Published in final edited form as:
Aliment Pharmacol Ther. 2009 July ; 30(1): 71–81. doi:10.1111/j.1365-2036.2009.04000.x.

NSAID-induced Antral Ulcers are Associated with Distinct Changes in Mucosal Gene Expression

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Abstract

Aims—The basis for individual variation in gastroduodenal vulnerability to NSAIDs is not well understood. We aimed to assess whether a gene expression signature was associated with susceptibility to gastroduodenal ulcerations.

Methods—Twenty-five *H pylori* negative adults were treated for 7 days with naproxen 500 mg BID. Subjects underwent baseline and post-treatment endoscopy, during which biopsies were taken from antrum and duodenum. RNA extraction and cDNA synthesis were performed, followed by PCR of 23 genes relevant to mucosal injury and repair. Fold changes in gene expression were compared between subjects who developed ulcers and those who did not.

Results—Compared to subjects who did not develop ulcers (n=18), subjects who developed antral ulcers (n=7) had significantly greater mucosal up-regulation of interleukin-8 [Fold change = 33.5 (SEM = 18.5) versus −7.7 (3.2)] and of cyclo-oxygenase-2 [2.3 (1.7) versus −10.8 (2.2)]. Conversely, non-ulcer subjects had significantly greater up-regulation of toll-like receptor-4, cyclo-oxygenase-1, and hepatocyte growth factor [14.0 (2.2) vs. −0.8 (1.0), 9.8 (2.4) vs. 0.0 (0.7), and 8.2 (2.6) vs. −2.2 (0.3), respectively].

Conclusions—NSAID-induced antral ulcers are associated with a specific pattern of gastroduodenal mucosal gene expression. These patterns may provide insight into the molecular basis of individual susceptibility to mucosal injury.

INTRODUCTION

NSAID-induced gastroduodenal injury remains an important cause of morbidity and mortality (1). Strategies to minimize NSAID-related adverse events include the use of lower drug doses or of less toxic NSAIDs such as COX-2-selective inhibitors, the co-administration and co-

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Statement of Interests:
1. Author's declaration of personal interest
(i) Dr Aisenberg has performed sponsored research and consulting for Pfizer, Inc. and for NiCox.
2. Declaration of funding interests:
(i) This study was funded in part by in part by an investigator initiated grant by Pfizer Inc. and in part by a grant from the Digestive Diseases Research Foundation.

packaging of NSAIDs with gastro-protective agents, and the stratification of patients based on risk factors for bleeding (2–8). Unfortunately, these strategies have been only partially effective: patient and physician adherence with experts' prescribing recommendations is only fair (9), while the GI-safety advantage of the COX-2 inhibitors is mitigated by such factors as concurrent aspirin use (10) and cardiovascular safety concerns (11).

It has been observed across different studies and in clinical practice that individuals vary in their vulnerability to gastroduodenal NSAID injury. Although certain individuals tolerate many different non-selective NSAIDs, others experience repeated adverse events with different agents. Clinical characteristics such as age and co-morbidity only partly explain these differences. Animal models have suggested several molecular pathways that may influence NSAID-related mucosal injury and repair (12–15). In humans, certain polymorphisms in common cytochrome P450 genes may increase NSAID-related adverse events (16). However, there exist few studies in humans regarding the molecular mechanisms underlying the observed differences in individual vulnerability to NSAID injury (16–18).

We hypothesized that mucosal gene expression during NSAID treatment differs between individuals who develop mucosal injury and those who do not, and that these differences may help explain ulcer pathogenesis. To test our hypothesis, we preselected for analysis a panel of 23 genes that encode proteins that are important in mucosal inflammation and repair. The primary aim of this study was to compare changes in mucosal expression of these 23 genes during one week of naproxen treatment in healthy volunteers who developed antral ulcers versus those who did not.

SUBJECTS AND METHODS

Study Population

The current gene expression study was performed in a sub-group of patients who participated in a clinical study of the effectiveness of omeprazole in preventing naproxen-induced injury (19).

In the clinical study, 70 subjects were divided into two treatment arms (naproxen plus placebo, naproxen plus omeprazole), and 4 subjects were treated with placebo alone. Major inclusion criteria included: age 50 to 75 years; generally good health; willingness to sign the informed consent. Major exclusion criteria included: (1) use of any NSAID (including aspirin) within past 2 weeks, or history of chronic NSAID use; (2) use of antacids, H-2 blocker within past 2 weeks, or PPI within past 30 days; (3) use of any corticosteroid within past 60 days; (4) history of bleeding tendencies or warfarin use within past 60 days; (5) history of previous bleeding ulcer; (6) consumption of 3 or more alcoholic beverages a day; (7) hypersensitivity or allergy to NSAIDs or other contraindications to their use; (8) baseline abdominal pain, nausea and/or cramping; and (9) the presence of one or more gastroduodenal mucosal breaks (erosions or ulcerations) at a baseline endoscopy. As part of the clinical trial, patients provided consent for biopsies. The demographics for the study population are presented in Table 2.

