

2. Patients and methods

2.1. Survey for CNSU

We reviewed the diagnosis, the prevalence, and the management of inflammatory bowel diseases diagnosed during a period 1964–2009 at Kyushu University Hospital, Fukuoka University Chikushi Hospital, and their satellite hospitals, and collected data for clinicopathologic features of patients with CNSU. The two referral centers have been treating approximately 600 patients with Crohn's disease and 800 patients with ulcerative colitis.

2.2. Diagnosis of CNSU

The diagnosis of CNSU was made on the basis of clinical manifestations and small-bowel lesions.[4] As for clinical manifestations, patients with CNSU should have iron deficiency anemia and hypoproteinemia in their adolescence.[4] Small-bowel lesions should be multiple shallow ulcers in the ileum, with sharply demarcated margin and linear or oblique configuration (Fig. 1).[11] Furthermore, the repeated ascertainment of those clinical manifestations with time intervals for more than 5 years was inevitable for the diagnosis of CNSU.

2.3. Data collection

We focused on the demographic data regarding the initial clinical manifestation, which led to the identification of small-bowel ulcers, the age at the onset, and the laboratory values of serum protein, serum albumin, C-reactive protein (CRP), hemoglobin, and white blood cell count at the time of the initial diagnosis. We also reviewed histories and laboratory data presumably associated with other enteropathy. They included history of NSAID use, purified protein derivative (PPD) skin test, interferon-γ assays (IGRA) for *Mycobacterium* infection, anti-tissue transglutaminase (tTGA) antibodies, findings obtained by esophagogastroduodenoscopy with forceps biopsy, and histologic findings of the resected small bowel. In addition, medical and surgical treatments, response to the medication as determined by changes in serum protein value, and prognosis were retrospectively investigated. We also collected data of the final enteroscopic findings. The procedures for enteroscopy included retrograde ileoscopy (RI), double balloon endoscopy (DBE) and intraoperative endoscopy (IOE). The enteroscopic findings were evaluated with regard to the stage (open or scarred), the depth (deep or shallow), and the configuration (circular, linear, or their combination) of the representative lesion.[11]

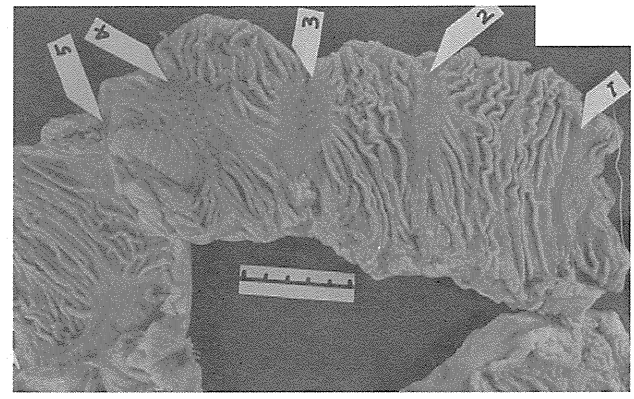

Figure 1 Typical macroscopic findings of the resected ileum in a case of CNSU (Case 9). There are shallow and clear ulcers in circular or linear configuration in the ileum. The intervening mucosa is not affected.

We directly contacted the patients and/or their relatives to obtain family histories. The items of special interest were consanguinity, anemia, malnutrition, abdominal surgery, and clinical diagnosis of enteropathy, if any, in the family pedigrees. Family history of enteropathy was regarded as positive in the case of surgical interventions for the small bowel, the established diagnosis of small-bowel ulcers or both. We examined the medical records of the relatives with enteropathy in the case that the records were available.

This retrospective study was approved by the ethical committee at Kyushu University Hospital, and it was undertaken in accordance with Helsinki Declaration.

3. Results

3.1. Clinical features and laboratory data

During a period from 1964 to 2009, 13 patients were diagnosed with CNSU. Table 1 summarizes the clinical features of the patients. There were 11 females and two males. All patients had anemia of obscure origin as the presenting symptom. In addition, three patients had edema and other two patients complained of abdominal pain. The age at the time of the onset ranged from 8 to 37 years. Eleven patients complained of the symptoms at the age of less than 20 years. The time interval

Table 1 Cases of CNSU diagnosed at our institution during 1964–2009.

