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Risk factors of symptomatic NSAID-induced small intestinal injury and diaphragm disease

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SUMMARY

Background
The aetiology for nonsteroidal anti-inflammatory drug (NSAID)-induced small intestinal injuries has not been well characterised.

Aim
To determine the risk factors of symptomatic NSAID-induced small intestinal injuries, including diaphragm disease.

Methods
Of the 1262 symptomatic patients who underwent videocapsule endoscopy and/or double-balloon enteroscopy, 156 consecutive patients were verified as having taken NSAIDs. Their *CYP2C9*2*, **3* and **13* single nucleotide polymorphisms (SNPs) were determined by allelic discrimination with Taqman 5'-nuclease assays.

Results
Of the 156 NSAIDs users, 31 patients (20%) were diagnosed with NSAID-induced small intestinal injury. Multivariate analysis indicated that the presence of comorbidities and the use of oxicams (meloxicam, ampiroxicam and lornoxicam) or diclofenac were associated with an increased risk of NSAID-induced small intestinal injury (adjusted OR: 2.97, 95% CI: 1.05–8.41, $P = 0.041$ and adjusted OR: 7.05, 95% CI: 2.04–24.40, $P = 0.002$, respectively). The combination of aspirin and non-aspirin NSAID was more damaging than aspirin alone. Age, sex, concomitant use of proton pump inhibitors, indications for NSAIDs use, duration of NSAIDs use and *CYP2C9*2*, **3* and **13* SNPs were unrelated. The use of meloxicam and *CYP2C9*3* SNPs were significantly associated with an increased risk for diaphragm disease (adjusted OR: 183.75, 95% CI: 21.34–1582.38; $P < 0.0001$ and adjusted OR: 12.94, 95% CI: 1.55–108.36, $P = 0.018$, respectively).

Conclusions
The use of specific NSAIDs and the factors interfering with NSAIDs metabolism might associate with small intestinal injury, especially with diaphragm disease.

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INTRODUCTION

Gastrointestinal bleeding associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs) is a common adverse drug reaction in developed countries. The risk factors of NSAID-induced gastroduodenal ulcers have been identified and include old age, history of peptic ulcer disease, concomitant use of corticosteroid and anticoagulants, higher doses of NSAIDs including the use of more than one NSAID, serious systemic disorder, concomitant infection with *Helicobacter pylori*, etc.[1] In addition, pharmacogenetic risk factors, such as cytochrome P450 2C9 (CYP2C9) polymorphisms, have been identified. Individuals with the loss-of-function alleles *CYP2C9*3* (Ile359Leu, A1075C) and *CYP2C9*2* (Arg144Cys, C430T) are exposed to higher plasma concentrations of the drugs[2] and to a higher risk of NSAIDs-related acute upper gastrointestinal bleeding.[3, 4] *CYP2C9*13* (Leu90Pro, T269C) is also associated with the decreased metabolism of an NSAID of the oxicam class with a long plasma half-life, meloxicam.[5] Not only in the upper gastrointestinal tract but also in the small intestine, NSAID-induced mucosal injury has been reported at a post-mortem examination and latest enteroscopy, videocapsule endoscopy (VCE) and double-balloon endoscopy (DBE).[6–11] In addition, diaphragm-like small intestinal stenosis, so-called diaphragm disease, is a relatively rare NSAID-induced complication that is notable because it can cause small bowel obstruction and VCE retention.[12–14] The risk factors of these NSAIDs injuries in the small intestine have not been well identified. A selective cyclooxygenase-2 (COX-2) inhibitor was reported to be significantly less injurious than traditional NSAIDs in the small bowel,[9] but another report did not find this difference.[11] Proton pump inhibitors (PPIs) exacerbate NSAID-induced small intestinal injury in a rat model by inducing dysbiosis,[15] but whether this relationship also occurs in humans remains unknown.

Therefore, we determined the risk factors of symptomatic NSAIDs injuries, including diaphragm disease in the small intestine, in 156 NSAID users and 1106 non-users who underwent DBE and/or VCE. We further evaluated the genetic effect of *CYP2C9*2*, **3* and **13* polymorphisms on their risk in a case–control study.

