NSAID-induced gastrointestinal damage and the design of GI-sparing NSAIDs

John L Wallace* & Linda Vong

Address
University of Calgary, Department of Pharmacology & Therapeutics, 3330 Hospital Drive Northwest, Calgary, Alberta T2N 4N1, Canada
Email: wallacej@ucalgary.ca

*To whom correspondence should be addressed

NSAIDs are among the most widely used medications, but the side effects of these drugs frequently include gastrointestinal (GI) ulceration and bleeding. COX-2 inhibitors were designed that were claimed to be devoid of ulcer-promoting effects; however, this promise has been unfulfilled, and there are concerns about the cardiovascular safety of COX-2 inhibitors. Several novel approaches to developing GI-sparing NSAIDs have been used, with promising preclinical and clinical results. Selective inhibition of terminal PG synthases, and NSAIDs modified to slowly release gastroprotective gaseous mediators (ie, nitric oxide or hydrogen sulfide) may offer renewed hope for developing anti-inflammatory therapies with greatly reduced GI toxicity.

Keywords Arthritis, hydrogen sulfide, inflammation, nitric oxide, prostaglandin, ulcer

Introduction

NSAIDs represent one of the most widely used classes of drugs, and are used primarily to alleviate the symptoms (eg, pain and swelling) of osteoarthritis, rheumatoid arthritis and other inflammatory disorders; however, the use of NSAIDs is significantly limited by their ability to induce the formation of erosions and ulcers in the gastrointestinal (GI) tract [1]. Approximately 50% of individuals who use NSAIDs develop gastric erosions, while an estimated 2 to 4% of these individuals develop clinically significant GI ulcers and bleeding, sometimes leading to death [1]. There is therefore a need for anti-inflammatory and analgesic drugs that will provide patients with symptom relief without causing GI injury.

The effectiveness of NSAIDs in reducing pain and swelling lies in their ability to inhibit PG synthesis. COX-1 and COX-2 are the key enzymes for the synthesis of PGs, which have hyperalgesic effects and can augment edema formation. PGs also mediate many components of the GI mucosal defense, including mucus and bicarbonate secretion, mucosal blood flow, epithelial cell turnover, and mucosal immunocyte function [2]. In the healthy stomach, most of the PGs that are produced are derived from COX-1; however, COX-2 activity can be rapidly induced in the stomach in response to subtle challenges, including the administration of aspirin [3] or a short period of ischemia [4]. In these and other circumstances, COX-2-derived PGs make an important contribution to gastric mucosal defense and facilitate the repair of pre-existing ulcers in the stomach [5-7].

The dual contribution of PGs to inflammation and mucosal defense presents a challenge: can symptomatic relief of inflammation be achieved without markedly diminishing the ability of the GI mucosa to defend itself against injury and repair? This review focuses on attempts to solve this problem, particularly highlighting advances during the past 4 years. The reasons for the failure of one approach to developing GI-sparing NSAIDs, the selective COX-2 inhibitors, are also summarized.

Selective COX-2 inhibitors: A promise unfulfilled

By suppressing mucosal PG synthesis (as well as through direct topical irritant effects on the epithelium), NSAIDs can impair mucosal defense and render the stomach more susceptible to injury (Figure 1). While conventional NSAIDs inhibit both COX-1 and COX-2, selective inhibition of the COX-2 isoform was considered more therapeutically desirable – the hypothesis being that suppression of COX-2 activity would reduce the production of PGs at sites of inflammation while sparing COX-1-mediated PG synthesis in the stomach. However, COX-2 significantly contributes to mucosal defense and repair, and the two COX isoforms appear to act in a cooperative manner in physiological responses to tissue injury [7]. NSAID-induced gastric damage requires suppression of both COX-1 and COX-2 [8]. The observation that selective inhibition of COX-1 does not result in significant gastric damage, at least in healthy animals, has led to the proposal that selective COX-1 inhibitors may be gastrointestinal-sparing analgesic compounds [9]. Clinically, selective COX-2 inhibitors, at least in some studies, produced severe GI complications less frequently than conventional NSAIDs [10]; however, COX-2 inhibitors produced clinically significant GI injury more frequently than placebo [11].
Results from animal studies of the GI safety of selective COX-2 inhibitors suggested that these drugs might fall short of predicted GI-sparing properties [12]. Shortly after administration of NSAIDs, leukocytes (primarily neutrophils) adhere to the vascular endothelium in the GI microcirculation [13]. This event appears to be critical in the pathogenesis of NSAID gastropathy, because inhibition of the adhesion, through various means, results in the prevention of gastric ulceration in laboratory animals [13]. Neutrophil adhesion to the vascular endothelium likely contributes to NSAID-induced gastropathy in at least two ways: physical obstruction of capillary flow, and release of tissue-damaging proteases and oxygen-derived free radicals subsequent to activation of the neutrophils. The induction of neutrophil adherence by NSAIDs is due to suppression of COX-2 activity (Figure 1) [8,14]. Thus, selective inhibition of COX-2 activity may spare much of the total PG synthesis by the mucosa, but it also triggers a key event in the pathogenesis of NSAID gastropathy.

