

# GI-Sparing Anti-inflammatory Drugs: A Promising Future

John L. Wallace and Andre G. Buret

*Inflammation Research Network, University of Calgary, Calgary, Alberta, Canada*

## INTRODUCTION

The promise of effective anti-inflammatory drugs that do not cause gastrointestinal (GI) injury has been oft-repeated in the past 40 years, but not yet delivered on. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and edema by suppressing the formation of prostaglandins.<sup>1</sup> They do this by inhibiting the activity of the enzymes cyclooxygenase (COX)-1 and -2. However, prostaglandins are key mediators of several components of GI mucosal defense, so suppression of their synthesis by NSAIDs greatly reduces the resistance of the mucosa to injury as well as interfering with repair processes.<sup>2</sup> Selective COX-2 inhibitors were thought to be the solution to this conundrum, as they were proposed to suppress prostaglandin synthesis at sites of inflammation, but not in the GI tract.<sup>3</sup> However, it is now clear that both COX-1 and COX-2 contribute to mucosal defense.<sup>4</sup> Selective COX-2 inhibitors elicit less clinically significant GI damage and bleeding than conventional NSAIDs,<sup>5,6</sup> although the magnitude of this reduction continues to be contested in the literature.<sup>7,8</sup> As widely reported in the lay-press, there are also significant adverse effects of selective COX-2 inhibitors in the renal and cardiovascular systems, possibly more serious than those caused by conventional NSAIDs.<sup>9,10</sup>

The world market for NSAIDs, including the selective COX-2 inhibitors, exceeds \$8 billion per year. The market for NSAIDs is expanding rapidly because of an aging population in developed countries and the associated increase in the prevalence of diseases like arthritis. Aspirin use is also increasing because of its utility in reducing the incidence of a number of common disorders, including stroke, myocardial infarction, Alzheimer's disease, and various cancers.

In recent years, several novel approaches to reducing the GI toxicity of NSAIDs have been taken, with promising results. These mainly involve modification of existing NSAIDs such that inhibition of COX is maintained, but other attributes are added that diminish GI (and other) toxicity, and in some cases boost efficacy and/or potency. The rationale for these approaches, the preclinical and clinical evidence that these approaches are rational, and the potential utility of these new classes of drugs are reviewed below.

## NITRIC OXIDE-RELEASING NSAIDS

Nitric oxide (NO) is an endogenous gaseous mediator that is involved in a wide variety of physiological processes, including vascular and nonvascular smooth muscle relaxation and neurotransmission. It is also recognized as a critical mediator of GI mucosal defenses,<sup>11</sup> exerting many of the same actions as prostaglandins in the GI tract. Like prostaglandins, NO modulates mucosal blood flow, mucus and bicarbonate secretion, and repair of mucosal injury.<sup>11</sup> NO is also a very potent inhibitor of neutrophil adherence to the vascular endothelium.<sup>12</sup> This observation was critical to the development of NO-releasing NSAIDs

(NO-NSAIDs), since it had been discovered in the early 1990s that adherence of neutrophils to the vascular endothelium in the gastric microcirculation was a critical event in the pathogenesis of NSAID-induced gastric damage.<sup>13-18</sup> Moreover, NO suppresses the release from mast cells of several inflammatory mediators that are known to contribute to gastric ulceration,<sup>19</sup> including platelet-activating factor.<sup>20</sup> Not surprisingly, given these actions, nitric oxide donors reduce the severity of gastric injury in experimental models<sup>21,22</sup> and can accelerate healing of experimental gastric ulcers.<sup>23,24</sup> It is noteworthy that use of NO donors (for cardiovascular indications) has been shown to be associated a significant reduction in GI bleeding in patients who are also taking aspirin.<sup>25</sup>

The development of NO-NSAIDs was based on the premise that slow release of NO would suppress NSAID-induced neutrophil adherence to the vascular endothelium, thereby preventing damage to the gastric mucosa.<sup>26-29</sup> Other experimental interventions that prevented NSAID-induced neutrophil adherence, such as antibodies against adhesion molecules, had been found to prevent gastric damage.<sup>13,17</sup> Moreover, as NO is a potent vasodilator, NO-NSAIDs would not reduce mucosal blood flow as conventional NSAIDs do.<sup>29</sup> The challenge, from a chemistry standpoint, was to create an NSAID that delivered NO at concentrations sufficient to produce these desired effects without causing systemic effects, such as hypotension, and without producing vascular tolerance, as is seen with some conventional NO-releasing dilators (e.g., glyceryltrinitrate). Of course, it was also essential that the chemical modification of the NSAID did not interfere with its ability to inhibit cyclooxygenase activity, as this would diminish the anti-inflammatory and analgesic activity.