METHODS

Patients and controls

Of the 1262 symptomatic patients who underwent VCE and/or DBE at Nagoya University Hospital between June 2003 and December 2011, 156 patients (NSAIDs users) were verified as having been given NSAIDs during a 4-week period prior to VCE or DBE, and 1106 patients (NSAIDs non-users) were identified as not having been treated with NSAIDs (Table 1). Out-patient section nurses and doctors checked out-patients' compliance of prescribed drugs in the first consultation, and ward pharmacists checked in-patients' compliance of prescribed drugs on admission. NSAIDs included traditional NSAIDs, selective COX-2 inhibitors and aspirin. The definition of NSAID-induced small intestinal injury was (i) having a history of NSAIDs use; (ii) having endoscopic findings of erosion, ulcer or typical diaphragm-like strictures; (iii) having symptoms, such as obscure gastrointestinal bleeding or small bowel obstruction; (iv) showing improved clinical findings and/or endoscopic findings after the cessation of NSAIDs, except for diaphragm-like strictures; and (v) having no other causes, such as malignant tumour, inflammatory bowel disease or infectious disease. The controls for *CYP2C9* genotyping were apparently healthy individuals and were recruited from health check-up examinees who had undergone gastroscopy and/or double-contrast barium meal radiography as part of a screening programme for gastric carcinoma, as previously described.[16] All these control subjects (326 men and 96 women, 53.1 ± 10.0 years of age) were Japanese, and none of them had anaemia. Genotype frequencies of polymorphisms in the *MDM2* and four cytokine genes did not deviate significantly from those expected under the Hardy–Weinberg equilibrium in these controls. These studies were reviewed and approved by the Institutional Review Boards and Ethics Committees of Nagoya University School of Medicine.

Double-balloon enteroscopy and videocapsule endoscopy

DBE (EN-450T5 or P5; FUJIFILM Medical Co., Ltd., Tokyo, Japan) and VCE (PillCam SB and SB2; Given Imaging, Ltd., Yokneam, Israel, or Endocapsule, Olympus Medical Systems Corp., Tokyo, Japan) were performed as previously described.[17, 18] Bioptic specimens at DBE were reviewed histologically unless the patients took antiplatelets or anticoagulants. A specific pathological finding of NSAID-induced injuries included both crypt apoptosis and tissue eosinophilia.[19] Endoscopic pictures of all patients were reviewed by two gastroenterologists with more than 5 years of experience with DBE and VCE. The endoscopic morphologies of the NSAID-related injuries were classified into four categories according to their annular ulcers/scars including ulcers/

Table 1 | Clinical characteristics of patients who underwent DBE and/or VCE

	NSAIDs users			NSAIDs injury-positive vs. negative users			NSAIDs injury-positive users vs. non-users		
	NSAIDs injury-positve	NSAIDs injury-negative	NSAIDs non-users	*P*	Crude OR	95% CI	*P*	Crude OR	95% CI
No. of patients	31	125	1106						
Men/women	18/13	62/63	684/422	0.399	1.407	0.635–3.115	0.669	0.854	0.414–1.761
Age (years) at examinations	68.3 ± 10.9	68.2 ± 12.2	54.7 ± 19.4	0.756	–	–	<0.001	–	–
Comorbidities, no. of patients (%)	21 (68%)*	65 (52%)†	396 (36%)	0.115	1.939	0.845–4.490	<0.001	3.765	1.756–8.075
Indications for examinations, no. of patients (%)									
OGIB	26 (84%)	108 (86%)	616 (56%)	0.454	0.819	0.277–2.423	0.001	4.136	1.577–10.851
SBO	5 (16%)	4 (3%)	134 (12%)	0.315	1.525	0.504–4.612	0.159	1.893	0.712–5.036
Others	0 (0%)	13 (10%)	356 (32%)	0.005	0.000	–	<0.001	0.000	–
Use of PPI, no. of patients (%)	15 (48%)	44 (35%)	126 (11%)	0.175	1.726	0.780–3.819	<0.001	7.292	3.520–15.107
NSAIDs, no. of patients (%)									
NSAIDs other than LDA	16 (52%)	50 (41%)	–	0.241	1.600	0.726–3.526	–	–	–
LDA	6 (19%)	68 (54%)	–	<0.001	0.201	0.077–0.524	–	–	–
Both	9 (29%)	7 (5%)	–	<0.001	6.896	2.324–20.461	–	–	–
Species of NSAIDs									
Enteric-coated aspirin, 100 mg	13	60	–	0.545	0.782	0.353–1.733	–	–	–
Regular aspirin, 81 mg	2	15	–	0.300	0.506	0.109–2.338	–	–	–
Loxoporofen	9	33	–	0.767	1.141	0.477–2.727	–	–	–
Diclofenac	9	11	–	0.006	4.240	1.572 – 11.435	–	–	–
Meloxicam	5	3	–	0.008	7.821	1.758–34.793	–	–	–
Others	2	10	–	0.561	0.793	0.165–3.819	–	–	–
Indications for NSAIDs use, no. of patients (%)									
Osteoarthropathy	13	28	–	0.027	2.502	1.093–5.727	–	–	–
Rheumatoid arthritis	7	7	–	0.008	4.917	1.590–15.310	–	–	–
Cardiovascular disease	8	46	–	0.249	0.597	0.247–1.444	–	–	–
Cerebro-vascular disease	7	28	–	0.983	1.010	0.394–2.590	–	–	–
Others	5	32	–	0.267	0.559	0.198–1.578	–	–	–
Duration of NSAIDs use (m), median (range)	48 (0.5–264)	36 (0.25–240)	–	0.102	–	–	–	–	–

