Research Overview

Gastrointestinal-Sparing Anti-Inflammatory Drugs: The Development of Nitric Oxide-Releasing NSAIDs

John L. Wallace,[1*] Susan N. Elliott,[1] Piero Del Soldato,[2] Webb McKnight,[1] Franco Sannicolo,[3] and Giuseppe Cirino[4]

[1]*Department of Pharmacology, The University of Calgary, Calgary, Alberta, Canada*
[2]*NicOx Ltd., Paris, France*
[3]*Department of Organic Chemistry, University of Milan, Milan, Italy*
[4]*Department of Experimental Pharmacology, University of Naples, Naples, Italy*

Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed medications, but their use continues to be limited by significant toxicity, particularly in the gastrointestinal tract and kidney. Better understanding of the pathogenesis of these adverse effects has led to the development of a series of derivatives of standard NSAIDs that are not only less toxic but more efficacious. The coupling of a nitric oxide-releasing moiety to a range of NSAIDs greatly reduces their ability to induce gastrointestinal damage, and greatly increases their tolerability in situations in which there is preexisting gastrointestinal inflammation. There is also evidence that these compounds are much better tolerated by the kidney. On the other hand, the analgesic and anti-thrombotic properties of NO-releasing NSAIDs significantly exceed those of the parent drugs. These compounds appear to represent a significant advance in the treatment of inflammation and pain and for prophylaxis of thrombotic conditions. Drug Dev. Res. 42:144–149, 1997.

Key words: anti-inflammatory; ulcer; analgesic; anti-thrombotic

INTRODUCTION

The class of drugs known as "NSAIDs" (nonsteroidal anti-inflammatory drugs) are among the most commonly used medications [Garner, 1992]. They are prescribed largely for their anti-inflammatory, antipyretic, and analgesic properties but are also very widely used in over-the-counter preparations for the same indications. Moreover, aspirin is increasingly used on a long-term basis for its well-documented anti-thrombotic effects. The major limitation to the use of NSAIDs is their ability to cause ulceration and bleeding in the gastrointestinal tract [Soll et al., 1991]. This effect of NSAIDs has been recognized for decades [Douthwaite and Lintott, 1938]. More recently, it has become apparent that NSAIDs can also exert significant toxicity in the kidney [Segasothy et al., 1994].

Over the past 40 years, numerous new NSAIDs have been marketed with the claim that they reduce gastrointestinal toxicity. These approaches have included enteric coating of the drug to prevent absorption in the stomach, formulation as a pro-drug, to prevent contact between the active drug and the gastric mucosa, and delivery by non-oral routes. The basis for these approaches was the observation that some NSAIDs, particularly those which are acidic, can directly damage gastric epithelial cells. Reducing the topical irritancy should therefore prevent the epithelial damage and, in turn, prevent ulceration and bleeding. However, each of these approaches has failed in terms of reducing the truly significant ad-

*Correspondence to: Dr. John L. Wallace, Department of Pharmacology and Therapeutics, The University of Calgary, 3330 Hospital Drive, NW, Calgary, AB T2N 4N1, Canada. E-mail:wallacej@acs.ucalgary.ca

verse effects of NSAIDs in the gastrointestinal tract [Graham, 1990]. This is now recognized as being attributable to the fact that a central component of the mechanism underlying NSAID-induced ulceration and bleeding is their ability to inhibit prostaglandin synthesis in the gastrointestinal mucosa. Since the desired effects of NSAIDs are also dependent upon suppression of prostaglandin synthesis [Vane, 1971], delivery of the drug to the systemic circulation at a dose that is effective invariably resulted, in some patients, in development of ulcers.

WHY NITRIC OXIDE?

