Increased Gastric Mucus Secretion Alleviates Non-Steroidal Anti-Inflammatory Drug-Induced Abdominal Pain

Toshimitsu Iwabuchi,[1] Katsunori Iijima,[1] Nobuyuki Ara,[1] Tomoyuki Koike,[1] Hirohiko Shinkai,[1] Takafumi Ichikawa,[2] Yayoi Kamata,[2] Kazuhiko Ishihara[3] and Tooru Shimosegawa[1]

[1]Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan
[2]Department of pathological biochemistry, Kitasato University School of Allied Health Sciences, Sagamihara, Kanagawa, Japan
[3]Kitasato Junior College of Health and Hygienic Sciences, Minamiuonuma, Niigata, Japan

Non-steroidal anti-inflammatory drugs (NSAIDs) can cause dyspeptic symptoms, including abdominal pain. Gastric mucus is important as the first line of defense against luminal irritants. In the present study, we investigated whether gastric mucus secretion could influence the severity of gastric mucosal injuries or NSAID-induced dyspeptic symptoms. Fifteen *Helicobacter pylori*-negative, healthy males were administered two types of NSAIDs, a non-selective cyclooxygenase inhibitor, naproxen (300 mg, twice a day), or a cyclooxygenase-2-selective inhibitor, etodolac (200 mg, twice a day), for 1 week in a crossover study, with an interval of ≥ 4 weeks. Study participants underwent endoscopic examinations before and after treatment. Pentagastrin-stimulated gastric secretions were collected for 10 min during endoscopic examinations, and were analyzed for gastric acid levels (mEq/10 min) and mucus output (mg hexose/10 min). The grade of gastric mucosal injury was assessed endoscopically. Among 29 subjects who completed the crossover study, 11 individuals reported abdominal pain following the administration of naproxen or etodolac for 1 week, as judged by elevated pain scores, while 18 individuals did not report abdominal pain. The occurrence of symptoms was not associated with the type of NSAIDs administered or the occurrence of erosive injury visualized by endoscopy. Gastric mucus secretion was significantly increased in subjects without drug-induced abdominal pain ($P < 0.05$), whereas it was significantly reduced in those with drug-induced abdominal pain ($P < 0.05$). In conclusion, the occurrence of NSAID-induced abdominal pain is associated with reduced levels of gastric mucus secretion rather than the occurrence of endoscopic mucosal injury.

Keywords: dyspepsia; etodolac; gastric mucus secretion; naproxen; prostaglandin

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used as effective antipyretic analgesics in the treatment of a wide spectrum of conditions and diseases, ranging from the common cold to rheumatoid arthritis. In Western countries, the use of NSAIDs is reportedly the most important cause of upper gastrointestinal (GI) injury (Lassen et al. 2006; Cai et al. 2009; Musumba et al. 2012). A similar trend has become apparent in Japan, where the prevalence of drug-induced peptic ulcers should soon surpass that of conventional *Helicobacter pylori*-infected peptic ulcers (Ootani et al. 2006). The mechanisms underlying gastroduodenal mucosal damage are not fully understood, but it has been primarily attributed to NSAID-induced sup-pression of mucosal prostaglandin (PG) synthesis through the inhibition of the cyclooxygenase (COX) enzyme, which is involved in the rate-limiting step in PG synthesis (Wallace 2008).

Dyspeptic symptoms are also induced by NSAIDs, and it is well recognized that the extent and severity of endoscopic mucosal damage are not directly associated with an increased risk for dyspeptic symptoms (Larkai et al. 1987; Straus et al. 2002; Wildner-Christensen et al. 2006). NSAID-induced dyspepsia, although not life-threatening, is an important clinical issue because it can reduce a patient's quality of life considerably (Hawkey et al. 2005), and it could be an important cause of NSAID withdrawal (Lanas et al. 2012).

Stimulating gastric mucus secretion is one of the most

important functions of PGs because this enhances the defense of the gastroduodenal mucosa (Seidler et al. 1988; Quadros and Wilson 1991). Gastric mucus is important as the first line of defense against noxious luminal acid by creating an unstirred layer on the mucosal surface (Allen and Flemström 2005; Laine et al. 2008; Wallace 2008). Numerous experimental animal studies have demonstrated that NSAID-induced upper GI mucosal damage is mediated by a reduction in the production of gastric mucus (Azuumi et al. 1980; Morris et al. 1984; Asada et al. 1990). However, few human studies have investigated the effects of NSAIDs on gastric mucus secretion in relation to gastric PG levels (Marcinkiewicz et al. 1996; Jaworski et al. 2005).

There are two COX enzyme isoforms, the constitutively expressed COX-1 and the highly inducible COX-2. The anti-inflammatory benefits of NSAIDs are primarily derived from COX-2 inhibition, while COX-1 inhibition is often associated with GI toxicity. In comparison with the non-selective COX inhibitors (conventional NSAIDs), selective COX-2 inhibitors have been reported to possess a safer upper GI side-effect profile (Langman et al. 1999). Previous studies have consistently reported that gastric PG synthesis was inhibited by selective COX-2 inhibitors, if at all, to a lesser extent compared with conventional NSAIDs (Wight et al. 2001; Hawkey et al. 2001). In the present study involving healthy individuals, we compared the pharmacological actions of a selective NSAID, etodolac, and a non-selective NSAID, naproxen, on gastric secretory functions, including mucus secretion, to investigate whether changes in these parameters could be related to gastric mucosal damage. Moreover, we investigated the potential association of such changes in gastric secretory function with NSAID-induced dyspeptic symptoms.