For the current primary analysis, subjects treated with omeprazole and/or infected with *H pylori* (n=41) were excluded in order to eliminate confounding by these two variables. All other subjects from the clinical study were eligible for inclusion in the current study.

In the exploratory analysis of gene regulation at the ulcer margin, we studied tissue from 6 subjects from the sub-population described above, plus tissue from 1 *H pylori* infected subject.

Study Design

The study was approved by an independent institutional review board. The study consisted of three visits:

Visit 1, Screening

During Visit 1 (Day -10 to -1), subjects read and signed the informed consent, underwent a physical exam, and were screened for inclusion and exclusion criteria.

Visit 2, Baseline Endoscopy

During Visit 2 (Day 0), upper GI endoscopy was performed using standard methods by one of three investigators (JA, LC, and KM). Biopsies were taken for *H pylori* analysis (HPFast, GI SUPPLY, Windcrest, TX). If the subject had no evidence of gastroduodenal mucosal breaks (erosions or ulcers, see definition below) or of *H pylori* infection the subject was eligible for the study.

To assess baseline gene expression, 4 forceps biopsies were obtained from normal-appearing mucosa in the duodenal bulb, gastric antrum, and the gastric body (12 biopsies in total). In order to help distinguish healing biopsy sites from NSAID-related lesions during the final endoscopy, the antral biopsies were taken approximately one cm proximal to the pyloric orifice at the 12, 3, 6 and 9 o'clock positions. In addition, a "washout" period of 7 days was provided before the study drug was started in order to allow the biopsy sites heal. All biopsies were obtained utilizing a standard biopsy forceps (Olympus FB 36K-1). Samples were immediately submerged in Trizol, snap frozen with liquid nitrogen, and stored at −80° C until processing for RNA extraction.

Treatment

Study medication was started 7 days after baseline endoscopy (Day 8). Subjects included in the primary analysis underwent a 6.5 day treatment of naproxen (NPX) 500 mg BID (n=25). In order to provide an indication of background gene variation, four subjects were treated with placebo. Investigators were blinded to which treatment subjects received. Subjects were given a dosing diary to record daily dosages of medication taken. We chose the one-week duration of treatment based on considerations regarding patient safety, patient compliance, and the known kinetics of naproxen-related mucosal injury.

Visit 3, Post-Treatment Endoscopy

Subjects returned within 2–4 hours of the last study drug dose (day 14) for clinical and endoscopic evaluation. Compliance was assessed by counting the number of pills returned and by reviewing the subject's dosing diary. The follow-up endoscopy was then performed.

Ulcers, defined as any mucosal break ≥ 3mm with unequivocal depth (20), were counted individually and localized to the duodenum, gastric antrum, or gastric body. When necessary for scoring, the endoscopist performed a side-by-side comparison of the lesion's size with the size of a biopsy forceps passed through the endoscope working channel during the procedure.

At the post-treatment endoscopy, 4 biopsies for molecular analysis were again obtained from the duodenal bulb, gastric antrum, and the gastric body. In order to minimize the potential for tissue degradation, biopsies for the primary analysis were obtained from endoscopically normal-appearing mucosa (i.e. *not* from endoscopically inflamed or ulcerated mucosa). In an exploratory analysis, a group of subjects also underwent biopsy of ulcer margins. To assess background variability in intra-individual gene expression, we took biopsies from healthy-appearing antrum in 4 subjects who received only placebo.

At the conclusion of the study, a committee comprised of one gastroenterologist and two research assistants adjudicated the endoscopic findings by reviewing high-resolution digital video-recordings of each subject's final endoscopy. For the purposes of the primary analysis, each subject was adjudicated as either "ulcer" or "non-ulcer." The judgments of the committee were binding.