DBE, double-balloon enteroscopy; VCE, videocapsule endoscopy; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; CI, confidence interval; OGIB, obscure gastrointestinal bleeding; SBO, small bowel obstruction; PPI, proton pump inhibitor; LDA, low-dose aspirin.

* Heart disease (n = 11), collagen disease (n = 6), chronic kidney disease (n = 5), malignancy (n = 5) and chronic obstructive pulmonary disease (n = 2) that were overlapped.

† Heart disease (n = 51), chronic kidney disease (n = 14), malignancy (n = 6), collagen disease (n = 6), malignancy (n = 6), chronic obstructive pulmonary disease (n = 4), liver cirrhosis (n = 1) that were overlapped.

scars that extended in the circumferential direction, small round or oval ulcers/scars or erosions, longitudinal ulcers/scars, and diaphragm-like strictures (Figure 1A). A diaphragm-like stricture (diaphragm disease) was designated as a stricture with a thin concentric, diaphragm-like septum that could not be traversed by therapeutic DBE (EN-450T5), the outer diameter of which is 9.4 mm. Capsule retention was designated as a capsule remaining in the digestive tract for a minimum of 2 weeks or a capsule remaining in the bowel lumen unless medical, endoscopic or surgical intervention was instituted. Enteroscopic balloon dilation by using