NSAIDs inhibit prostaglandin synthesis by inhibiting the activity of the enzyme cyclo-oxygenase. Prostaglandins play an important role in the gastrointestinal tract in that they mediate several components of mucosal defense (blood flow, mucus and bicarbonate secretion, mucosal immunocyte function) [Wallace and Tigley, 1995]. Inhibition of gastrointestinal prostaglandin synthesis leads to decreased mucus and bicarbonate secretion, decreased blood flow, impaired repair of epithelial injury, and an increase in the number of leukocytes adhering to the vascular endothelium in the gastrointestinal microcirculation. It is this latter observation [Wallace, 1993] that first suggested that nitric oxide (NO) might be capable of preventing NSAID-induced gastric injury. NO can inhibit leukocyte adherence to the vascular endothelium [Kubes et al., 1991]. We had previously observed that prevention of NSAID-induced leukocyte adherence resulted in near-complete protection against gastric injury associated with these drugs in animals [Wallace et al., 1990, 1991, 1993]. Moreover, NO also exhibits many of the same actions in the stomach as prostaglandins; namely, stimulation of mucus secretion [Brown et al., 1992] and maintenance of mucosal blood flow [Whittle, 1993]. Thus, it seemed logical that if NO could be delivered in a controlled manner (i.e., slowly, so that systemic arterial blood pressure would not be affected), the detrimental effects of NSAIDs in the gastrointestinal tract might be prevented (Fig. 1). The rate of delivery of NO, in terms of effectiveness in preventing NSAID-induced gastric damage, was underscored by our observations that some doses of standard NO donors (glyceryl trinitrate, sodium nitroprusside) were capable of reducing the extent of gastric damage induced by an NSAID [Wallace et al., 1994c], but small increases in the dose led to an exacerbation of injury (unpublished observations). Moreover, it was difficult to identify doses of these drugs which were effective in preventing gastric injury without altering systemic blood pressure. Nevertheless, the concept that NO was capable of reducing the severity of NSAID-induced gastric injury was proven [Wallace et al., 1994c]. Protective effects of NO in other models of gastric injury had previously been demonstrated [MacNaughton et al., 1989], but again, increasing the dose of the NO donor led to an exacerbation of injury and profound effects on systemic blood pressure [Lopez-Belmonte et al., 1993].

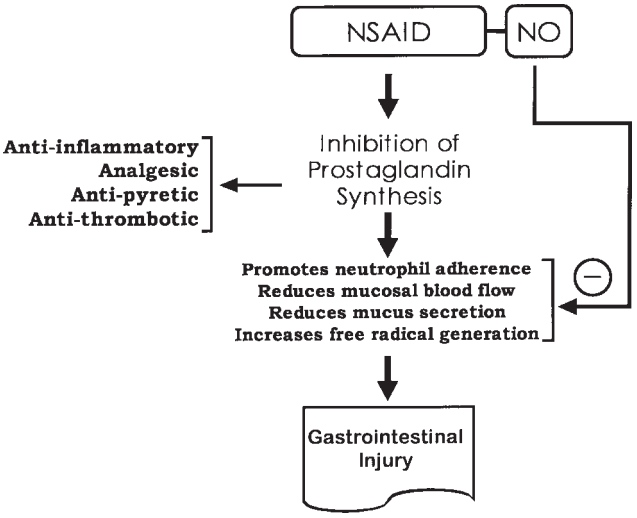

Fig. 1. Schematic diagram illustrating the rationale behind the development of NO-releasing NSAIDs. As the compounds retain the ability to inhibit cyclo-oxygenase, they inhibit prostaglandin synthesis as effectively as the parent drugs. However, the release of NO counteracts the detrimental effects of inhibition of prostaglandin synthesis in the gastrointestinal tract. The release of NO also appears to enhance the analgesic and anti-thrombotic efficacy of the drugs over that of the parent drugs.