NSAID use is also associated with toxicity in tissues other than the GI tract. NSAIDs can elevate blood pressure and cause detrimental effects in the kidney. In the case of selective COX-2 inhibitors, cardiovascular toxicity led to the withdrawal of the largest selling drug of this class, rofecoxib [15]. Recent data suggest a similar association of severe cardiovascular events with the use of celecoxib [16]. There is also emerging evidence that an increase in the incidence of myocardial infarction and stroke is associated not only with the use of selective COX-2 inhibitors, but also with the use of non-selective NSAIDs (the possible exception being naproxen) [15,17]. The underlying mechanism for NSAID-associated cardiovascular events is controversial, and is the subject of ongoing investigations [18-20].

**New approaches: Terminal PG synthase inhibitors**

The cardiovascular toxicity of selective COX-2 inhibitors is possibly a consequence of the inhibition of the synthesis of prostacyclin (PGI₂), which has anti-thrombotic properties, while sparing the synthesis of thromboxane A₂ (TXA₂), a pro-thrombotic substance [21]. However, with the discovery that all NSAIDs may exhibit the cardiovascular toxicity originally ascribed to selective COX-2 inhibitors, this hypothesis will need to be carefully re-evaluated (alternative hypotheses have been investigated previously [19,20]). PGE₂ is the PG primarily associated with inflammation. Therefore, selective inhibition of PGE₂ synthesis could be a rational approach to reducing inflammation without producing the cardiovascular and GI toxicity associated...
with NSAIDs [22]. There is a presumption in this theory that PGs other than PGE\(_2\) will be sufficient to maintain GI mucosal defense. COX-1 and COX-2 metabolize arachidonic acid to PGH\(_2\), which can then be converted via the action of several terminal PG synthase enzymes into the various species of prostanoids (eg, PGE\(_2\), PGI\(_2\) and TXA\(_2\)). Selective inhibition of PGE\(_2\) synthesis at sites of inflammation may be achievable through the inhibition of one of the PG synthases, namely mPGES-1. This enzyme is regarded as inductive, and exhibits a preference for metabolizing PGH\(_2\) that is derived from COX-2 [23,24]. Selective inhibitors of mPGES-1 are in development by several pharmaceutical companies, but there is relatively little published data on their effects in animal models of inflammation. There have been, however, studies of mice genetically deficient for mPGES-1 that provide some insights into this enzyme and its potential as a therapeutic target. Cheng et al reported that mice deficient for mPGES-1 did not exhibit altered thrombogenesis or blood pressure, presumably because synthesis of PGI\(_2\) would be unaltered by deletion of the gene for mPGES-1 [25]. Accompanying the expected decrease in PGE\(_2\) synthesis was a significant increase in PGI\(_2\) synthesis, suggesting that a shunting of PGH\(_2\) to at least one other terminal synthase pathway occurred. If this were to occur in animals or humans chronically ingesting selective inhibitors of mPGES-1, there could be significant therapeutic consequences. PGI\(_2\) has been implicated as an important mediator of pain, therefore elevations in the production of PGI\(_2\) may counteract any analgesic benefit of selectively inhibiting PGE\(_2\) synthesis [26]. Elevated production of PGI\(_2\) could provide benefit through its potent anti-thrombotic effects, although there would also be the possibility of detrimental effects on hemostasis.

In a study of a non-selective inhibitor of mPGES-1 (MK-886), Shinji et al reported that in addition to significant inhibition of mPGES-1 activity, a suppression of VEGF production by gastric fibroblasts was observed [24]. This latter finding is potentially significant given the important role of VEGF in the healing of gastric ulcers [27]. VEGF release is stimulated by PGs produced via COX-2 and mPGES-1 [28]. In addition to these observations, the fact that COX-2-derived PGE\(_2\) synthesis occurs primarily via mPGES-1 and the observation that mice deficient in one of the PGE\(_2\) receptors (EP4) also exhibit a marked impairment of ulcer healing [29], suggest that there is a strong possibility that selective inhibition of mPGES-1 would produce a similar delay in ulcer healing to that observed with selective COX-2 inhibitors [5,7]. Further research is needed, particularly in relevant animal models, to determine if these concerns about selective inhibitors of terminal PG synthases are justified.