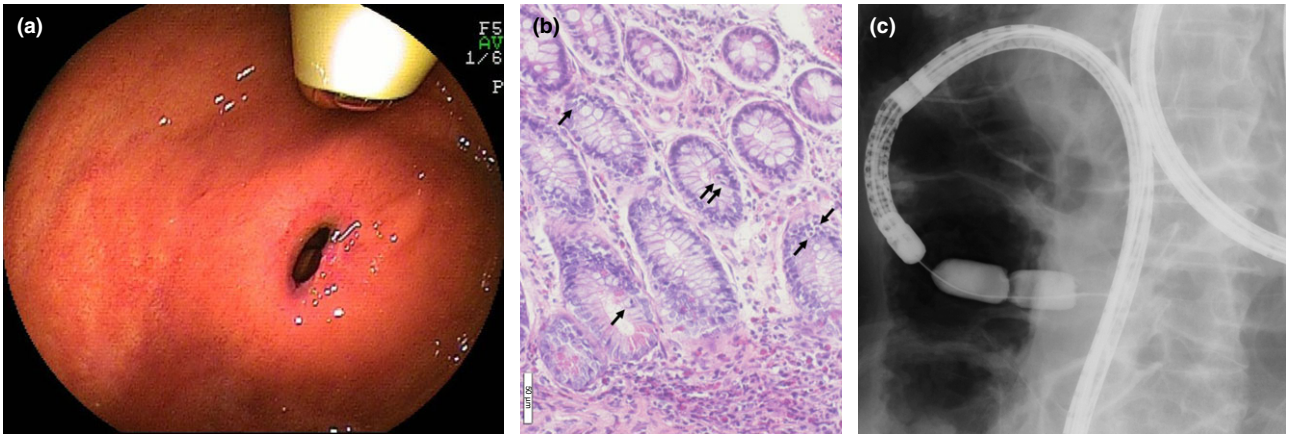

Figure 1 | Diaphragm disease in a patient heterozygous for *CYP2C9*3* who had taken meloxicam for 6 years. (a) Antegrade enteroscopic view of a stricture in the ileum where a capsule endoscope was lodged. (b) A biopsy specimen from a circumferential erosion on a stricture showing apoptotic bodies (arrows) in the crypts of Lieberkuhn and tissue eosinophilia. (c) Roentgenogram showing the balloon dilation of a stricture.

through-the-scope balloon catheters (Boston Scientific Corp, Natick, MA, USA)[20] or surgical resection was performed for diaphragm disease.

CYP2C9 genotyping

Genomic DNA was extracted from peripheral blood leucocytes, as previously described.[16] The *CYP2C9*2*, **3* and **13* single nucleotide polymorphisms (SNPs) were determined by allelic discrimination with Taqman 5'-nuclease assays. Subjects were genotyped for *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910) and *CYP 2C9*13* (rs72558187) SNPs in the CYP2C9 gene by using a StepOne Real-Time PCR System (Applied Biosystems, Tokyo, Japan). Standardised TaqMan assays were used to detect *CYP2C9*2* (C_25625805-10), *CYP2C9*3* (C_27104892-10) and *CYP2C9*13* (C_34816136-20) in the *CYP2C9* gene.

Statistical analyses

The numbers in the text are expressed as the means ± standard deviations or median (range), and comparisons were analysed with the Mann–Whitney *U*-test. Categorical variables are expressed as percentages and compared among the groups using χ^2 statistics. Multivariate analysis of the clinical risks of symptomatic NSAID-induced small intestinal injury in 161 NSAIDs users was performed using unconditional logistic regression models to adjust for age (< or ≥65 years of age), sex, comorbidities (presence of heart disease, chronic kidney disease, cirrhosis, chronic obstructive pulmonary disease, collagen disease or malignant tumours), concomitant use of PPI, use of aspirin alone, use of diclofenac or oxicams, indications for NSAIDs use (rheumatoid arthritis or not) and duration of NSAIDs use (< or ≥42 months). Adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were computed. Multivariate analysis of the clinical risks for diaphragm disease was performed using unconditional logistic regression models to adjust for age and sex. An OR for the genetic effect of *CYP2C9*3* polymorphisms, adjusted for sex and age, and the 95% CIs were computed using unconditional logistic regression models. The Hardy–Weinberg equilibrium of alleles at individual loci was assessed by χ^2 statistics or Fisher's exact probability test. Differences were considered significant when the *P* values were less than 0.05, except in comparisons of age, sex and the proportions of patients among the three different groups, in which *P* values less than 0.0167 were considered significant.

RESULTS

Clinical characteristics of patients with NSAID-induced small intestinal injury

Of the 156 NSAIDs users, 31 consecutive patients (20%) were diagnosed with NSAID-induced small intestinal injury. The clinical characteristics are shown in Table 1. Of these 31 patients, 26 with OGIB underwent VCE followed by DBE, and five with SBO underwent DBE only. Of the remaining 125 NSAID-induced injury-negative users, 55 with OGIB, four with other indications

such as protein-losing enteropathy, abdominal pain and distention underwent VCE followed by DBE; 24 with OGIB, four with small bowel obstruction, three with other indications such as Crohn's disease and abdominal pain underwent DBE only; and 29 with OGIB, six with other indications such as diarrhoea, abdominal pain and distention underwent VCE only. Univariate analysis is shown in Table 1. The use of NSAIDs was associated with advanced age, but the frequency of NSAID-induced small intestinal injury was not associated with advanced age among NSAIDs users. PPIs were used to treat or prevent NSAID-induced gastroduodenal damage in 15 (48%) NSAIDs injury-positive NSAIDs users and 44 (35%) NSAIDs injury-negative NSAIDs users, and the difference was not significant. NSAID-induced gastroduodenal ulcers were observed in 24 (15%) NSAIDs users, three (13%) of whom had concomitant small intestinal injuries.