Derivatives of a number of different NSAIDs were made, using ester linkages to NO-releasing groups, and several of the desired features were proven to have been attained in animal models:

**Feature 1.** Despite suppressing gastric prostaglandin synthesis as effectively as the parent drugs,<sup>26,28,30-32</sup> NO-NSAIDs do not reduce gastric mucosal blood flow,<sup>28</sup> nor cause leukocyte adherence to the vascular endothelium.<sup>26</sup> NO-NSAIDs elicit significantly less gastric and small intestinal damage than the parent NSAIDs, even when given repeatedly over a number of weeks.<sup>26,31,32</sup> With prolonged administration, there is no evidence of the development of tolerance. NO-NSAIDs were also assessed in rats with pre-existing gastric ulcers. Conventional NSAIDs and selective COX-2 inhibitors significantly retard gastric ulcer healing.<sup>24,33-35</sup> In contrast, NO-NSAIDs did not impair gastric ulcer healing, and in some cases, actually accelerated it.<sup>24,35,36</sup> NSAIDs have also been reported to exacerbate inflammatory bowel disease,<sup>37-39</sup> and the same has been shown in experimental colitis in rats.<sup>40,41</sup> In contrast, an NO-NSAID was well tolerated by rats with colitis.<sup>31</sup>

**Feature 2.** NSAIDs, including selective COX-2 inhibitors, can exacerbate hypertension and interfere with the antihypertensive

effects of some agents.<sup>42</sup> Hypertension is a predisposing factor for myocardial infarction and stroke,<sup>43</sup> and even elevations in systemic blood pressure of only 3–5 mm Hg can significantly increase the risk of serious cardiovascular events.<sup>44</sup> While inhibiting prostaglandin synthesis to the same extent as the parent drugs, NO-NSAIDs exhibited increased analgesic potency.<sup>32</sup> NO-NSAIDs, in some models, also exhibited an expanded anti-inflammatory profile as compared to the parent drugs.<sup>45</sup> For example, NO-NSAIDs potently suppressed synthesis of several proinflammatory cytokines (e.g., TNF $\alpha$ , IL-1 $\beta$  and IFN $\gamma$ ).<sup>45,46</sup>

**Feature 3.** NO-NSAIDs do not markedly alter systemic arterial blood pressure in healthy laboratory animals or in healthy human volunteers,<sup>30,47</sup> but produce significant reductions in systemic blood pressure when given to hypertensive rodents.<sup>48–52</sup> NO-NSAIDs do not produce the small, but clinically significant, increases in blood pressure in arthritis patients that are seen with both conventional NSAIDs and selective COX-2 inhibitors.<sup>53</sup> Pharmacokinetic studies have confirmed the prolonged release of small amounts of NO from NO-NSAIDs.<sup>47</sup>

**Feature 4.** Long-term use of NSAIDs has been shown to cause renal papillary necrosis and other forms of renal injury as well as reducing renal perfusion in patients in whom there is a greater dependency on local prostaglandin synthesis for maintenance of renal blood flow.<sup>55</sup> The NO-NSAIDs can be clearly distinguished from conventional and COX-2-selective NSAIDs in terms of renal and cardiovascular toxicity. In a model of extensive renal ablation, treatment with an NO-NSAID resulted in significant recovery of renal structure and function, in sharp contrast to the effects of the conventional NSAID.<sup>56</sup> The NO-NSAID markedly reduced albuminuria and systemic blood pressure, without causing anemia.