**New approaches: Gaseous mediator-releasing NSAIDs**

The hypothesis that a deficiency of PGs in the GI mucosa leads to ulceration has led to several new approaches to drug development, involving supplementation of either PGs or mediators that carry out similar functions. In the 1980s, PG analogs were developed for use in the prophylaxis of NSAID-induced GI injury; however, a high rate of side effects (eg, diarrhea and abdominal pain) limited the widespread use of these drugs. The discovery that other endogenous mediators produced many of the same effects of PGs in terms of mucosal defense has led to the development of novel NSAIDs that slowly release ‘gastroprotective’ substances. Nitric oxide (NO) and hydrogen sulfide (H\(_2\)S) are endogenous gaseous mediators that exhibit many PG-like effects in the GI tract [30]. They are vasodilators and can inhibit leukocyte adherence to the vascular endothelium [30]. Inhibition of mucosal synthesis of NO or H\(_2\)S renders the stomach more susceptible to the damaging effects of NSAIDs and impairs the healing of pre-existing ulcers [31-33]. Conversely, administration of NO or H\(_2\)S donors increases the resistance of the gastric mucosa to injury induced by NSAIDs or other noxious substances [33,34], and accelerates the healing of pre-existing ulcers [7,32,35].

The protective properties of NO and H\(_2\)S in the GI tract make them attractive candidates for coupling to NSAIDs. The premise for this approach is that the release of small amounts of one of these gaseous mediators over prolonged periods of time might compensate, in terms of mucosal defense, for the inhibition of gastric PG synthesis. By counteracting the detrimental effects of the NSAID portion of the molecule, this may be an effective method for reducing GI toxicity.

Because NO and H\(_2\)S are also potent anti-inflammatory agents [36,37], there is the possibility of boosting the anti-inflammatory activity of NSAIDs (and other drugs) by coupling NSAIDs with these gaseous mediators. In the case of ‘NO-NSAIDs’, there is a substantial body of evidence that these drugs produce less GI injury than the parent NSAIDs, both in animal studies [38] and clinical trials [39]. NO-NSAIDs have demonstrated anti-inflammatory effects at least as potent as naproxen and rofecoxib in osteoarthritis patients [40]. Phase III clinical trials of naproxcinod (AZD-3582, NicOx SA; Figure 2), an NO-releasing derivative of naproxen, are scheduled for completion by the end of 2008 [41]. These trials are primarily focused on demonstrating that naproxcinod elevates systemic blood pressure to a significantly lower degree than naproxen, as has been observed in phase II clinical trials [41]. Naproxcinod significantly lowered systemic blood pressure in hypertensive rats [42]. One potential concern associated with the use of naproxcinod is the possibility

![Figure 2. The structure of naproxcinod.](image)
of eliciting brief periods of hypotension that could lead to dizziness, as was observed in a small percentage of patients (6 and 12% of those in the 500- and 750-mg naproxinod groups, respectively, compared with 3 and 5% in the rofecoxib and placebo groups, respectively) in a clinical trial of acute postoperative dental pain [43].

H$_2$S-releasing NSAIDs were developed more recently than NO-NSAIDs, and therefore the published data regarding their actions are more limited. Nevertheless, there is considerable evidence of the important role of H$_2$S in many physiological processes in the GI tract and liver [44], and substantial emerging data to suggest that coupling an H$_2$S-releasing moiety to NSAIDs and other anti-inflammatory drugs can reduce their toxicity and boost efficacy [30]. For example, H$_2$S-releasing derivatives of NSAIDs suppress gastric PG synthesis as effectively as their parent NSAIDs, without causing any detectable gastric damage [45,46]. While administration of an NSAID (diclofenac) resulted in significant increases in mucosal granulocyte infiltration and expression of the adhesion molecules ICAM-1 and lymphocyte function-associated antigen-1, equimolar doses of an H$_2$S-releasing diclofenac (ATB-337, Antibe Therapeutics Inc) did not trigger granulocyte infiltration or upregulate adhesion molecule expression [45]. Moreover, whereas the parent NSAID elicited a significant elevation in gastric TNFα expression, no such effect was observed with ATB-337 [45]. It is hypothesized that some of the additional anti-inflammatory effects observed with H$_2$S-releasing NSAIDs may be attributable to inhibition of NFκB activity by the H$_2$S-releasing moiety [46].