Multivariate analysis is shown in Table 2. The presence of comorbidities and the use of oxicams or diclofenac were associated with increased risks of NSAID-induced small intestinal injuries, and the use of both aspirin and non-aspirin NSAIDs was associated with an increased risk compared with the use of aspirin alone.

At enteroscopy, low-dose aspirin injuries were multiple in four of six users (67%), and injuries induced by non-aspirin NSAIDs and both aspirin and non-aspirin NSAIDs were multiple in 24 of 25 users (96%, $P = 0.09$). Of the 185 injuries observed at VCE and/or DBE, the most common morphology was annular ulcers/scars ($n = 128$), followed by round ulcers or erosions ($n = 42$), diaphragm-like strictures ($n = 14$) and longitudinal ulcers ($n = 1$). Table 3 shows the numbers of lesions detected at VCE and DBE. Overall, 3, 42, 31 and 85 lesions were located in the proximal jejunum, the distal jejunum, the proximal ileum and the distal ileum respectively. Of the 17 low-dose aspirin injuries observed in six patients, the most common morphology was round ulcer or erosions ($n = 11$), followed by annular ulcers or scars ($n = 6$); two, three, four, and eight lesions were located in the proximal jejunum, the distal jejunum, the proximal ileum and the distal ileum respectively. Annular, longitudinal ulcers/scars and diaphragm-like strictures were more frequently observed in injuries induced by both non-aspirin NSAIDs and both types (89%) than in low-dose aspirin injuries (35%, $P < 0.0001$). Especially, annular ulcers or scars (23 cases, 128 lesions) were significantly associated with diclofenac or oxicams (9 cases/57 lesions, 5 cases/28 lesions, respectively, $P < 0.0001$). The location of the lesions was not significantly different between low-dose aspirin injuries and injuries induced by non-aspirin NSAIDs or both ($P > 0.999$).

The clinical characteristics of the seven patients with diaphragm disease are shown in Tables 4, 5, and Figure 1A. With respect to drug species, meloxicam was used in five of seven patients with diaphragm-like strictures (71%), while it was used in none of the 23 patients without diaphragm-like strictures (0%) ($P = 0.001$). The use of meloxicam was significantly associated with an increased risk of diaphragm disease (adjusted OR: 183.75, 95% CI, 21.34–1582.38, $P < 0.0001$).

Table 2 | Multivariate analysis in NSAIDs users

	NSAIDs injury-positive vs. negative users		
	P	Adjusted OR	95% CI
Men/women	0.676	1.233	0.462–3.295
Age (≥ or <65 years of age)	0.107	2.408	0.828–7.008
Comobidities	0.041	2.965	1.045–8.414
Use of PPI	0.350	1.590	0.601–4.208
Species of NSAIDs (diclofenac or oxicams)	0.002	7.047	2.035–24.400
NSAIDs combination			
Both	0.010	7.913	1.635–38.295
Non-aspirin NSAID alone	0.799	1.203	0.289–5.013
Aspirin alone	–	–	–
Indications for NSAIDs use	0.082	3.637	0.848–15.588
Duration of NSAIDs use	0.092	2.808	0.845–9.335

NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; CI, confidence interval; PPI, proton pump inhibitor.

Pathological characteristics of NSAID-induced small intestinal injury

DBE-directed biopsies and surgical specimens were obtained from 49 lesions in 22 patients. Crypt apoptosis and tissue eosinophilia was pathologically confirmed only in four lesions (8%) from four patients (18%). Of these patients, three had diaphragm disease (Figure 1B), and the other had multiple annular ulcers presenting with small bowel obstruction. Two patients received meloxicam; one, loxoprofen; and the other, both loxoprofen and aspirin. Nonspecific inflammation was observed in the remaining lesions.