Quantitative Real Time PCR for Measuring Gene Expression (QPCR)

Total RNA was isolated from gastric and duodenal samples using RNA Bee (Tel-Test, Inc., Friendwood, TX) according to the manufacturer's instructions. RNA quality from all samples was evaluated using the Agilent Bioanalyzer 2100 with a RNA Integrity Score (RIN) assigned to each sample tested. Samples with a RIN score of less than 6.5 were removed from analysis based on degradation based bias in the QPCR results (< 5% of antral samples).1 μg of total RNA was used as the template for single-strand complementary DNA (cDNA) synthesis using the Transcriptor Fast Strand cDNA Synthesis Kit (Roche, Indianapolis, IN) according to the manufacturer's instructions. Quantitative real-time PCR (QPCR) reactions were performed in triplicate for 23 genes using TaqMan chemistry (Applied Biosystems [ABI], Foster City, CA) on preconfigured TaqMan Low Density Arrays (TLDA) utilizing ABI Assay on Demand assays. The 23 targets chosen by the investigators (Table 1) represent a panel of genes that from previous studies have demonstrated to be important in the mucosal response to injury. (21). More specifically, these individual gene targets have found to be either up- or down-regulated in rodent models of gastro-duodenal ulcer formation or healing (21). The primers and probes are commercially available through ABI's Assay on Demand program (Applied Biosystems, Foster City, CA), and can be found online. Quantitative real time PCR was performed using ABI Master Mix on an ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA), programmed for 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The plates were configured to provide duplicate reactions for each sample (for every assay) to account for pipetting and well-to-well variation.

All raw data was analyzed using sequence detection systems (SDS) 2.3 software (Applied Biosystems). Differential gene expression was calculated using the "delta-delta C_T (cycle threshold)" method (22). In brief, first the average of the triplicate results for the cycle threshold (C_T) for each gene was calculated. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression C_T value (also run in triplicate) was then used to calculate a normalized Ct (delta C_T) value for each target gene. The fold change (FC) of expression between baseline and post-treatment biopsy specimens was then calculated as previously described for each test gene in each subject (22). For each of the 23 genes studied, the differences between baseline and post-treatment expression were compared between the ulcer population and the non-ulcer populations.

Real time PCR data is represented both numerically (Tables 3 and 4) and using a "heat map" (Figure 1), which visually depicts patterns in gene expression for individual subjects across multiple genes. Briefly, a color scale is created using the largest gene expression fold changes as the low and high settings of the color scale. Red colors represent genes that are up-regulated, with the warmer color corresponding to more strongly up-regulated signals; green colors represent genes that are down-regulated, with the cooler colors corresponding to more strongly down-regulated signals. Each individual subject is depicted in one column and each individual gene is depicted in one row. This representation allows for the visual detection of gene expression patterns in individual subjects that otherwise might not be observed if represented in table format as statistical average of all subjects being measured.

Endpoints

For the primary endpoint, we calculated fold changes in individual target genes in normal-appearing antral mucosa in healthy subjects treated with 7 days of naproxen.

Our primary objective was to compare gene fold changes in naproxen-treated healthy subjects in the antral ulcer versus the non-ulcer populations. Our secondary objectives were to evaluate: (1) fold changes in subjects treated with placebo; (2) fold changes in duodenal mucosa in patients with antral ulcers compared to fold changes in the duodenum in non-ulcer subjects; and (3) fold changes at the ulcer margin.

Statistical Analysis

The statistical methods utilized for the clinical study have been previously reported (19). The sample size for the current pilot study was determined by available resources and by the number of the subjects in the clinical study who qualified for inclusion. Statistical analysis of the real time PCR data was performed using methodology described previously (23–25). For all C_T value triplicates, a mean and standard error was calculated. For each gene analyzed, a student's T-test was used in order to test the significance of the differences in regulation in response to treatment (normalized delta C_T value) between the ulcer and non-ulcer populations. The descriptive statistics for only the genes for which the fold change differences between the ulcer and non-ulcer populations had a p-value <0.05 by the student's T-test are reported. Aggregate statistics for each gene (mean and standard error of mean) across subjects were calculated, and compared between the ulcer and non-ulcer groups. Additionally, a Wilcoxon matched pairs signed ranks test was performed to address the small and unequal sample sizes for the groups compared. The results of this analysis yielded a p-value of 0.042 in the naproxen treated baseline vs. post-treatment group and a p-value of 0.038 in the same treatment group in duodenal samples (see Results). The significant p-value rejects the hypothesis that the differences measured are coincidence.

RESULTS

Demographics and Endoscopic Findings

A total of 25 naproxen-treated subjects met the inclusion and exclusion criteria. Of these subjects, 7/25 (28%) developed an antral ulcer. Two of these 7 subjects developed a duodenal ulcer in addition to an antral ulcer. No subject developed a duodenal ulcer alone. All subjects in the antral ulcer and the non-ulcer groups developed small erosions. No patient developed clinically evident bleeding or other significant adverse events. The demographic characteristics were similar between the groups (Table 2).