Case no.	Age (yrs)/gender		Presenting symptoms	Laboratory data		
	Onset	Diagnosis of CNSU		Hemoglobin (g/dl)	Serum protein (g/dl)	CRP (mg/dl)
1.	20/F	27	Anemia, edema	8.2	4.9	−*
2.	15/F	24	Anemia, edema	3.5	5.0	−*
3.	10/M	26	Anemia, growth retardation	4.4	4.5	−*
4.	15/F	28	Anemia, edema	4.7	5.3	−*
5.	12/F	27	Anemia, abdominal pain	9.7	5.8	0.3
6.	17/F	34	Anemia	9.6	4.6	0.5
7.	10/F	13	Anemia, abdominal pain	7.4	5.4	0.1
8.	37/F	38	Anemia	9.5	6.7	0.5
9.	15/M	30	Anemia, edema	7.4	8.2	0.1
10.	13/F	29	Anemia	5.9	4.6	0.2
11.	16/F	52	Anemia	5.3	6.3	0.1
12.	13/F	40	Anemia	9.4	4.1	1.1
13.	8/F	33	Anemia, edema	8.6	4.5	0.6

* CRP was determined to be negative under semi-quantitative measurement.

from the onset until diagnosis of CNSU ranged from 1 to 27 years (median; 15 years). NSAID use was not verified in any patient at the time of the initial diagnosis. We further confirmed possible use of NSAID in seven patients who had been under observation. Those patients again clearly denied any continuous use of NSAID or other medications at the time of their first diagnosis of CNSU.

Laboratory data at the initial diagnosis showed hypochromic anemia and hypoproteinemia. The hemoglobin value ranged from 3.5 to 9.7 g/dl and serum protein value from 4.1 to 8.2 g/dl. In four patients (Cases 1–4) with the diagnosis of CNSU in 1970s, CRP value was not quantified. In the remaining nine patients, there were slight increases in CRP with values from 0.1 to 1.1 mg/dl.

Eleven patients were treated by surgery. The remaining two patients (Cases 11 and 13) were diagnosed with CNSU on the basis of the clinical and enteroscopic findings. Results of the diagnostic work-up are summarized in Table 2. PPD skin test and IGRA showed none of the patients to be positive for *Mycobacterium* infection. Anti-tTGA antibodies were measured

Table 2 Results of diagnostic work up for patients with CNSU.

Case no.	PPD skin test	IGRA test	Anti-tTGA antobody	Gastroduodenal lesions			Surgically removed ileal lesions			Final enteroscopic findings			
										Concentric stenosis		Non-stricturing ulcers	
				Endoscopy	Granuloma	Villous atrophy	Maximal depth of ulcer	Granuloma	Villous atrophy	Number	Open ulcer at stenosis	Circular	Linear
1.	−	NE	NE	NS	−	−	Submucosa	−	−	NE	NE	NE	NE
2.	−	NE	NE	Gastric ulcer	−	−	Submucosa	−	−	Multiple	+	+	+
3.	−	NE	NE	NS	−	−	Submucosa	−	−	NE	NE	NE	NE
4.	−	NE	NE	NS	−	−	Submucosa	−	−	Single	+	−	−
5.	+	−	NE	Duodenal ulcer	−	−	Submucosa	−	−	NE	NE	NE	NE
6.	+	−	NE	NS	−	−	Submucosa	−	−	Multiple	+	+	+
7.	−	−	NE	NS	−	−	Submucosa	−	−	Single	+	+	−
8.	±	−	NE	Stomal ulcer	−	−	Submucosa	−	−	-		+	+
9.	−	NE	NE	Stomal ulcer	−	−	Submucosa	−	−	Single	+	+	−
10.	−	NE	NE	NS	−	−	Submucosa	−	−	Multiple	+	+	+
11.	−	NE	NE	NS	−	−	NE	NE	NE	Single	+	−	−
12.	−	NE	NE	NS	−	−	Submucosa	−	−	Multiple	+	−	+
13.	−	−	−	NS	−	−	NE	NE	NE	Single	+	−	+

PPD; purified protein derivative. IGRA; interferon-γ release assays for tuberculosis. tTGA; tissue transglutaminase.
NS; no significant finding. NE; not examined.

in only one patient, who showed a negative result. Two patients had a prior history of gastrectomy for gastroduodenal ulcers. Both patients had stomal ulcers. Two patients had gastric or duodenal ulcer. However, duodenal biopsies performed in all the patients were negative for villous atrophy. Also, villous atrophy of the ileum was not evident in any patient treated by ileal resection. The depth of the ileal ulcer was restricted to the submucosa in those patients. There was not any patient who had caseating or non-caseating granuloma in the biopsy or surgical specimens.