Table 3 | Enteroscopic findings of NSAID-induced small intestinal injury

	VCE (21 patients)		DBE (31 patients)	
	No. of patients	No. of lesions	No. of patients	No. of lesions
Annular ulcers/scars	13 (62%)	57	23 (74%)	128
Round ulcers or erosions	10 (48%)	42	4 (13%)	18
Diaphragm-like strictures	3 (14%)	3	8 (26%)	14
Longitudinal ulcers	1 (5%)	1	1 (3%)	1

NSAID, nonsteroidal anti-inflammatory drug; VCE, videocapsule endoscopy; DBE, double-balloon enteroscopy.

CYP2C9 polymorphism

Because *CYP2C9*2* and **13* polymorphisms were observed in no patients with NSAID-induced small intestinal injuries, further genotyping was not performed in the controls. *CYP2C9*3* polymorphisms in patients with diaphragm-like strictures are shown in Table 5. A patient homozygous for this polymorphism had six strictures; a heterozygous patient had three strictures; and the remaining wild-type patients had only one stricture. The genotypic frequencies of *CYP2C9*3* polymorphisms between patients with diaphragm disease and controls are shown in Table 6. The genetic risk of the diaphragm disease for *CYP2C9*3* polymorphism (**3/*3* and **1/*3*) was significantly increased, with a crude OR of 8.98 (95% CI: 1.63–49.46, $P = 0.038$) and an adjusted OR of 12.94 (95% CI: 1.55–108.36, $P = 0.018$) when compared with wild type (**1/*1*). The genotypic frequencies of *CYP2C9*3* polymorphisms in patients and controls did not deviate significantly from the expected frequencies under Hardy–Weinberg equilibrium. The frequency of NSAID-induced small intestinal injuries was not significantly associated with *CYP2C9*3* polymorphisms, with a crude OR of 2.09 (95% CI: 0.46–9.55, $P = 0.285$) and an adjusted OR of 4.23 (95% CI: 0.59–30.5, $P = 0.153$).

Treatment and prognosis of NSAID-induced small intestinal injury

The therapeutic interventions during or after enteroscopy included the cessation of NSAIDs in 23 patients, a change to other NSAIDs in four patients, the use of misoprostol in six patients, enteroscopic balloon dilation

Table 4 | Clinical characteristics of small intestinal diaphragm disease

Patient	Age at onset	Sex	DBE indications	Hb level (g/dL)	Serum albumin (g/dL)	NSAIDs used			
						Type	Dose/day	Duration (months)	Indications
1	71	M	SBO	14.5	4.3	Meloxicam	15 mg	36	OA
2	76	M	OGIB with capsule retention	7.9	3.3	Meloxicam Loxoprofen	10 mg 180 mg	72 1	OA
3	73	F	OGIB with abdominal pain	7.3	3.0	Meloxicam	10 mg	24	RA
4	80	M	OGIB with capsule retention	5.8	3.5	Meloxicam	10 mg	43	RA
5	64	M	OGIB with abdominal pain	7.3	3.9	Meloxicam Aspirin	5 mg 100 mg	24 144	OA post-PCI for AMI
6	74	F	SBO	8.1	3.0	Diclofenac*	25-50 mg	264	RA
7	78	M	OGIB with capsule retention	8.5	4.0	Loxoprofen Aspirin	60 mg 100 mg	12 92	OA post-CABG for AP

DBE, double-balloon enteroscopy; Hb, haemoglobin; NSAIDs, nonsteroidal anti-inflammatory drugs; SBO, small bowel obstruction; OGIB, obscure gastrointestinal bleeding; OA, osteoarthropathy; RA, rheumatoid arthritis.

* Rectal diclofenac suppository.

Table 5 | Location and *CYP2C9* SNPs of small intestinal diaphragm disease

Patient	Lesions: Location	Lesions: No. of diaphragms balloon-dilated/resected	SNPs: *CYP2C9*
1	Jejunum-Ileum	5/1†	*3/*3
2	Ileum	3/0	*1/*3
3	Ileum	1/0	*1/*1
4	Ileum	1/0	*1/*1
5	Ileum	1/0	*1/*1
6	Ileum	0/1‡	*1/*1
7	Ileum	1/0	*1/*1

SNPs, single nucleotide polymorphisms; *CYP2C9*, cytochrome P450 enzyme 2C9.

† One diaphragm-like stricture surgically resected for the pathological diagnosis at the bladder reconstruction using ileal conduit after radical cystectomy for bladder cancer.