Antral Gene Expression in Placebo-Treated Subjects

None of the 4 placebo-treated subjects had mucosal breaks at the follow-up endoscopy. When comparing gene expression in antral and duodenal tissue obtained at the baseline and post-treatment endoscopies in subjects who received placebo, no significant changes were detected for the 23 genes tested (selected data included in Table 3). These data demonstrate that changes in gene expression are not due to background intra-individual variation due to other causes.

Antral Gene Expression in the Naproxen-Treated Subjects

The primary endpoint was the change in gene expression in tissue obtained from biopsies of normal-appearing antral mucosa after 7 days of naproxen treatment. Significant differences in gene expression between the ulcer and non-ulcer groups were detected for five of the 23 genes tested (Table 3). Specifically, IL-8 and COX-2 were significantly up-regulated in the ulcer group compared to the non-ulcer group. Indeed, the expression of these two genes was down-

regulated in the non-ulcer group. Conversely, COX-1, TLR4 and HGF were significantly up-regulated in the non-ulcer group relative to the ulcer group.

Gene Expression in Tissue Obtained from the Antral Ulcer Margin

In an exploratory analysis, gene expression at the ulcer margins was tested in 7 subjects who developed antral ulcers after 7 days of treatment. RNA quality in tissue obtained from biopsies of the ulcer margins was found to be comparable to RNA quality from tissue obtained from biopsies of normal-appearing mucosa. In the biopsies from the ulcer margins, up-regulation of IL-8 (FC=14.7, SEM=4.1) and COX-2 (FC=3.8, SEM=1.0) was detected. Significant fold changes were not detected for the other 21 genes tested (data not shown).

Gene Expression in Duodenum after Naproxen Treatment

Based on the antral results, we subsequently analyzed expression of IL-8, COX-2, COX-1, TLR-4, and HGF genes in normal-appearing duodenal mucosa after naproxen treatment. In 6 of 25 cases, RNA quality was inadequate.

In naproxen-treated patients, significant up-regulation of IL-8 and COX-2 was observed (Table 4). Unlike in the antrum, there were no significant differences between the ulcer and non-ulcer groups. In 3 subjects who also developed duodenal ulcers, the trends in duodenal mucosal gene expression were similar to results in subjects who did not develop duodenal ulcers (data not shown).

Gene Expression in Individual Subjects after Treatment with Naproxen

In order to detect patterns of gene expression, fold changes were next analyzed subject-by subject. In the non-ulcer group, it was noted that the 7 subjects who had strong up-regulation of HGF also had strong up-regulation of COX-1 and TLR4 (Figure 1a). Conversely, none of the other non-ulcer patients (n=11) had up-regulation (FC>2.0) of any of these three genes. Of the 7 subjects who strongly up-regulated HGF, COX-1 and TLR-4, 5 strongly down-regulated COX-2. In the subject-by-subject analysis, we did not observe any significant correlation between gene expression in the antrum and duodenum (data not shown).

When NSAID-associated antral expression of different genes was compared within individual subjects in the ulcer group, it was noted that that 4/5 subjects who had antral up-regulation of IL-8 also had strong up-regulation of COX-2 (Figure 1b). Of the other two ulcer patients, one had up-regulation of COX-2 but not IL-8, while the other had up-regulation of neither.

DISCUSSION

NSAIDs injure the gastrointestinal mucosa by direct topical toxicity and by blocking the activity of mucosal COX, the rate-limiting enzyme in prostaglandin production (26). Prostaglandins mediate mucosal protection by decreasing acid production, increasing mucus and bicarbonate secretion, enhancing mucosal blood flow, and regulating genes that are important to mucosal homeostasis (27). In addition to injuring the mucosa, NSAIDs inhibit mucosal healing (28,29). The chemistry, duration and dose of the NSAID as well as clinical characteristics of the patient influence the susceptibility to ulcer development (3–7). However, the biological basis for individual-to-individual differences in vulnerability to NSAID injury is poorly understood. Rodent studies suggest that analyzing mucosal gene expression during NSAID treatment may yield insights into the pathogenesis of injury (30,31).

Given the frequency of NSAID-related ulcers and the limitations to methods of ulcer prevention, we aimed to identify molecular markers associated with ulceration. Ideally, we could identify patterns of gene expression that predict who will develop an ulcer. The principal

finding in the current study is that healthy human subjects who develop antral ulcers during treatment with a non-selective COX inhibitor display a distinct pattern of mucosal gene expression. Specifically, subjects who developed antral ulcers up-regulated COX-2 and IL-8, compared with subjects who did not develop ulcers. Conversely, individuals who did not develop NSAID injury showed increased expression of TLR-4, COX-1, and HGF.