Table 3 summarizes the treatments applied for the patients. During the follow-up periods, prednisolone, aminosalicylates, combined anti-*Mycobacterium* agents, azathioprine and infliximab were used for nine patients, seven patients, six patients, two patients and a patient, respectively. The serum protein did not respond to any of those medications. In nine patients, the malnutrition transiently improved after total parenteral nutrition. Eleven patients were treated by ileal resection because of small-bowel stricture. Ten of those 11 patients, however, required repeated surgery after the recurrence of strictures. As indicated in Table 3, two patients were lost to follow up, while other four patients died. The remaining seven patients have been under observation. They still have hypoproteinemia and anemia, which require iron supplementation and total parenteral or enteral nutrition.

3.2. Final enteroscopic findings

We attempted enteroscopy in 11 patients during the clinical course. The time interval from the initial diagnosis until the final enteroscopy ranged from 0.5 to 44 years. In a patient (Case 5), however, enteroscopy was unavailable because of a duodenal stenosis.

The enteroscopic findings are indicated in Table 2. Nine patients had single or multiple concentric stenoses. In those patients, shallow and clearly demarcated ulcers were seen at the most severe stenosis (Figs. 2A and 3A). In addition, shallow ulcers accompanied by faint mucous exudates were seen in eight patients (Figs. 2B and 3B). A patient had a single stenosis without any accompanying mucosal defects.

3.3. Family history

Family histories of the patients are indicated in Table 4. The interviews to the patients and their relatives revealed that four patients were offspring of consanguineous marriage of 3 degrees, which means that their parents were cousins. In addition, other two patients were those of 5 degrees, indicating that their maternal and paternal grandparents were cousins. Four patients denied any such consanguinity in their family pedigrees. In the remaining three patients, we were not able to confirm their family pedigrees.

Information with regard to family histories of enteropathy was available in 11 patients. None of the patients commented on enteropathy in their parents or in their offspring. However, three patients commented on enteropathy in their siblings. The enteropathy included small intestinal stenoses of obscure origin (an elder sister of Case 4), CNSU (a younger sister of Case 10) verified in her medical record, and protein-losing enteropathy of obscure origin (an elder sister in Case 13). Two of the three family pedigrees were siblings of consanguineous marriage, while consanguinity was not evident in the remaining pedigree.

4. Discussion

We could confirm in this report that 1) CNSU is an enteropathy characterized by persistent anemia and hypoproteinemia occurring in childhood or in adolescence, 2) patients with CNSU had life-long illness, and 3) more than half of the patients had consanguinity and/or family history of enteropathy in their siblings even though vertical heredity was not obvious. These clinical observations suggest that CNSU is possibly a chronic enteropathy, which segregates in offspring from consanguinity. Even though most autosomal recessive disorders of the human bowel occur in infancy,[12] there have been recently reported two gastrointestinal disorders with such a hereditary trait, one being adenomatous polyposis with homozygous mutations of *MUTYH*[13–15] and the other chronic colitis with homozygous mutations of *IL10R*.[16]

Table 3 Treatment and prognosis of patients with CNSU.

Case no.	Medication		Efficacy of total parenteral nutrition	Number of ileal resection	Prognosis
	Species	Efficacy			
1.	PSL, cAMA	Not effective	Effective	2	Lost to follow-up
2.	PSL, cAMA, SASP	Not effective	Effective	3	Died of liver cirrhosis at age of 49 years
3.	PSL, cAMA	Not effective	(Not performed)	6	Lost to follow-up
4.	PSL, cAMA, SASP	Not effective	Effective	3	Died of pancreas cancer at age of 73 years
5.	PSL	Not effective	Effective	2	Alive at age of 59 years
6.	PSL, 5ASA, AZA	Not effective	Effective	1	Alive at age of 58 years
7.	PSL, cAMA	Not effective	Effective	6	Died of thyroid cancer at age of 58 years
8.	PSL, 5ASA	Not effective	Effective	2	Alive at age of 75 years
9.	PSL, cAMA, SASP	Not effective	(Not performed)	2	Alive at age of 67 years
10.	5ASA	Not effective	Effective	2	Died of stroke at age of 46 years
11.	IFX	Not effective	(Not performed)	0	Alive at the age of 60 years
12.	(None)		Effective	3	Alive at the age of 50 years
13.	5ASA, AZA	Not effective	(Not performed)	0	Alive at the age of 35 years

PSL; prednisolone, cAMA; combined anti-*Mycobacterium* agents, SASP; sulfasalazine, 5ASA; 5-aminosalicylate, AZA; azathioprine, IFX; infliximab.