‡ One diaphragm-like stricture surgically resected due to complicating deep ulcers, which pathologically reached the muscle layer.

for diaphragm disease in six patients (Figure 1C), surgical resection in two patients, and enteroscopic capsule retrieval in two patients (cases #2 and #7) (Table 4). A capsule endoscope lodged above diaphragm-like strictures was excreted on the 15th day after ingestion in one patient (case #4). Laboratory testing detected no relapsing anaemia in 26 patients with obscure gastrointestinal bleeding during the follow-up period after examination (median, 35 months; range, 6–84 months). The small bowel obstruction had not relapsed after the enteroscopic balloon dilation in one patient with diaphragm disease, both enteroscopic balloon dilation and surgical resection in one patient with diaphragm disease, discontinuance of NSAIDs in three patients, or a change to other NSAIDs in two patients during the follow-up period after examination (median, 76 months; range, 16–102 months).

DISCUSSION

The diagnostics of NSAID-induced small intestinal injuries has evolved since the advent of VCE and deep enteroscopy. In this study, the presence of comorbidities and the use of oxicams or diclofenac were associated with increased risks of symptomatic NSAID-induced small intestinal injury, whereas advanced age, sex, concomitant use of PPI and duration of NSAIDs use were unrelated. The risk for the development of NSAID-associated gastroduodenal ulcers was also high in patients with serious systemic disorders who took piroxicam and arylacetic acid derivatives, such as indomethacin and tolmetin.[1] In this study, the use of both aspirin and non-aspirin NSAIDs was more injurious to the small bowel with respect to the frequency and severity of mucosal breaks compared with aspirin alone. The Japanese Study Group for DBE reported that mucosal breaks were more frequent in patients taking non-aspirin NSAIDs (25/41, 61%) than in patients taking aspirin (6/20, 30%).[7] The faecal excretion of ^{111}In white cells, which represent intestinal inflammation, was significantly increased among arthritic patients taking conventional NSAIDs, except among those patients taking aspirin; the rate of inflammation in this subset of patients did not differ significantly from controls.[21] In this study, however, non-aspirin NSAIDs was as injurious as aspirin. No patients had injuries associated with a selective COX-2 inhibitor in this study. A selective COX-2 inhibitor may be significantly less injurious than traditional NSAIDs in the small bowel, but further study is needed because celecoxib is the only COX-2 inhibitor used in Japan. It was approved for

Table 6 | *CYP2C9* polymorphisms between patients with small intestinal diaphragm disease and controls

	Patients (n = 7)* *N* (%)	Controls (n = 422)* *N* (%)	Crude analysis: OR	95% CI	*P*	Adjusted analysis: OR	95% CI	*P*
Genotype								
*CYP2C9*1/*1*	5 (71.4)	404 (95.7)	1.00	Reference	–	1.00	Reference	–
*CYP2C9*3 carriers*	2 (28.6)	18 (4.3)	8.98	1.63–49.46	0.038	12.94	1.55–108.36	0.018
Variant allele								
*CYP2C9*1*	11 (78.6)	826 (97.8)	1.00	Reference	–	1.00	Reference	–
*CYP2C9*3*	3 (21.4)	18 (2.2)	9.02	3.15–47.36	0.0002	16.3	2.85–94.0	0.002

OR, odds ratios; CI, confidence intervals.

* Hardy–Weinberg equilibrium, $P > 0.9999$.

patients with rheumatoid arthritis and osteoarthropathy in 2007 and for patients with diseases such as lumbagos and scapulohumeral periarthritis in 2009.