Methods

Subjects

Fifteen male asymptomatic healthy subjects, all of whom were non-smokers, with a mean age of 26.7 years (range: 19-42 years), were enrolled in this study. All subjects were *Helicobacter pylori*-negative, as determined by the ^{13}C-urea breath test, and had normal endoscopic findings at the time of their inclusion into the study. None of the subjects took any drugs before they entered the study or during the study period. All subjects received naproxen sodium (300 mg twice a day) for 1 week or etodolac (200 mg twice a day) for 1 week, in a crossover format. The daily dose of each drug was chosen based on the recommended daily doses for osteoarthritis in Japan. The 2 studies were conducted in a random sequence with an interval of at least 4 weeks that comprised the drug-washout period.

During each study, endoscopic examination was performed before and after treatment, and the final dose of the drug was administered 2 h before the examination. Gastric secretory function was evaluated during each endoscopic examination using the endoscopic gastrin test (EGT) technique, and the extent of gastric mucosal injury was assessed. In addition, 3 biopsy specimens were obtained from the mucosa that appeared normal in the greater curvature of the antrum, and were immediately frozen in liquid nitrogen for subsequent measurement of the gastric PG concentration.

The study was approved by the Tohoku University School of Medicine Ethics Committee (No. 2009-165) and each subject gave written informed consent.

Evaluation of gastrointestinal symptoms

GI symptoms were assessed using the gastrointestinal symptom rating scale (GSRS) (Svedlund et al. 1988). The GSRS contains 15 items rated on a 7-point Likert scale, where a score of 1 represents "no discomfort" and a score of 7 represents "very severe discomfort". The items are divided into 5 major GI symptoms as follows: abdominal pain, reflux, indigestion, diarrhea, and constipation. The GSRS was applied before the drugs were administered and after the study drugs had been administered for 1 week, and the scores were averaged for each major GI symptom.

Gastric aspiration using the endoscopic gastrin test technique

The details of the EGT have been reported previously (Iijima et al. 1998, 2009). Briefly, subjects were injected intramuscularly with pentagastrin (Sigma, St. Louis, MO, USA) at a dose of 6 μg/kg at approximately 15 min before endoscopy. After entering the stomach with the endoscope, the gastric fluid that had pooled in the stomach was aspirated and discarded. Thereafter, the newly secreted gastric fluid produced 20-30 min after the pentagastrin injection was aspirated and collected under direct visualization during the routine endoscopic examination, during which the subjects were asked not to swallow their saliva. After the collection of the gastric fluid, the endoscope was removed. The volume of gastric juice collected in the 10-min period was recorded, the sample was divided, and half of the sample was analyzed for gastric acid secretion and the other half was analyzed for gastric mucus secretion. The investigators performing the laboratory work were blinded to the subjects' medical information.

Assessment of gastric acid secretions

The H^+ concentration of the gastric juice was determined by titration. The acid output in the 10-min period was calculated by multiplying the volume by the H^+ concentration, and the EGT value was expressed as mEq/10 min. We previously determined that the EGT values correlate very well with the peak acid outputs, as determined by conventional methods (correlation coefficient = 0.92), and have a high reproducibility (coefficient of variation = 5.6%) (Iijima et al. 1998).

Extraction and isolation of mucin in gastric juice

The gastric juice was centrifuged at 1,500 × g for 30 min at room temperature to remove the contaminating debris. The mucin in the gastric juice samples was extracted and isolated using a previously described method. This method successfully isolates and condenses the mucin from the gastric juice without contamination by non-mucin glycoproteins, including serum-type glycoproteins. Absolute ethanol (6 mL) was added to the supernatant (2 mL) obtained from the gastric juice, thus producing a 75% ethanol concentration (v/v). The resultant suspension was maintained at 4°C overnight to complete the precipitation, after which the precipitate was collected by centrifugation (8,000 × g for 30 min at 4°C). The pellet was dissolved in distilled water (2 mL) and its hexose content was measured using the phenol-sulfuric acid method. The mucin content of the gastric juice was expressed as the amount of hexose in the solution obtained by precipitation (μg/mL). The total mucus out-

put was determined by multiplying the mucin concentration by the volume of gastric juice collected during the 10-min period (Iijima et al. 2009, 2012).

Endoscopic evaluation of gastric mucosal injury

The grade of gastric mucosal injury was assessed based on the modified Lanza score (MLS) (Lanza et al. 1990; Iijima et al. 2011). In the present analysis, the scoring system was based on the number of erosions and/or ulcers regardless of the presence or absence of hemorrhaging, because petechial hemorrhages are generally of little clinical significance (Feldman et al. 2001). Thus, the gastric mucosal injuries were graded as follows: (a) Grade 0: no erosions; (b) Grade 1: 1-2 erosions localized in 1 area of the stomach; (c) Grade 2: 3-5 erosions localized in 1 area of the stomach; (d) Grade 3: 6-9 erosions localized in 1 area of the stomach, or no more than 10 lesions in 2 areas of the stomach; (e) Grade 4: erosions in 3 areas of the stomach, or no fewer than 10 lesions in the whole stomach; and (f) Grade 5: a gastric ulcer defined as a mucosal defect larger than 5 mm in diameter.