IL-8, a member of the CXC family of chemokines, induces neutrophils to adhere to the endothelium at areas of tissue injury, translocate across the endothelium, and perform phagocytosis and local tissue destruction (32). Crabtree et al have shown that in rodents neutrophils mediate *H pylori*-related gastroduodenal injury (33). Wallace et al have shown that NSAID-related injury causes adherence of neutrophils to gastric venules, and that NSAID injury is decreased in neutropenic rats (34,35). Several signals have been proposed to mediate neutrophil recruitment by IL-8, including prostaglandins, TNF-alpha, and leukotrienes (36). In humans, up-regulation of IL-8 may be associated with *H pylori*-related ulcer disease (37), while proton pump inhibitors suppress IL-8 production by cell lines and abrogate IL-8-mediated actions (38). Our data demonstrating striking IL-8 up-regulation in individuals who developed ulcers suggest that IL-8 dependent pathways are also important in human NSAID-related injury and repair.

TLR-4, an innate immune receptor, has been identified in non-inflamed human gastric epithelium and in gastric mucosal leukocytes and dendritic cells (39,40). Hold et al demonstrated that in *H pylori* infection a polymorphism in the TLR-4 gene increases risk of gastric carcinoma (41). Rodent studies suggest that TLR-4 activation increases intestinal susceptibility to NSAID-related injury (15,42). Up-regulation of TLR-4 in the stomach during NSAID treatment may result from topical injury (43), or from exposure of injured mucosa to luminal bacteria or to the by-products of tissue injury (44). In the current study subjects who did not develop ulcers up-regulated TLR-4. In previous studies, we have shown that TLR-4 is required for colonic mucosal healing, and that a relationship exists between TLR-4 activation and COX activation (45), Although is plausible that a similar pathway exists during NSAID-induced damage, the mechanism by which TLR-4 activation favors in this setting is unknown.

After gastric injury, damaged cells are sloughed and replaced by healthy cells that proliferate, migrate, and organize into glands. *In vitro* studies have shown that HGF mediates restitution (46,47). *In vivo* human studies have suggested that HGF expression is increased in hypertrophic gastritis, ulcerative colitis, and *H pylori* gastritis (48–50). Production of HGF by lamina propria macrophages, neutrophils, and fibroblasts has been shown to be up-regulated by mucosal prostaglandins (13,46). In our study, HGF was up-regulated in response to naproxen in individuals who did not develop ulcers, suggesting that it may mediate protection or repair in certain individuals. In the *H pylori* infected stomach, IL-1β has been shown to increase HGF release (48), but in the current study IL-1β was not found to be up-regulated. Unlike *in vitro* studies by Takahashi (46), our experiments did not detect HGF up-regulation at the ulcer margin, which may be relevant to failure of restitution.

Previous studies investigating the impact of NSAID administration on COX biosynthesis and enzymatic activity have produced heterogeneous results (51–53). In electron microscopic studies, Jackson et al showed strong COX-1 and COX-2 staining in parietal cells of the normal human stomach, and increased COX-2 at the rims of ulcers (54). In rats, “compensatory” up-regulation of COX-2 in the stomach mucosa has been shown in response to COX-1 inhibition (12,55). McCarthy et al showed that COX-2 immunostaining was present in epithelial cells and parietal cells in *H pylori* infected individuals (56). In our study subjects who did not develop ulcers up-regulated COX-1 and down-regulated COX-2. The finding of COX-1 up-regulation in a feedback response to blockade of COX-1 by naproxen corroborates the finding of Bhandari et al (57). Although early studies suggested that in the normal stomach COX-2 is not

constitutively expressed (58), our findings and those of several other groups suggest that in certain populations COX-2 is constitutively present (54,59). Our data demonstrating up-regulation of COX-2 at the margin of ulcers, presumably promoting ulcer healing, is consistent with previous studies (54,57). In our study the non-ulcer population down-regulated COX-2, suggesting that resistance to injury was not COX-2-mediated. Further studies measuring COX-2 protein, prostaglandins, and RNA copy number will address this question further.