During cardiac ischemia, COX-2 is up-regulated in the myocardium and the production of prostacyclin from this enzyme acts to preserve myocardial blood flow.<sup>57–59</sup> NO-NSAIDs, by virtue of the release of NO, can protect the myocardium in ischemia-reperfusion.<sup>60</sup> While aspirin and a number of selective COX-2 inhibitors were found to exacerbate ischemia-reperfusion-induced myocardial damage and dysfunction in a rabbit model,<sup>57</sup> an NO-releasing derivative of aspirin significantly reduced infarct size.<sup>61</sup> The potential clinical utility of NO-releasing aspirin as a cardioprotective agent was recently reviewed.<sup>62</sup>

**Feature 5.** GI safety of NO-NSAIDs in humans has been demonstrated in endoscopic studies and through measurement of small intestinal permeability.<sup>63,64</sup> In a recent trial of NO-releasing naproxen in patients with osteoarthritis of the knee, the NO-NSAID reduced the signs and symptoms to a similar extent as naproxen or rofecoxib.<sup>53</sup> (Figure 1) Interestingly, even at half the molar dose of naproxen, the NO-NSAID produced comparable reductions of pain, consistent with the observed increase in analgesic potency in animal studies.<sup>32</sup>

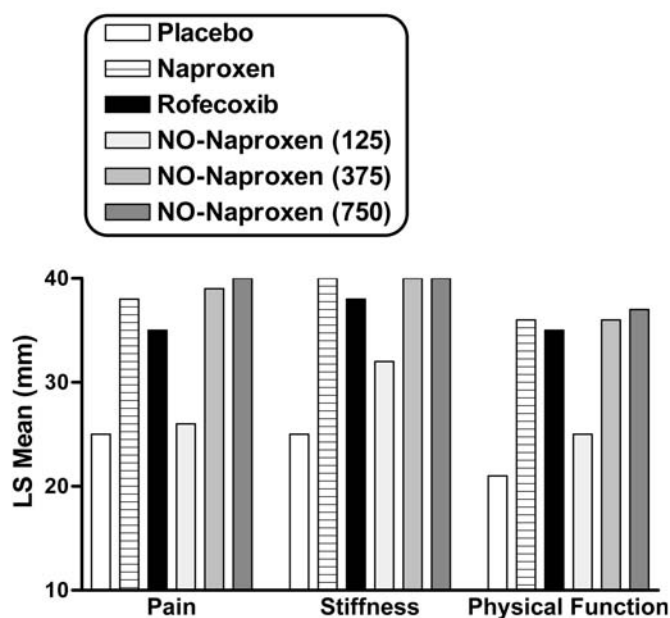
## HYDROGEN SULFIDE: ANOTHER GAS, ANOTHER SOLUTION

Hydrogen sulfide (H<sub>2</sub>S) is very well known for its “rotten egg” smell and its toxicity, but in recent years, it has become recognized as an important endogenous mediator sharing many actions in common with NO.<sup>65</sup> Several studies in recent years have highlighted roles for H<sub>2</sub>S in immune and inflammatory reactions. Given the commonality of actions of H<sub>2</sub>S and NO, it is possible that incorporating an H<sub>2</sub>S-releasing moiety into NSAIDs will result in a significant reduction in their ulcerogenic properties. Like NO, H<sub>2</sub>S is a vasodilator<sup>65</sup> and an

inhibitor of leukocyte adherence to the vascular endothelium.<sup>66</sup> H<sub>2</sub>S inhibits leukocyte adhesion, at least in part, through inhibition of adhesion molecule expression on leukocytes and on endothelial cells.<sup>66</sup> Systemic administration of an H<sub>2</sub>S donor at a dose as low as 10  $\mu$ mol/kg suppressed aspirin-induced leukocyte adherence in the rat.<sup>66</sup> Consistent with its vasodilator action in other tissues, H<sub>2</sub>S significantly elevates gastric mucosal blood flow.<sup>66</sup> Interestingly, the endogenous production of H<sub>2</sub>S by the stomach can be inhibited by NSAIDs,<sup>66</sup> possibly underscoring an important role of H<sub>2</sub>S in gastric mucosal defense.

The above-mentioned actions of H<sub>2</sub>S suggest that it, like NO, should be able to reduce the severity of NSAID-induced gastric injury. Indeed, proof-of-concept data have been reported.<sup>66</sup> (Figure 2) Administration of an H<sub>2</sub>S donor prior to various NSAIDs resulted in a marked reduction of gastric injury. The H<sub>2</sub>S donor also significantly reduced the granulocyte infiltration of the mucosa stimulated by NSAIDs.<sup>66</sup> The latter effect is likely attributable to the ability of H<sub>2</sub>S to inhibit leukocyte adherence to the vascular endothelium, but could also be related to the reported ability of H<sub>2</sub>S to induce apoptosis of neutrophils.<sup>67</sup> H<sub>2</sub>S exerts several other actions that could contribute to its gastroprotective effects, including interfering with hypochlorous acid- and peroxynitrite-mediated tissue injury.<sup>68,69</sup>