During endoscopy, more than 40 endoscopic images covering the whole area of the stomach were stored in a database, and the MLS was later graded independently by 2 endoscopists (KI and TI) who were blinded to any other information about the subjects. In cases of disagreement, a consensus was reached through joint review of the endoscopic pictures.

Measurement of gastric prostaglandin E_2 concentration

The concentration of PGE_2 in the gastric mucosa was measured in the biopsy specimens. The frozen tissue was homogenized in ice-cold T-PER buffer (Thermo SCIENTIFIC) and then centrifuged for 10 min at 12,000 rpm at 5°C. The supernatant of each sample was used to determine the total protein concentration using the BCA Protein Assay Kit, and to determine the PGE_2 concentration, expressed as ng/μg protein, by enzyme immunoassay using a PGE_2 kit (R&D systems) (Venerito et al. 2006).

Statistical analysis

Given the importance of the equilibrium between aggressive and protective factors in the pathogenesis of gastric mucosal injury (Iijima et al. 2012), we calculated the ratio of the total acid output relative to the total mucin output (acid/mucus ratio) in addition to the various gastric secretory parameters that were determined directly from the gastric aspirates. Since some of the raw data did not fit a normal distribution, continuous data as well as non-parametric data were expressed as medians (interquartile range), and the statistical significance of the differences was determined using the Wilcoxon rank sum test. A *P*-value of < 0.05 was considered to be statistically significant.

Results

All 15 subjects completed the study protocol without any problematic side effects. Gastric aspiration using the EGT technique yielded sufficient amounts of gastric fluid for gastric acid and mucus secretion analyses in all subjects. The data on gastric secretory function (etodolac study) from 1 subject were excluded from the analysis due to technical problems experienced with the measurement.

Comparisons between the effects of administration of naproxen and etodolac

Administration of naproxen or etodolac for 1 week induced divergent effects in terms of gastric erosive injury. The median MLS significantly increased with naproxen administration from 0 (1.0) before treatment to 4.0 (2.2) after treatment (*P* < 0.01). The administration of etodolac marginally increased the median MLS from 0 (1.0) before treatment to 0.5 (1.0) after treatment (*P* = 0.06) (Table 1).

Neither naproxen nor etodolac significantly affected levels of gastric acid secretion. Similarly, neither of the drugs significantly altered gastric mucus secretion, although etodolac administration induced a modest increase in gas-

Table 1. Changes in various parameters by 1-week administration of naproxen or etodolac.

Parameters	Naproxen (*n* = 15)			Etodolac (*n* = 14)		
	Pre-treatment	Post-treatment	*P* value	Pre-treatment	Post-treatment	*P* value
GSRS						
Abdominal pain	1.0 (0.2)	1.3 (1.8)	0.06	1.0 (0.3)	1.6 (1.0)	0.05
Reflux	1.0 (0)	1.0 (0)	N.S.	1.0 (0)	1.0 (0)	N.S.
Indigestion	1.0 (0.2)	1.0 (0)	N.S.	1.0 (0.8)	1.0 (0.3)	N.S.
Diarrhea	1.0 (0.5)	1.0 (0.2)	N.S.	1.0 (0.5)	1.0 (0.2)	N.S.
Constipation	1.0 (0)	1.0 (0)	N.S.	1.0 (0)	1.0 (0)	N.S.
Median MLS	0.0 (1.0)	4.0 (2.0)	< 0.01	0.0 (1.0)	0.5 (1.0)	0.06
Total acid secretion (mEq/10 min)	4.9 (1.9)	4.6 (1.9)	N.S.	4.8 (2.2)	4.5 (1.9)	N.S.
Total mucus secretion (mg hexose/10 min)	1.4 (2.1)	1.4 (1.7)	N.S.	1.0 (1.0)	1.4 (1.4)	N.S.
Acid/mucus ratio	4.2 (3.4)	3.5 (3.3)	N.S.	4.1 (4.5)	2.7 (4.3)	0.09
Gastric PGE_2 concentration (ng/μg protein)	2.2 (1.5)	0.7 (0.8)	< 0.01	2.3 (1.9)	2.6 (2.9)	N.S.

GSRS, gastro-intestinal symptom rating scale; MLS, modified Lanza score. All data represent median (inter-quatile range), and the statistical significance of differences the Wilcoxon rank sum test. N.S. represents *P* > 0.1.
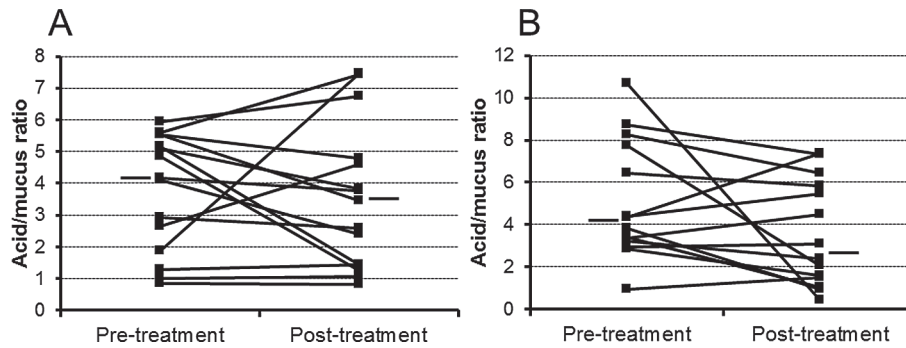
Fig. 1. Changes in gastric acid/mucus secretion ratio after administration of naproxen and etodolac.
Shown are changes in gastric acid/mucus secretion ratio after 1-week administration of naproxen ($n = 15$) (A) and etodolac ($n = 14$) (B). While acid/mucus ratio was unchanged by naproxen administration ($P = 0.5$), the ratio tended to decrease by etodolac administration ($P = 0.09$). Horizontal bars represent the median values.