Like prostaglandins, nitric oxide (NO) mediates mucosal protection, via its effects on mucosal blood flow, leukocyte adherence, and other properties (60). NO is released from vascular endothelial cells, epithelial cells and sensory nerves in gut mucosa, and is regulated by constitutive and inducible nitric oxide synthetase (NOS). Because of its importance in mucosal protection, NO donating NSAIDs are currently in human trials (61). Multiple studies have suggested that COX inhibition results in an increase in local production of NO. For example, two recent healthy human volunteer studies showed a local increase in inducible NOS at the protein level in the gastric corpus in subjects treated with ibuprofen or aspirin for three days (51,52). A parallel increase in constitutive NOS was not seen, and in one of these studies up-regulation of inducible NOS was noted only in subjects with adverse reactions or endoscopic lesions related to NSAIDs. Up-regulation was greater in H. pylori-infected subjects. We did not demonstrate up-regulation of inducible NOS in response to COX inhibition. This discrepancy likely reflects methodological differences. For example, we utilized 7-day rather than 3-day treatment, and naproxen rather than aspirin or ibuprofen. In addition, we obtained tissue from the gastric antrum, which is the predominant site of mucosal NSAID damage, rather than the gastric corpus. Lastly, we measured gene expression rather than protein expression. Focused and detailed molecular analysis of the impact of NSAID treatment on constitutive and inducible NOS under a variety of study conditions will be required to clarify these differences.

A subject-by-subject analysis of the non-ulcer population showed that the same individuals induced HGF, COX-1 and TLR4 (Figure 1a). It is unclear whether or not these changes are regulated dependently or independently. However, previous animal studies have shown a relationship between TLR-4 and COX gene regulation in colitis (45) as well as of prostaglandin biosynthesis and HGF regulation (13).

There are several limitations to this pilot study. First, the number of subjects in each arm is small, and the findings require confirmation in larger populations. Second, in order to minimize risk to our healthy volunteer subjects, we limited the treatment duration to 1 week. It is unproven that molecular changes seen after one week mirror changes occurring during long-term NSAID treatment. This question can be addressed in studies of patients requiring chronic analgesic use. Third, the value of endoscopic ulcers as a “surrogate marker” for adverse clinical events remains controversial, although widely accepted (20). Of note, the antral and duodenal ulcer rates were 28% and 8% respectively in our study, somewhat higher than those reported in the literature. We believe this reflects the fact that this was a single site study and that we enforced a strict width definition by sizing lesions with the biopsy forceps and by using blinded adjudication. As expected, no subjects in the placebo group developed ulcers. Also reassuringly, no significant gene changes occurred in the placebo-treated population, suggesting that neither meals nor other environmental factors on the gastric mucosa confounded our results.

The results of our pilot study suggest future directions for research. First, our findings require corroboration in other, larger populations. Second, studies of protein expression will help clarify the functional significance of the genetic changes that we observed. Third, studies of gene copy number can provide absolute quantitative data for gene activation. Fourth, studies of gene families (e.g. using microarrays) can help delineate molecular pathways of gene activation in the stomach in response to NSAIDs.

In conclusion, our results suggest that NSAID-induced antral ulcers are associated with a specific pattern of mucosal gene regulation. Up-regulation of HGF-1, COX-1, and TLR-4 are associated with decreased NSAID injury, while up-regulation of IL-8 may represent a mechanism of injury. Detailed analysis of pathways involving HGF-1, COX-1, COX-2, TLR-4, and IL-8 using tissue obtained from endoscopic biopsy specimens may yield useful information regarding the mechanisms of NSAID-related injury.

Acknowledgments

The authors thank Colleen D'Souza, Liliana Morales, Jennifer Page, and Maya Voynarovska, Joanna Sypula, Michael DiCola, without whom this study could not have been accomplished.

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Figure 1a.