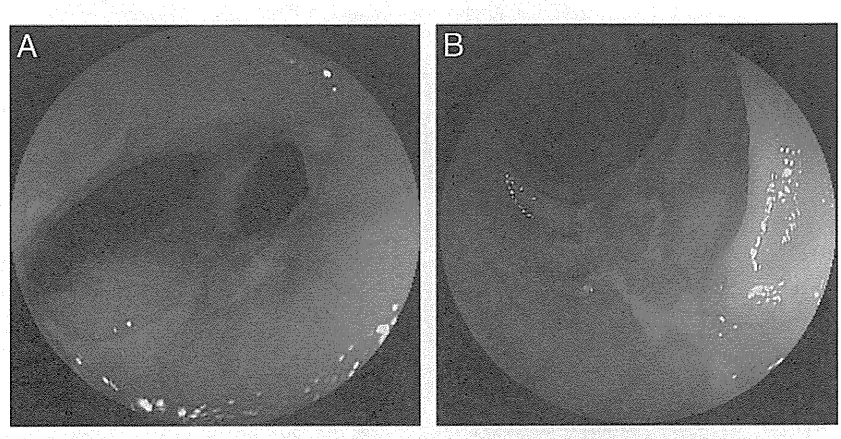

Figure 2 Enteroscopic findings of Case 13. This case is a daughter of a consanguineous marriage of 3 degrees, who has an elderly sister with protein-losing enteropathy. A; DBE reveals a severe concentric stenosis in the middle ileum. The stenostic area is accompanied by circular and sharply demarcated ulcer. B; DBE also shows a shallow, linear mucosal effect with clear margin in the distal ileum.

Small-bowel ulcers are known to occur in various types of chronic enteropathy of obscure etiology. These include Crohn's disease, chronic ulcerative duodenojejunoileitis,[17–19] cryptogenic multifocal ulcerous stenosing enteritis (CMUSE),[9] and diaphragm disease of the small bowel without apparent NSAID use.[8] CNSU shares common clinical manifestations with CMUSE and diaphragms unrelated to NSAID with respect to less severe inflammatory infiltrates and stenosing lesions of the ileum. We thus cannot conclusively distinguish CNSU from those two conditions. There also seems to be an argument that CNSU, together with CMUSE and diaphragms, belongs to a peculiar phenotype of Crohn's disease with less severe inflammation. The occurrence in adolescents with predominant involvement of the ileum in CNSU apparently mimics Crohn's disease, although the ileal phenotype is different between the two diseases.

In 1990s, data on the familial acquisition of Crohn's disease were accumulated. Analyses of those data from all over the world showed that the occurrence of Crohn's disease in the first-degree relatives of a proband ranged from 2.2% to 13.6%.[20–26] A common trend in those analyses was that the siblings of a proband were at the highest risk for the occurrence of the disease while parents have the lowest risk. Although a similar trend was also found in our patients with CNSU, the occurrence of enteropathy in the siblings was much higher, with a value of 23%. In contrast, the consanguinity has rarely been described in Crohn's disease. It thus seems likely that CNSU is genetically different from Crohn's disease.

So far as we reviewed the literature, two types of enteropathy are described in association of consanguinity. The first one is an intractable ulcerating enterocolitis of infancy characterized by diarrhea in the first year of life with large and deep ulcers in the colon.[27] The other enteropathy, referred to as intestinal epithelial dysphasia, has also been characterized by severe diarrhea in infants with disorganization of entrecotes in the epithelium and basement membrane abnormalities of the small-bowel.[28,29] The clinicopathologic features of the infantile enteropathy are obviously different from those of CNSU with respect to the age of onset and the clinical course.