Table 2. Changes in various parameters in subjects with or without the emergence of drug-induced abdominal pain.

Parameters	Without elevated abdominal pain score ($n = 18$)			With elevated abdominal pain score ($n = 11$)		
	Pre-treatment	Post-treatment	P value	Pre-treatment	Post-treatment	P value
Abdominal pain score	1.0 (0.9)	1.0 (0.6)		1.0 (0.2)	2.3 (1.4)	
Administered drugs (naproxen/etodolac) (n)	9/9			6/5		
MLS	0.0 (1.0)	1.5 (3.0)	< 0.01	0.0 (1.0)	2.0 (3.5)	< 0.05
Total acid secretion (mEq/10 min)	4.6 (2.0)	4.2 (1.7)	N.S.	5.0 (2.3)	4.8 (3.4)	N.S.
Total mucus secretion (mg hexose/10 min)	1.0 (0.7)	1.4 (2.5)	< 0.05	1.8 (1.8)	1.4 (0.8)	< 0.05
Acid/mucus ratio	5.0 (3.2)	2.2 (3.5)	< 0.01	3.4 (2.3)	4.5 (3.2)	0.07
Gastric PGE$_2$ concentration (ng/μg protein)	2.2 (1.1)	1.6 (2.5)	N.S.	2.3 (2.6)	0.6 (1.7)	0.05

MLS, modified Lanza score; PGE$_2$, prostaglandin E$_2$. All data represent median (inter-quatile range), and the statistical significance of differences the Wilcoxon rank sum test. N.S. represents $P > 0.1$.

tric mucus secretion, which was not observed with naproxen administration (Table 1). Consequently, the gastric acid/mucus ratio tended to decrease from 4.1 (4.5) before treatment to 2.7 (4.3) after etodolac administration ($P = 0.09$). Naproxen administration did not alter the gastric acid/mucus ratio ($P = 0.5$) (Table 1; Fig. 1).

Administration of naproxen for 1 week significantly suppressed the gastric PGE$_2$ concentration from 2.2 (1.5) ng/μg protein before treatment to 0.7 (0.8) ng/μg protein after treatment ($P < 0.01$). Etodolac administration did not significantly alter the gastric PGE$_2$ concentration, which was 2.3 (1.9) ng/μg protein before treatment and 2.6 (2.9) ng/μg protein after treatment (Table 1).

Comparison between individuals with and without elevated abdominal pain scores

Among 29 subjects who completed the crossover study, 11 subjects reported elevated abdominal pain scores following the administration of the drug for 1 week, and 18 subjects did not show elevated abdominal pain scores. The data obtained from both the naproxen and etodolac studies were considered together, and the changes in the different parameters before and after drug administration were com-

pared between the subjects who reported elevated abdominal pain ($n = 11$) and those who did not report elevated abdominal pain ($n = 18$). The results of this analysis are presented in Table 2.

Administration of either naproxen or etodolac tended to increase the abdominal pain scores (Table 1). Consequently, drug-induced abdominal pain developed in 6 of 15 (40%) individuals administered naproxen and 5 of 14 (36%) individuals administered etodolac (Table 2). Further, the occurrence of endoscopic erosive injury showed a similar pattern after drug administration irrespective of the occurrence of drug-induced abdominal pain, and the MLS significantly increased in both groups (Table 2). Nonetheless, we found marked differences in the gastric secretory parameters between the 2 subgroups. The median gastric mucus secretion significantly increased from 1.0 (0.7) mg hexose/10 min before treatment to 1.4 (2.5) mg hexose/10 min after treatment in those individuals who did not report drug-induced abdominal pain ($P < 0.05$). Individuals who reported drug-induced abdominal pain showed a significant decrease in the median gastric mucus secretion from 1.8 (1.8) mg hexose/10 min to 1.4 (0.8) mg hexose/10 min ($P < 0.05$) (Table 2; Fig. 2). There was no significant difference
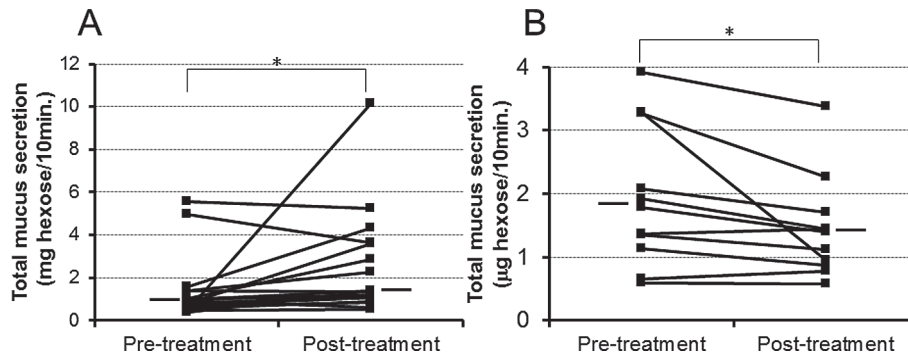
Fig. 2. Different changing patterns in total gastric mucus secretion in subjects with or without drug-induced abdominal pain.
Total mucus secretion was significantly increased by the drug administration in subjects ($n = 18$) without drug-induced abdominal pain (A). In contrast, it was significantly decreased by the drug administration in subjects ($n = 11$) with the symptoms (B). Horizontal bars represent the median values. *$P < 0.05$.
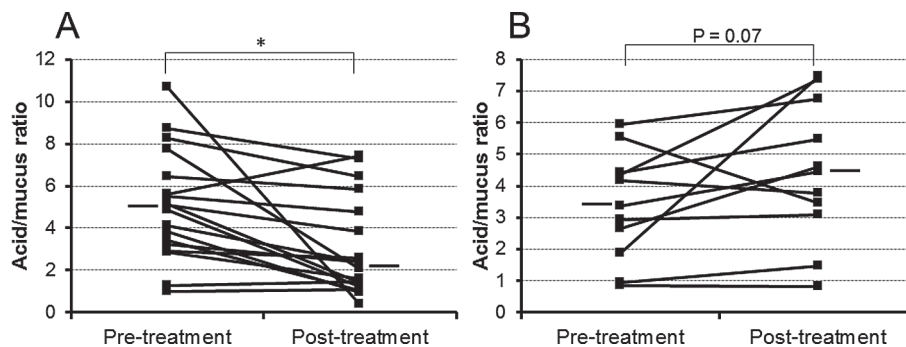