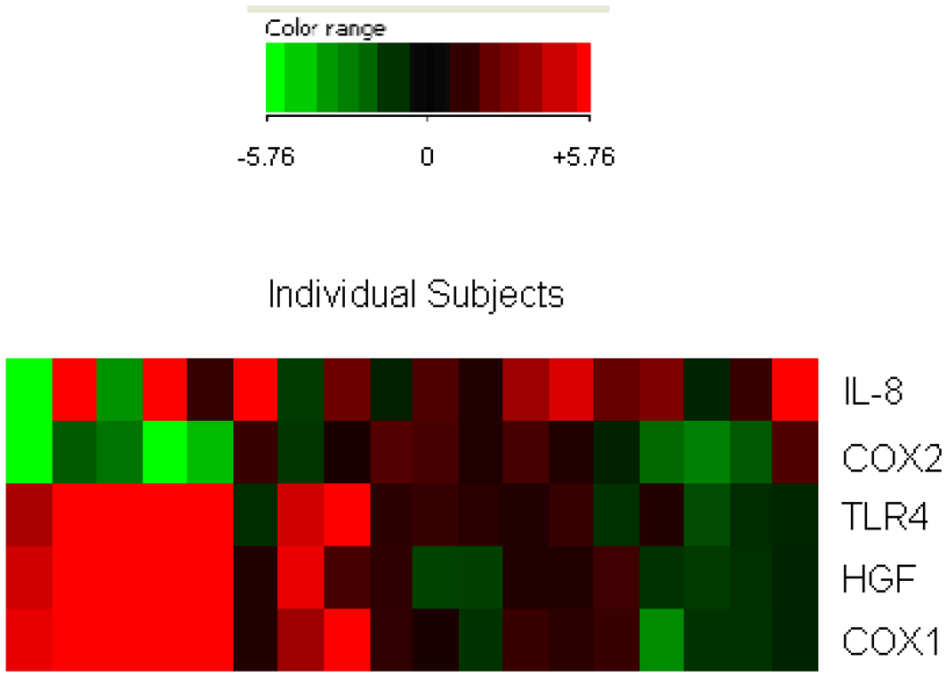

Figure 1b.

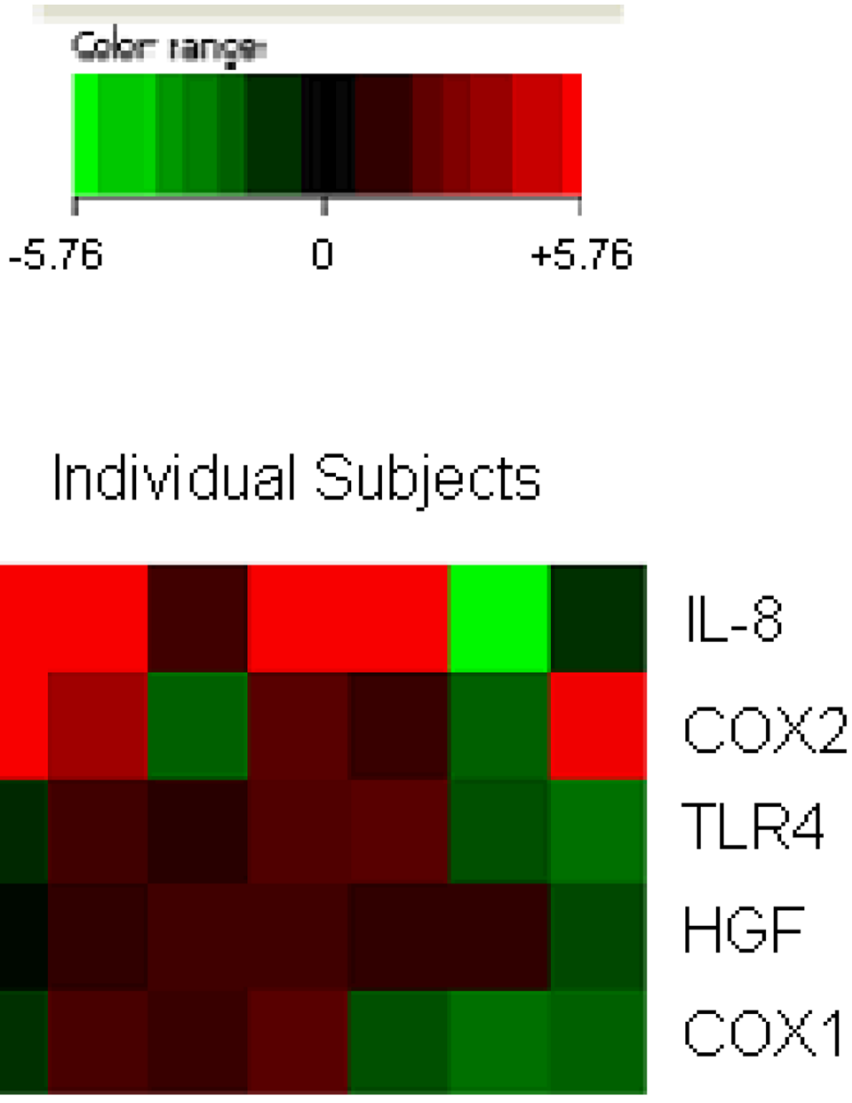

Figure 1.

"Heat maps" depicting a subject-by-subject comparison of antral gene fold changes. Each subject is depicted by one column. Each gene is depicted by one row. Only the five genes for which significant differences between ulcer and non-ulcer populations were noted are depicted. Data are presented on a scale from strong up-regulation (bright red) to strong down regulation (bright green) (see more detailed description in Methods).

Figure 1a. Non-ulcer subjects

Figure 1b. Ulcer subjects.

"Heat map" depicting a subject-by-subject comparison of gene fold changes for subjects who developed ulcers.