H<sub>2</sub>S-releasing NSAIDs are in development, but no data have yet been published regarding these compounds. In addition to reduced gastrointestinal toxicity, there is good reason to believe that these compounds will exhibit greater potency as analgesic agents than

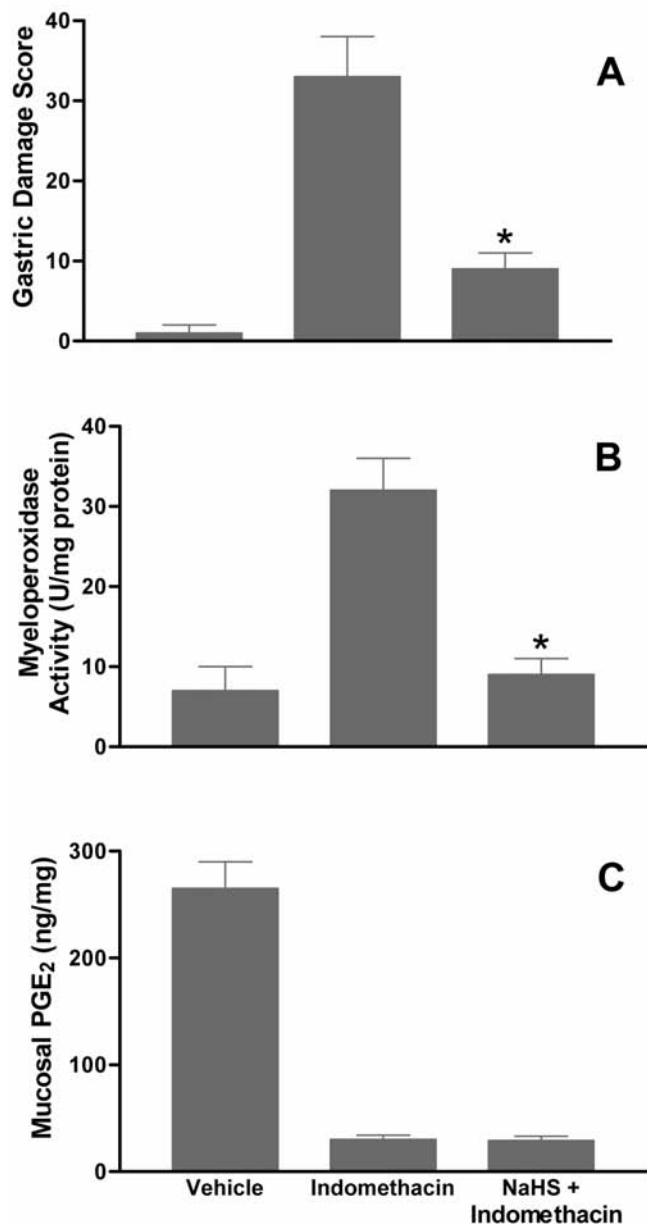


**Figure 1.** Effects of NO-naproxen versus naproxen and rofecoxib in patients with osteoarthritis of the knee. Data from weeks 4 and 6 of the study are shown as the least squares mean change from baseline in Western Ontario and McMaster Universities Osteoarthritis Index subscale scores (pain, stiffness, and physical function). Rofecoxib was given once daily at 25 mg. Naproxen was given at 500 mg/kg twice daily. NO-naproxen was given at a dose equimolar to that of naproxen (750 mg twice daily), at half that dose (375 mg twice daily), and at one quarter that dose (125 mg twice daily). NO-naproxen at 375 mg and 750 mg was statistically superior to rofecoxib in reducing pain ( $P < .001$ ). Further details of this study can be found in Schnitzer et al.<sup>53</sup>

conventional NSAIDs. H<sub>2</sub>S donors have recently been shown to exert analgesic effects in a model of colorectal distention-induced visceral pain.<sup>70</sup> These effects were shown to be mediated via K<sup>+</sup><sub>ATP</sub> channels.<sup>70</sup> Interestingly, NSAIDs have been shown to produce their analgesic effects, in part, through activation of K<sup>+</sup><sub>ATP</sub> channels.<sup>71</sup>