Fig. 3. Different changing patterns in gastric acid/mucus secretion ratio in subjects with or without drug-induced abdominal pain.
Gastric acid/mucus secretion was significantly decreased by the drug administration in subjects ($n = 18$) without drug-induced abdominal pain (A). Meanwhile, the ratio tended to increase in subjects ($n = 11$) with the symptoms ($P = 0.07$) (B). Horizontal bars represent the median values. *$P < 0.01$.

in the levels of gastric acid secretion before and after drug administration, regardless of the occurrence of symptoms. Consequently, the gastric acid/mucus ratio decreased in all but 2 of 18 subjects in whom abdominal pain did not develop, and the median value significantly decreased from 5.0 (3.2) before treatment to 2.2 (3.5) after treatment ($P < 0.01$). In contrast, the median acid/mucus ratio tended to increase from 3.4 (2.3) to 4.5 (3.2) in those with symptoms ($P = 0.07$) (Table 2; Fig. 3). Furthermore, gastric PGE_2 levels tended to reduce upon the administration of drugs, but only in those with symptoms ($P = 0.05$) (Table 2).

Discussion

We compared two types of NSAIDs in the present study, and noted that the effects of the COX-2-selective NSAID, etodolac, on the gastric PGE_2 concentration and gastric secretory function differed in comparison with the non-selective NSAID, naproxen. In particular, etodolac tended to reduce the gastric acid/mucus ratio, resulting in less injury to the gastric lumen, which could be related to reduced gastropathy. Furthermore, by combining the data from these studies with two drugs, we found that the occurrence of symptoms was associated with decreased levels of

gastric mucus secretion caused by drug administration rather than with the occurrence of endoscopic mucosal injury.

This study confirmed previous findings indicating that selective NSAIDs, such as etodolac, are less injurious to the gastric mucosa than naproxen (Laine et al. 1995; Lipscomb et al. 1995; Weideman et al. 2004). We also confirmed that gastric PGE_2 levels were not reduced in the subjects who were administered etodolac, whereas gastric PGE_2 levels were markedly suppressed in individuals who were administered naproxen (Lee and Dvornik 1985; Taha et al. 1990; Laine et al. 1995). COX-1 is consecutively expressed in the physiological settings of the stomach, whereas COX-2 has little or no expression at baseline but is induced in inflamed tissue (Laine et al. 2008). Thus, while a nonselective NSAID, naproxen inhibits both COX-1 and COX-2 resulting in a marked decrease in gastric PG contents, a COX-2 selective inhibitor, etodolac has little effect on gastric mucosal PG production in healthy volunteers. In turn, since gastric PG plays a critical role in controlling the normal mucosal defense mechanism (Wallace 2008), the different effects of the 2 NSAIDs on the gastric mucosal PGE_2 concentrations may explain the difference observed in the

magnitude of gastric mucosal injury produced by the 2 drugs.

Despite the marked decrease in the gastric PGE_2 concentration that followed 1 week of naproxen administration, we found no overall alteration in gastric secretory function (gastric acid or mucus secretion). Only a few studies have investigated the effects of NSAIDs on gastric mucus secretion in humans (Marcinkiewicz et al. 1996; Jaworski et al. 2005). Of these, Jaworski et al. (2005) investigated the effects of NSAID administration on mucus secretion by obtaining gastric aspirates under pentagastrin stimulation in a manner that was similar to the technique we used in the current study, and a more reproducible condition than the fasting, basal condition. Jaworski et al. (2005) reported that the administration of naproxen for 1 week decreased the total mucin output in healthy individuals. The differences between the daily naproxen doses administered in the two studies—500 mg twice a day in the study by Jaworski et al. (2005) and 300 mg twice a day in the present study—may explain the discrepancies in the results regarding the effects of naproxen administration on gastric mucus secretion.