Table 1

Genes Analyzed by Quantitative PCR of Tissue Obtained at Baseline and Post-Treatment Endoscopy

Functional Category	Gene	ABI Assay ID
Immune Response/Pro-Inflammatory	Chemokine (C-X-C motif) Receptor 4 (CXCR-4)	Hs00607978_s1
	Cyclo-oxygenase 2 (COX-2)	Hs00153133_m1
	Interleukin 1 beta (IL1B)	Hs00174097_m1
	Interleukin 8 (IL-8)	Hs00174103_m1
	Tumor Necrosis Factor (TNF)	Hs00174128_m1
Growth Factors/Mucosal healing	Cyclo-oxygenase 1(COX-1)	Hs00168776_m1
	Endothelial Growth factor	Hs00153181_m1
	Hepatocyte Growth Factor (HGF)	Hs00300159_m1
	Platelet Derived Growth Factor C (PDGFC)	Hs00211916_m1
	Platelet Derived Growth Factor D (PDGFD)	Hs00937332_m1
	Toll Like Receptor 2 (TLR-2)	Hs01872448_s1
	Toll Like Receptor 4 (TLR-4)	Hs00152939_m1
	Toll Like Receptor 5 (TLR-5)	Hs00152825_m1
	Transforming Growth Factor Alpha (TGFA)	Hs00177401_m1
	Transforming Growth Factor Beta (TGFB2)	Hs00234244_m1
	Vascular Epidermal Growth Factor (VEGF)	Hs00900054_m1
Miscellaneous	Angiopoietin 1 (ANGPT-1)	Hs00375823_m1
	Annexin-1 (ANXA-1)	Hs00167549_m1
	Annexin-2 (ANXA-2)	Hs00743063_s1
	Hydroxyprostaglandin Dehydrogenase (HPGD)	Hs00168359_m1
	Inducible Nitric Oxide Synthetase (INOS)	Hs00167257_m1
	Trefoil Factor 1 (TFF-1)	Hs00170216_m1
	Trefoil Factor 2 (TFF-2)	Hs00193719_m1

Table 2

Subject Demographics

Subject Characteristics	Ulcer (N=7)	No Ulcer (N=18)
Age, y		
Mean (SD)	57.4 (7.25)	57.6 (8.19)
Range	50–68	50–75
Sex, no. (%)		
Male	2 (28.6)	8 (44.4)
Race, no (%)		
White	5 (71.4)	14 (77.8)
Black	0 (0)	2 (11.1)
Hispanic	2 (28.6)	1 (5.6)
Asian	0 (0)	1 (5.6)

Table 3

A comparison of fold changes (baseline versus post-treatment) in genes in naproxen-treated patients between the ulcer and the non-ulcer populations. Tissue samples were obtained from normal-appearing antral mucosa. The 5/23 genes are presented for which significant differences between the ulcer and non-ulcer groups were observed. In one non-ulcer patient, RNA degradation in the post-endoscopy specimen prevented analysis of gene expression, and this patient was excluded from the analysis.

Gene	Ulcer (N = 7)	No Ulcer (N = 17)	Placebo (N=4)
Hepatocyte Growth Factor	−2.2 (0.3)	8.2 (2.6)	−0.9 (0.7)
Cyclo-oxygenase 1	0.0 (0.7)	9.8 (2.4)	0.3 (0.8)
Toll-Like Receptor-4	−0.8 (1.0)	14.0 (2.2)	−0.2 (1.4)
Cyclo-oxygenase-2	2.3 (1.7)	−10.8 (2.2)	−0.7 (1.2)
Interleukin-8	33.5 (18.5)	−7.7 (3.2)	−1.8 (1.1)

Data are expressed as mean (SEM). P= 0.042 by Wilcoxon matched pairs signed ranks test.

Table 4

A comparison of fold changes (baseline versus post-treatment) in genes in duodenal mucosa in naproxen-treated patients between antral ulcer and the non antral-ulcer populations. Unlike in antral tissue, differences between the ulcer and non-ulcer populations are not significant. All biopsies were taken from normal-appearing mucosa. Six subjects were excluded because of lost sample/poor RNA quality. PCR assays for inducible nitric oxide synthetase and endothelial growth factor (EGF) did not produce interpretable data.

Gene	Ulcer (N = 5)	No Ulcer (N = 14)
Hepatocyte Growth Factor	−3.40 (2.95)	−0.49 (2.15)
Cyclo-oxygenase 1	−0.27 (1.24)	2.10 (1.52)
Toll-Like Receptor-4	−0.34 (2.59)	−1.01 (1.76)
Cyclo-oxygenase-2	3.46 (0.57)	4.23 (1.53)
Interleukin-8	57.05 (38.46)	21.03 (10.7)

Data are expressed as mean (SEM). P= 0.038 by Wilcoxon matched pairs signed ranks test.