NO-NSAIDS: REDUCED TOXICITY

The hypothesis that NO-releasing derivatives of standard NSAIDs would have reduced toxicity has now been tested using derivatives of flurbiprofen, ketoprofen, naproxen, aspirin, and diclofenac [Wallace et al., 1994a,b, 1995a, 1997; Davies et al., 1996]. When given as a single dose, all of the NO-NSAIDs have been shown to produce significantly less gastric damage than standard NSAIDs [Wallace et al., 1994a,b, 1995a; Davies et al., 1996]. Figure 2 shows the effects of an NO-aspirin derivative (NCX-4016) in comparison to aspirin on the rat stomach. A similar reduction in toxicity is apparent if the compounds are given systemically rather than orally, indicating that the reduced injury observed with these compounds is not solely due to reduced "topical irritant" properties. With repeated administration for up to three weeks, these compounds were also shown to produce significantly less gastrointestinal injury [Wallace et al., 1994a; Reuter et al., 1994; Davies et al., 1996].

A major clinical problem associated with the use of NSAIDs is the ability of these drugs to interfere with the healing of preexisting ulcers. For this reason, we assessed the effects of NO-NSAIDs in three models. First, we studied the effects of an NO-NSAID in a model of colitis in the rat [Reuter et al., 1994]. In this model, standard

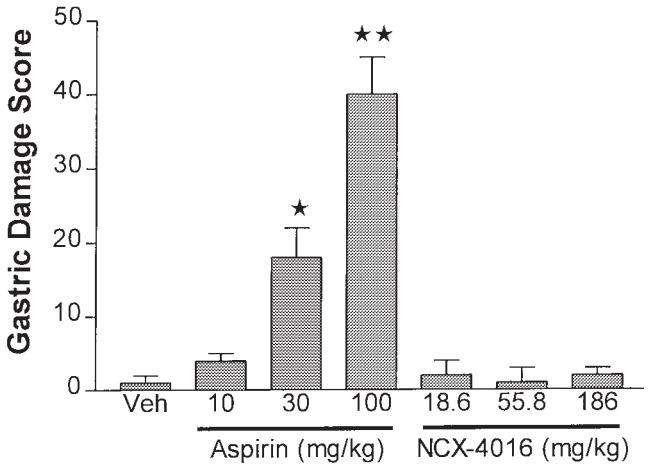

Fig. 2. Extent of gastric damage induced in the rat stomach 3 h after oral administration of aspirin or equimolar doses of an NO-aspirin derivative (NCX-4016). Aspirin caused significant damage at doses of 30 mg/kg or greater (★ = $P < 0.05$, ★★ = $P < 0.01$ vs. the vehicle-treated group), while NCX-4016 did not cause damage at any dose tested.

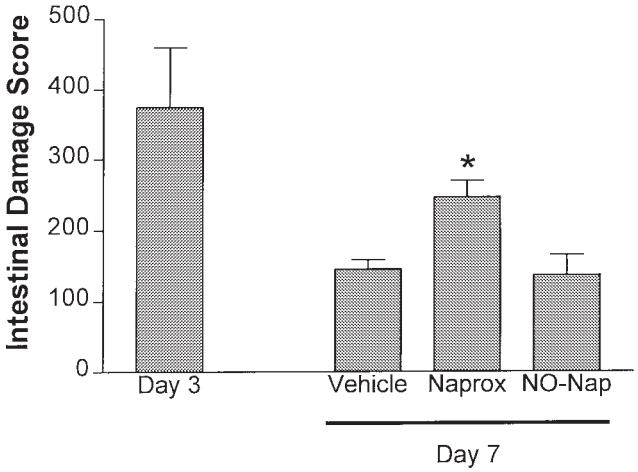

Fig. 3. Effects of naproxen and NO-naproxen on healing of intestinal ulcers induced by indomethacin. Indomethacin was injected subcutaneously (7.5 mg/kg) twice, separated by 24 h. 48 h after the second indomethacin injection (day 3), the rats were randomized to receive naproxen, an equimolar dose of NO-naproxen, or vehicle. These drugs were given orally every 12 h for 4 days. 12 h after the final dose, the damage was scored. * = $P < 0.05$ compared to the vehicle-treated group.