The effects of COX-2-selective NSAIDs on gastric secretory function in humans have not been reported previously. The present study showed that the administration of etodolac for 1 week modestly increased the secretion of gastric mucus, but it did not affect gastric acid secretion and, consequently, the gastric acid/mucus ratio tended to decrease. The observed differences in the gastric secretory function associated with the administration of naproxen and etodolac could be related to their effects on gastric PGE_2 levels, which could account for the differences in the manifestations of gastropathy noted between the 2 types of NSAIDs. However, since the change in mucus secretion and the acid/mucus ratio did not differ significantly between those individuals who had severe mucosal injury and those who did not (such as in those with MLS of ≥ 4) (data not shown), there could be other mechanisms underlying the apparent differences in the severity of the mucosal injury between individuals administered naproxen and those administered etodolac. Interestingly, a recent study reported that etodolac consists of 2 enantiomers, S- and R-etodolac, and that R-etodolac reduced HCl/ethanol-induced gastric damage in rats (Inoue et al. 2011). The gastroprotective effects of R-etodolac may also be associated with a modulation of the gastric secretory function.

Although it is well-recognized that dyspeptic symptoms are relatively common in users of NSAIDs (Larkai et al. 1987; Straus et al. 2002; Wildner-Christensen et al. 2006), the clinical factors that can be associated with the development of NSAID-induced dyspepsia are largely unknown. The present findings, that NSAID-induced abdominal pain developed irrespective of the type of NSAID (naproxen and etodolac) administered or the degree of apparent endoscopic injury, were consistent with previous studies showing that both COX-2-selective NSAIDs and non-selective NSAIDs induce upper GI symptoms (Langman et al. 1999; Hawkey et al. 2005), and that GI symptoms during the use of NSAIDs do not correlate with visible gastroduodenal injury (Larkai et al. 1987; Hollenz et al. 2006). A well-recognized fact that pain is masked in most NSAID-induced ulcers (Skander and Ryan 1988; Taha et al. 1994) may also support the irrelevance of drug-induced abdominal pain with gastric mucosal injury. Nonetheless, we found an intriguing association between the occurrence of NSAID-induced dyspepsia and the level of gastric mucus secretion. More specifically, gastric mucus secretion significantly decreased upon NSAID administration in subjects that developed dyspeptic symptoms, whereas it significantly increased in those with no symptoms.

Gastric mucus is important as the first line of defense against noxious luminal acid because it creates an unstirred layer on the mucosal surface and acts as a protective physical barrier (Allen and Flemström 2005; Laine et al. 2008; Wallace 2008). Given that gastric sensory nerves terminate within the mucosal layer and transmit pain signals to the brain (Leek 1977; Holzer et al. 1991), it is reasonable to assume that the gastric mucus layer may also shield the sensory nerve terminals within the mucosa from luminal irritants such as HCl, thus preventing the activation of intramucosal pain receptors. Considering this information along with the lack of an association between the occurrence of abdominal pain and gastric mucosal injury observed in the present study, we speculate that suppressed gastric mucus secretion across the whole stomach, as opposed to the presence of a tiny mucosal erosion, is more important in the extensive stimulation of gastric sensory nerves, thus provoking NSAID-induced abdominal pain. Interestingly, a previous study demonstrated that pepper may induce gastric pain by removing the stomach's hydrophobic lining and that bismuth subsalicylate may reduce the pain by restoring the mucus gel layers, suggesting a fundamental role of the surface hydrophobic mucus layer in limiting gastric pain (Lichtenberger et al. 1998). In addition, a more recent study showed that rebamipide, a cytoprotective drug, had an additive effect to therapy with proton pump inhibitors in the prevention of low-dose aspirin-induced GI symptoms (Mizukami et al. 2012). These results seem to be consistent with our present results since we previously showed that rebamipide increased gastric mucus secretion in humans (Iijima et al. 2009). Further studies incorporating a placebo arm are required to confirm the present association between NSAID-induced dyspepsia and gastric mucus secretion.

Another limitation of this study is that it investigated healthy individuals rather than the patient population at risk. In clinical practice, NSAID users mainly comprise elderly patients with different underlying diseases; hence, the application of the present findings from young healthy individuals to NSAID users in general should be carefully considered.

In conclusion, this study demonstrated that decreased gastric mucus secretion rather than gastric mucosal injury

was associated with the occurrence of NSAID-induced abdominal pain, suggesting a pivotal role of the level of gastric mucus secretion in the etiology of NSAID-induced dyspepsia.

Conflict of Interest

The authors declare no conflict of interest.

References

Allen, A. & Flemström, G. (2005) Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Am. J. Physiol. Cell Physiol.*, **288**, C1-19.

Asada, S., Okumura, Y., Matsumoto, A., Hirata, I. & Ohshiba, S. (1990) Correlation of gastric mucous volume with levels of five prostaglandins after gastric mucosal injuries by NSAIDs. *J. Clin. Gastroenterol.*, **12 (12 Suppl 1)**, S125-130.

Azuumi, Y., Ohara, S., Ishihara, K., Okabe, H. & Hotta, K. (1980) Correlation of quantitative changes of gastric mucosal glycoproteins with aspirin-induced gastric damage in rats. *Gut*, **21**, 533-536.

Cai, S., García Rodríguez, L.A., Massó-González, E.L. & Hernández-Díaz, S. (2009) Uncomplicated peptic ulcer in the UK: trends from 1997 to 2005. *Aliment. Pharmacol. Ther*., **30**, 1039-1048.

Feldman, M., Cryer, B., Mallat, D. & Go, M.F. (2001) Role of Helicobacter pylori infection in gastroduodenal injury and gastric prostaglandin synthesis during long term/low dose aspirin therapy: a prospective placebo-controlled, double-blind randomized trial. *Am. J. Gastroenterol.*, **96**, 1751-1757.