Glocker et al.[16] recently analyzed two unrelated consanguineous families with an early onset of colitis, and they identified homozygous mutations in *IL10RA* and *IL-10RB* genes in the families. Even though the predominant site of involvement and other phenotypes are different between the cases reported by Glocker et al.[16] and those of CNSU, *IL-10R* may be one of the candidate genes associated with CNSU. Adler et al.[10] reported on another peculiar form of enteropathy with a life-long history of occult gastrointestinal

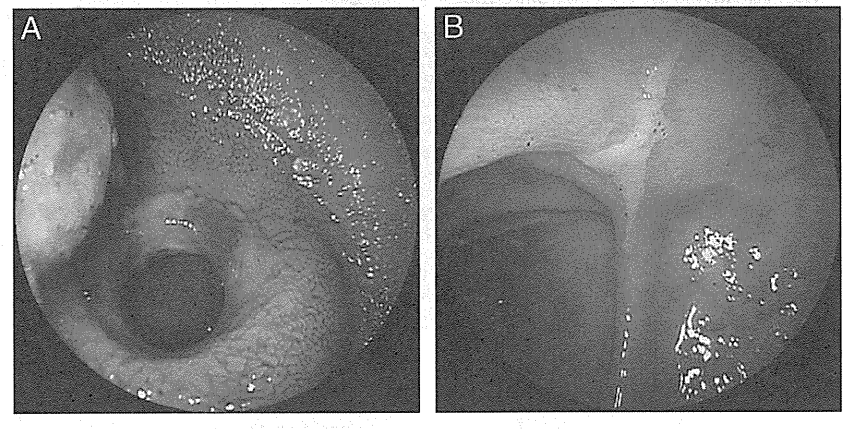

Figure 3 Enteroscopic findings of Case 6. This case is a daughter of a consanguineous marriage of 5 degrees. A; DBE shows a concentric stenosis with a clear ulcer in the ileum. B; in the distal ileum, sharply demarcated and linear mucosal defects are also seen.

Table 4 Consanguinity and family history of patients with CNSU.

Case no.	Consanguinity (degrees)	Family history of enteropathy
1.	Present (3)	None
2.	Absent	None
3.	Unknown	Unknown
4.	Absent	None
5.	Absent	A sibling
6.	Present (5)	None
7.	Present (5)	None
8.	Present (3)	None
9.	Unknown	Unknown
10.	Present (3)	A sibling
11.	Unknown	None
12.	Absent	None
13.	Present (3)	A sibling

blood loss, iron deficiency anemia and relapsing abdominal pain. The male patient had multiple, sharply demarcated ulcers and stenoses in the jejunum and in the ileum during his middle-aged period. Histological examination of the resected small-bowel disclosed nonspecific ulcers with minimal inflammatory infiltrates. Furthermore, Adler et al.[10] confirmed that the patient had inherited compound heterozygosity in *cPLA2α* gene, which resulted in a reduction in eicosanoid biosynthesis in platelets and leukocytes. Based on these observations, it was suggested that homozygous or compound heterozygous mutations of *cPLA2α* gene and a consequent reduction in substrates for arachidonic acids result in an enteropathy with recurrent small-bowel ulcers. It thus seems possible that *cPLA2α* is another candidate gene for CNSU. This hypothesis is under investigation.

The present case series has some limitations due to a retrospective analysis of historically accumulated patients. First, we cannot completely deny undisclosed use of NSAID, because we did not measure its metabolites in blood or urine samples.[8,30] However, we believe the enterosopic findings and the extra-ordinary long-term clinical course of CNSU to be completely different from NSAID enteropathy.[3] Second, we could not serologically deny chronic jejunoileitis complicating celiac disease in 12 of 13 patients. However, we consider celiac disease to be unlikely, because the patients did not have any villous atrophy, and furthermore, the disease is extremely rare among Asians.

In conclusion, a retrospective analysis of patients with CNSU revealed that the disease is possibly an enteropathy segregating in offsprings from consanguineous marriage. This concept may explain the rarity of the disease, and suggests that CNSU is a disease distinct from Crohn's disease. Further accumulation of the patients together with genetic analyses will be needed to conclude that CNSU is an autosomal recessive disorder.

Acknowledgment

TaM contributed to the analysis of the data and the writing of the manuscript. NK collected all the demographic and endoscopic data. ToM, MI and TY contributed to the concept of the manuscript and the management of the study subjects.

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