### PHOSPHATIDYLCHOLINE-ASSOCIATED NSAIDs

While suppression of gastric prostaglandin synthesis, through inhibi-



**Figure 2.** Protection of the gastric mucosa of the rat against indomethacin-induced damage by a hydrogen sulfide donor (NaHS; 100  $\mu$ mol/kg IP). Rats were given vehicle or NaHS 30 minutes prior to indomethacin (10 mg/kg PO). Gastric damage (panel A) was blindly scored and tissue samples were taken for measurement of myeloperoxidase activity (panel B) and prostaglandin E<sub>2</sub> (panel C). Myeloperoxidase is a biochemical marker for granulocytes. Pretreatment with the hydrogen sulfide donor significantly reduced the severity of gastric damage and granulocyte infiltration induced by indomethacin, but did not interfere with its ability to suppress gastric prostaglandin synthesis. Further details of these experiments can be found in Fiorucci et al.<sup>66</sup> \**P* < .05 versus the indomethacin group.

tion of COX-1 and COX-2 is often regarded as the primary mechanism through which NSAIDs cause gastric injury, there is strong evidence suggesting that COX-independent mechanisms are also important.<sup>72</sup> In particular, acidic NSAIDs are known to cause damage to the gastrointestinal epithelium through “topical” effects.

Lichtenberger and colleagues have been particularly active in demonstrating the ability of NSAIDs to interact with phospholipids within the mucus on the surface of the mucosa, thus rendering this barrier less hydrophobic.<sup>73</sup> They reasoned that if NSAIDs were preassociated with phosphatidylcholine (PC), they would cause less disruption of the hydrophobic barrier, and thus cause less gastric injury.<sup>74</sup>

Several “PC-NSAIDs” have been developed and tested in animal models and have been shown to cause significantly less gastric and intestinal damage.<sup>74</sup> A PC-aspirin derivative significantly accelerated healing of pre-existing ulcers in rat.<sup>75</sup> In a small clinical trial, healthy subjects receiving PC-aspirin exhibited about one third the number of erosions as those receiving an equivalent dose of aspirin, despite both drugs suppressing gastric prostaglandin synthesis to the same extent.<sup>76</sup> Moreover, likely due to enhanced absorption, PC-NSAIDs were found to have increased analgesic and anti-inflammatory activity in rodent models of inflammation.<sup>77,78</sup>

### R-ENANTIOMERS OF CHIRAL NSAIDs

Chiral NSAIDs are generally sold as racemic mixtures, but several studies have demonstrated that the S-enantiomer often exhibits much greater capacity to inhibit COX-1 and COX-2 than the R-enantiomer.<sup>79</sup> The R-enantiomers generally exhibit greatly reduced GI toxicity. However, the R-enantiomers are not without activity; they can exhibit significant beneficial effects, including anti-inflammatory and antiproliferative activities. NSAIDs have been suggested to reduce the incidence of various cancers<sup>80</sup> and of Alzheimer’s disease.<sup>81</sup> The gastrointestinal toxicity of these drugs limits their usefulness for these indications, particularly since the drugs would be taken for very long periods of time. R-enantiomers of some NSAIDs have been shown to be effective in animal models of Alzheimer’s<sup>82</sup> and colon cancer.<sup>83,84</sup> There is one cautionary note: bioconversion from an R-enantiomer to an S-enantiomer can occur in vivo,<sup>85</sup> thereby diminishing the benefits of this approach.

### FUTURE DIRECTIONS

There are several very promising approaches being taken to develop NSAIDs that are at least as effective as those presently available, but with markedly reduced toxicity. The withdrawal of two selective COX-2 inhibitors from the marketplace in recent years, and the increasing awareness of the cardiovascular and renal toxicity of NSAIDs, is fueling the efforts to develop novel anti-inflammatory drugs. NO-NSAIDs are in advanced clinical trials, as are PC-NSAIDs; the development of both of these novel classes of NSAIDs was slowed somewhat by the early success of the selective COX-2 inhibitors. H<sub>2</sub>S has only recently been identified as an important mediator of mucosal defense. Early data suggest that H<sub>2</sub>S-releasing anti-inflammatory drugs offer similar advantages as the NO-NSAIDs, in terms of reducing toxicity and increasing potency and efficacy. The R-enantiomers of NSAIDs are particularly attractive candidates for long-term use in chemoprevention of cancer and Alzheimer’s disease. Advanced clinical trials of these compounds are ongoing.

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Address requests for reprints to: Debra Raden, Assistant Managing Editor, at draden@gastro.org or mail request to 4930 Del Ray Avenue, Bethesda, Maryland 20814.

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