Hawkey, C.J., Jackson, L., Harper, S.E., Simon, T.J., Mortensen, E. & Lines, C.R. (2001) Review article: the gastrointestinal safety profile of rofecoxib, a highly selective inhibitor of cyclooxygenase-2, in humans. *Aliment. Pharmacol. Ther.*, **15**, 1-9.

Hawkey, C., Talley, N.J., Yeomans, N.D., Jones, R., Sung, J.J., Långström, G., Naesdal, J. & Scheiman, J.M.; NASA1 SPACE1 Study Group. (2005) Improvements with esomeprazole in patients with upper gastrointestinal symptoms taking non-steroidal antiinflammatory drugs, including selective COX-2 inhibitors. *Am. J. Gastroenterol.*, **100**, 1028-1036.

Hollenz, M., Stolte, M., Leodolter, A. & Labenz, J. (2006) NSAID-associated dyspepsia and ulcers: a prospective cohort study in primary care. *Dig. Dis.*, **24**, 189-194.

Holzer, P., Livingston, E.H., Saria, A. & Guth, P.H. (1991) Sensory neurons mediate protective vasodilatation in rat gastric mucosa. *Am. J. Physiol.*, **260**, G363-370.

Iijima, K., Ara, N., Abe, Y., Koike, T., Iwai, W., Iwabuchi, T., Ichikawa, T., Kamata, Y., Ishihara, K. & Shimosegawa, T. (2012) Association of gastric acid and mucus secretion level with low-dose aspirin-induced gastropathy. *J. Gastroenterol.*, **47**, 150-158.

Iijima, K., Ara, N., Abe, Y., Koike, T., Iwai, W., Uno, K., Asano, N., Imatani, A., Ohara, S. & Shimosegawa, T. (2011) Gastric acid secretion level modulates the association between Helicobacter pylori infection and low-dose aspirin-induced gastropathy. *J. Gastroenterol.*, **46**, 612-619.

Iijima, K., Ichikawa, T., Okada, S., Ogawa, M., Koike, T., Ohara, S. & Shimosegawa, T. (2009) Rebamipide, a cytoprotective drug, increases gastric mucus secretion in human: evaluations with endoscopic gastrin test. *Dig. Dis. Sci.*, **54**, 1500-1507.

Iijima, K., Ohara, S., Sekine, H., Koike, T., Kubota, Y., Kato, K., Asaki, S. & Toyota, T. (1998) A new endoscopic method of gastric acid secretory testing. *Am. J. Gastroenterol.*, **93**, 2113-2118.

Inoue, N., Nogawa, M., Ito, S., Tajima, K., Kume, S. & Kyoi, T. (2011) The enantiomers of etodolac, a racemic anti-inflammatory agent, play different roles in efficacy and gastrointestinal safety. *Biol. Pharm. Bull.*, **34**, 655-659.

Jaworski, T., Sarosiek, I., Sostarich, S., Roeser, K., Connor, M., Brotze, S., Wallner, G. & Sarosiek, J. (2005) Restorative impact of rabeprazole on gastric mucus and mucin production impairment during naproxen administration: its potential clinical significance. *Dig. Dis. Sci.*, **50**, 357-365.

Laine, L., Sloane, R., Ferretti, M. & Cominelli, F. (1995) A randomized double-blind comparison of placebo, etodolac, and naproxen on gastrointestinal injury and prostaglandin production. *Gastrointest. Endosc.*, **42**, 428-433.

Laine, L., Takeuchi, K. & Tarnawski, A. (2008) Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*, **135**, 41-60.

Lanas, A., Polo-Tomás, M., Roncales, P., Gonzalez, M.A. & Zapardiel, J. (2012) Prescription of and adherence to nonsteroidal anti-inflammatory drugs and gastroprotective agents in at-risk gastrointestinal patients. *Am. J. Gastroenterol.*, **107**, 707-714.

Langman, M.J., Jensen, D.M., Watson, D.J., Harper, S.E., Zhao, P.L., Quan, H., Bolognese, J.A. & Simon, T.J. (1999) Adverse upper gastrointestinal effects of rofecoxib compared with NSAIDs. *JAMA*, **282**, 1929-1933.

Lanza, F.L., Graham, D.Y., Davis, R.E. & Rack, M.F. (1990) Endoscopic comparison of cimetidine and sucralfate for prevention of naproxen-induced acute gastroduodenal injury. Effect of scoring method. *Dig. Dis. Sci.*, **35**, 1494-1499.

Larkai, E.N., Smith, J.L., Lidsky, M.D. & Graham, D.Y. (1987) Gastroduodenal mucosa and dyspeptic symptoms in arthritic patients during chronic nonsteroidal anti-inflammatory drug use. *Am. J. Gastroenterol.*, **82**, 1153-1158.

Lassen, A., Hallas, J. & Schaffalitzky de Muckadell, O.B. (2006) Complicated and uncomplicated peptic ulcers in a Danish county 1993-2002: a population-based cohort study. *Am. J. Gastroenterol.*, **101**, 945-953.

Lee, D. & Dvornik, D. (1985) Etodolac: effect on prostaglandin concentrations in gastric mucosa of rats. *Life Sci.*, **36**, 1157-1162.

Leek, B.F. (1977) Abdominal and pelvic visceral receptors. *Br. Med. Bull.*, **33**, 163-168.