NSAIDs can also cause significant damage to the small intestine. With repeated administration to rats, diclofenac caused extensive intestinal damage, and a 50% decrease in hematocrit (which is indicative of extensive bleeding) [45]. In contrast, repeated administration of ATB-337 resulted in intestinal damage that was reduced in extent by > 90% compared with that induced by diclofenac, and there was no significant change in hematocrit [45]. As mentioned above, H$_2$S donors and H$_2$S-releasing anti-inflammatory drugs significantly accelerate the healing of pre-existing ulcers in rodents [35], whereas NSAIDs and selective COX-2 inhibitors impair the healing of ulcers [5,7,32].

H$_2$S suppressed edema formation, leukocyte adherence and emigration, and demonstrated analgesic effects in a rat model of visceral pain [37,47]. It is perhaps not surprising then that H$_2$S-releasing NSAIDs exhibit greater anti-inflammatory effects than their parent drugs. For example, in a rat model of carrageenan-induced paw edema, ATB-337 inhibited edema formation to the same extent as a 3-fold greater dose (on a molar basis) of diclofenac [45]. Another H$_2$S-releasing NSAID, S-diclofenac (ACS-15, CTG Pharma Srl; Figure 3), significantly inhibited endotoxin-induced cytokine expression, while the parent drug had no effect [46]. S-diclofenac also reduced lung inflammation more efficiently than its parent drug (diclofenac) [48].

Figure 3. The structure of S-diclofenac.

In the aftermath of the withdrawal of several selective COX-2 inhibitors (ie, rofecoxib, lumiracoxib, etoricoxib and parecoxib), regulatory agencies will be increasingly examining the cardiovascular safety of new anti-inflammatory agents, perhaps even more so than their GI safety. It is noteworthy in this regard that both NO- and H$_2$S-releasing NSAIDs protect the heart from ischemia/reperfusion injury in animal models [49,50], in sharp contrast to the detrimental effects of selective COX-2 inhibitors [51].

Conclusions
Over the past two decades, significant advances have been made in understanding the pathogenesis of NSAID-induced GI injury. The identification of key events in the development of ulcers after NSAID administration has provided important clues for designing new anti-inflammatory drugs with reduced toxicity. Selective inhibitors of COX-2, while exhibiting an improved GI safety profile, provoke GI ulceration and bleeding, and their use is also associated with significant cardiovascular toxicity. The coupling of NSAIDs to moieties that slowly release gastroprotective gaseous mediators, such as NO and H$_2$S, appears to offer a novel and promising approach to significantly reducing the toxicity of this widely used class of drugs.

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References


- The first demonstration of rapid upregulation of COX-2 in the stomach after exposure of the mucosa to an agent that suppresses COX-1. This suggested that the upregulation of COX-2 was a compensatory response to diminished mucosal PG levels.


- This was the first paper to report that COX-2 was strongly induced along the margins of gastric ulcers, and selective inhibition of COX-2 led to a significant inhibition of ulcer healing.


- A demonstration that selective inhibition of either COX isoform alone is insufficient to induce gastric damage. This paper highlighted an important contribution of COX-2 to mucosal defense in the healthy GI tract.


- This paper provided evidence that a selective COX-2 inhibitor, rofecoxib, produced significantly more ulcers and bleeding than placebo, which contradicted earlier claims about the GI safety of rofecoxib.


- This analysis of cardiovascular events among NSAID users strongly suggested that all NSAIDs, not just selective COX-2 inhibitors, had the capacity to increase the incidence of serious cardiovascular events (naproxen being a possible exception).


- This provocative and elegant study provided evidence to support the theory that NSAIDs may affect COX-1 activity differently in the endothelium compared with platelets and this may underlie the ability of NSAIDs to increase the incidence of serious cardiovascular events.


- This paper reports data suggesting that PG derived from mPGES-1 may play an important role in ulcer healing. This highlights the importance of examining the safety of selective inhibitors of mPGES-1 in animal models of ulcer healing.


• This was the first study to demonstrate that H₂S can suppress leukocyte adherence to the endothelium, leukocyte emigration, and edema formation. It also provided evidence that endogenous H₂S tonically downregulates inflammatory processes.


• First clinical evidence that an NO-releasing NSAID derivative could reduce the symptoms of osteoarthritis at least as effectively as the parent NSAID (naproxen) and a selective COX-2 inhibitor (rofecoxib).