Diaphragm disease is a unique small intestinal pathology associated with NSAIDs, but its aetiology remains unknown. To our knowledge, this study is the first to demonstrate that the use of meloxicam and *CYP2C9*3* polymorphism were significantly associated with increased risks of diaphragm disease. Based on 42 cases with small intestinal diaphragm disease from the previously published literature[12–14, 22] and the seven cases from this study, the most frequent NSAIDs used were the oxicams (27%; piroxicam in eight and meloxicam in five), followed by the arylacetic acid derivatives (24%; diclofenac in nine and indomethacin in three), the coxibs (16%; rofecoxib in five and celecoxib in three), and the propionic acid derivatives (16%; loxoprofen in three, naproxen in two, ibuprofen in two, and flurbiprofen in one). The oxicams are enolic acids, and their main advantage is a long $t_{1/2}$ (the time it takes for the plasma concentration to be reduced by 50%), which permits once-a-day dosing except in the case of lornoxicam.[23] Although the information on the second most frequently causative agents, the arylacetic acid derivatives, was not detailed, some drugs used were of a slow release-type, with a long $t_{1/2}$ or t_{max} (the time to the peak plasma concentration after dosing).[13, 24, 25] Thus, these causative NSAIDs have relatively longer plasma half-lives. Hepatic biotransformation followed by renal excretion is the principal route of elimination for the majority of NSAIDs. NSAIDs are principally metabolised by CYP2C9 in liver microsomes. The $t_{1/2}$ can increase in the elderly and in subjects with renal insufficiency, advanced hepatic damage and *CYP2C9* polymorphisms.[2, 23] In this study, crypt apoptosis was pathologically confirmed chiefly in patients with diaphragm disease, which suggests a longer exposure to a higher concentration of NSAIDs.[23] Taken together, the chronic use of NSAIDs with long plasma half-lives and *CYP2C9* polymorphisms are likely to induce higher plasma concentrations of NSAIDs over time and may eventually lead to diaphragm disease. Meloxicam and diclofenac show a degree of selectivity for COX-2 that is similar to that of celecoxib. Piroxicam, however, is a nonselective COX inhibitor; thus, it is unlikely that this COX-2 isoform selectively associates with the development of diaphragm disease. The genetic frequencies of *CYP2C9* polymorphisms differ by ethnicity.[26, 27] At least one variant allele of *CYP2C9*2* and **3* is present in ~25% of European-Americans, but these variants are relatively uncommon in African-American and Asian populations. The *CYP2C9*2* and **3* genotype frequencies are 0% and 5% (**1/*3*, 4%; **3/*3*, 1%) in Asians, respectively,[28] which is consistent with this study. Generally, the **3* allele has a greater effect than the **2* allele. For the diagnosis of diaphragm disease, radiographic studies are inaccurate because thin-walled diaphragms could be mistaken for numerous Kerckring's folds and peristalses. Patients with early stages of diaphragm disease present without typical small bowel obstruction but with only abdominal pain or obscure gastrointestinal bleeding, as shown in this study. If the patients recovered after the cessation of causative NSAIDs or the change to another NSAID, they may not have been diagnosed with diaphragm disease. VCE is diagnostically effective, but may become lodged above a diaphragm-like stricture. If our findings are corroborated in a larger series that samples other ethnicities, appropriate surveillance and preventive measures could be formulated according to *CYP2C9* polymorphisms, especially in populations with high genetic frequencies. The elderly and patients with comorbidities should also avoid taking large amounts of NSAIDS with long half-lives. Patients with small intestinal diaphragm disease are often surgically treated,[22] but most cases indicate for enteroscopic balloon dilations because ulcers and fibrosis are mostly limited to the submucosal layer.[20, 25]

This study has some inherent bias because the database used direct referrals for DBE and VCE, and it was a retrospective study. The frequency of symptomatic small intestinal injury in NSAIDs users was 20% in this study. Previous VCE studies have identified NSAID-induced lesions in 50–71% of NSAIDs users,[8–11] but these studies analysed healthy subjects or asymptomatic patients and indicated that most lesions were not clinically pathogenic. In a 6-month double-blind, randomised trial comparing gastrointestinal toxicity between celecoxib and diclofenac combined with omeprazole in 4484 arthritic patients, definite small bowel haemorrhage was diagnosed in no patients, and acute haemorrhage of unknown origin, including presumed small bowel haemorrhage, was observed only in one patient.[29] Therefore, the present study still might overrepresent its incidence.

In conclusion, the presence of comorbidities and the use of oxicams (meloxicam, ampiroxicam and lornoxicam) or diclofenac were associated with an increased risk of symptomatic NSAID-induced small intestinal injury. Especially in patients with diaphragm diseases, meloxicam and *CYP2C9*3* polymorphisms were considered risk factors.

AUTHORSHIP

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