Lichtenberger, L.M., Romero, J.J., Carryl, O.R., Illich, P.A. & Walters, E.T. (1998) Effect of pepper and bismuth subsalicylate on gastric pain and surface hydrophobicity in the rat. *Aliment. Pharmacol. Ther.*, **12**, 483-490.

Lipscomb, G.R., Wallis, N., Armstrong, G., Goodman, M.J. & Rees, W.D. (1995) Gastric mucosal adaptation to etodolac and naproxen. *Aliment. Pharmacol. Ther.*, **9**, 379-385.

Marcinkiewicz, M., Peura, D.A. & Sarosiek, J. (1996) The relationship between the content of aggressive and protective components in gastric juice and endoscopic findings after naproxen sodium and acetaminophen administration. *Am. J. Gastroenterol.*, **91**, 360-365.

Mizukami, K., Murakami, K., Hirashita, Y., Hisamatsu, A., Ogawa, R., Uchida, M., Nakagawa, Y., Okimoto, T., Kodama, M. & Fujioka, T. (2012) Efficacy of rebamipide for low-dose aspirin-related gastrointestinal symptoms. *J. Clin. Biochem. Nutr.*, **51**, 216-220.

Morris, G.P., Harding, R.K. & Wallace, J.L. (1984) A functional model for extracellular gastric mucus in the rat. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.*, **46**, 239-251.

Musumba, C., Jorgensen, A., Sutton, L., Van Eker, D., Moorcroft, J., Hopkins, M., Pritchard, D.M. & Pirmohamed, M. (2012) The relative contribution of NSAIDs and Helicobacter pylori to the aetiology of endoscopically-diagnosed peptic ulcer disease: observations from a tertiary referral hospital in the UK between 2005 and 2010. *Aliment. Pharmacol. Ther.*, **36**, 48-56.

Ootani, H., Iwakiri, R., Shimoda, R., Nakahara, S., Amemori, S., Fujise, T., Kikkawa, A., Tsunada, S., Sakata, H. & Fujimoto, K. (2006) Role of Helicobacter pylori infection and nonste-

roidal anti-inflammatory drug use in bleeding peptic ulcers in Japan. *J. Gastroenterol.*, **41**, 41-46.

Quadros, E. & Wilson, D.E. (1991) Role of prostaglandins in mucus synthesis in rat gastric mucosa. *Adv. Prostaglandin Thromboxane Leukot. Res.*, **21B**, 767-770.

Seidler, U., Knafla, K., Kownatzki, R. & Sewing, K.F. (1988) Effects of endogenous and exogenous prostaglandins on glycoprotein synthesis and secretion in isolated rabbit gastric mucosa. *Gastroenterology*, **95**, 945-951.

Skander, M.P. & Ryan, F.P. (1988) Non-steroidal anti-inflammatory drugs and pain free peptic ulceration in the elderly. *BMJ*, **297**, 833-834.

Straus, W.L., Ofman, J.J., MacLean, C., Morton, S., Berger, M.L., Roth, E.A. & Shekelle, P. (2002) Do NSAIDs cause dyspepsia? A meta-analysis evaluating alternative dyspepsia definitions. *Am. J. Gastroenterol.*, **97**, 1951-1958.

Svedlund, J., Sjödin, I. & Dotevall, G. (1988) GSRS - a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig. Dis. Sci.*, **33**, 129-134.

Taha, A.S., Dahill, S., Sturrock, R.D., Lee, F.D. & Russell, R.I. (1994) Predicting NSAID related ulcers - assessment of clinical and pathological risk factors and importance of differences in NSAID. *Gut*, **35**, 891-895.

Taha, A.S., McLaughlin, S., Holland, P.J., Kelly, R.W., Sturrock, R.D. & Russell, R.I. (1990) Effect on gastric and duodenal mucosal prostaglandins of repeated intake of therapeutic doses of naproxen and etodolac in rheumatoid arthritis. *Ann. Rheum. Dis.*, **49**, 354-358.

Venerito, M., Treiber, G., Wex, T., Kuester, D., Roessner, A., Di Mario, F. & Malfertheiner, P. (2006) Effects of low-dose aspirin on gastric erosions, cyclooxygenase expression and mucosal prostaglandin-E2 do not depend on Helicobacter pylori infection. *Aliment. Pharmacol. Ther.*, **23**, 1225-1233.

Wallace, J.L. (2008) Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiol. Rev.*, **88**, 1547-1565.

Wight, N.J., Gottesdiener, K., Garlick, N.M., Atherton, C.T., Novak, S., Gertz, B.J., Calder, N.A., Cote, J., Wong, P., Dallob, A. & Hawkey, C.J. (2001) Rofecoxib, a COX-2 inhibitor, does not inhibit human gastric mucosal prostaglandin production. *Gastroenterology*, **120**, 867-873.

Wildner-Christensen, M., Hansen, J.M. & De Muckadell, O.B. (2006) Risk factors for dyspepsia in a general population: non-steroidal anti-inflammatory drugs, cigarette smoking and unemployment are more important than Helicobacter pylori infection. *Scand. J. Gastroenterol.*, **41**, 149-154.

Weideman, R.A., Kelly, K.C., Kazi, S., Cung, A., Roberts, K.W., Smith, H.J., Sarosi, G.A., Little, B.B. & Cryer, B. (2004) Risks of clinically significant upper gastrointestinal events with etodolac and naproxen: a historical cohort analysis. *Gastroenterology*, **127**, 1322-1328.