NSAIDs cause a marked exacerbation of colonic injury, leading to perforation and death. For example, in the case of diclofenac, treatment for one week at doses of 1, 5, and 10 mg/kg led to the death of 29%, 86%, and 100% of the rats, respectively. In contrast, treatment with equimolar doses of nitrofenac resulted in markedly less mortality (0%, 0%, and 33%, respectively). The second model was one in which the effect of nitrofenac on healing of gastric ulcers in the rat were examined [Elliott et al., 1995]. While daily diclofenac administration for a week caused a significant reduction in body weight gain and a marked decrease in hematocrit, nitrofenac did not alter either of these parameters in comparison to the vehicle-treated control group. Moreover, nitrofenac actually accelerated ulcer healing. The third model we utilized was one in which intestinal injury is established by treatment with indomethacin, and the rats are then switched to a different NSAID, to the corresponding NO-NSAID, or to vehicle. This model mimics the clinical situation in which a patient develops gastrointestinal damage while taking an NSAID, and has to be switched to a second NSAID in the hope that it will be less injurious. The results from one study performed with this model are shown in Figure 3. Intestinal injury was induced by two subcutaneous injections of indomethacin (7.5 mg/kg) 24 h apart. The rats were left for 48 h, after which they were randomized to receive naproxen (20 mg/kg), an equimolar dose of NO-naproxen, or vehicle. The test drugs or vehicle were administered orally every 12 h for 4 days, after which time the degree of intestinal ulceration was blindly scored. Indomethacin induced widespread intestinal ulceration. Considerable healing was observed in the group subsequently treated with vehicle. In contrast, naproxen significantly delayed healing. On the other hand, NO-naproxen, which suppresses prostaglandin synthesis in the intestine to the same extent as naproxen, did not cause any retardation of ulcer healing.

The fact that gastrointestinal toxicity was not observed following repeated administration of the NO-NSAIDs over periods of up to three weeks further supports the contention that these derivatives are not merely pro-drugs. Moreover, recent pharmacokinetic studies have demonstrated that the intact NO-NSAIDs can be detected in the plasma of rats for many hours after administration [Davies et al., 1996; Reuter et al., 1997]. Despite evidence that these agents generate NO in the rat in vivo, they do not significantly affect systemic blood pressure [Wallace et al., 1994a,b, 1995a].

There is also evidence suggesting that NO-NSAIDs spare the renal system. We tested the effects of administration of an NSAID (diclofenac) or the NO-releasing derivative (nitrofenac) on renal blood flow in both healthy and cirrhotic rats. In both subgroups, diclofenac caused a significant reduction in renal blood flow, while nitrofenac had no effect. The effects of the two drugs on renal blood flow in healthy rats are shown in Figure 4. As the renal toxicity of NSAIDs is believed to be attributable to reduced blood flow secondary to suppression of prostaglandin synthesis [Segasothy et al., 1994], these results suggest that NO-NSAIDs may not produce the renal toxicity associated with the parent NSAIDs. This conclusion is supported by recent studies in which NO-NSAIDs were found to be significantly better tolerated in a rat model of kidney failure than native NSAIDs [Fujihara et al., 1995].

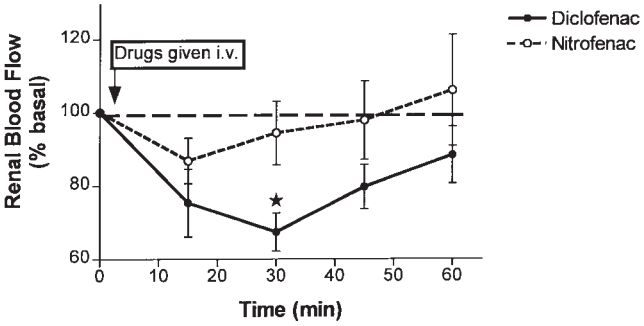

Fig. 4. Effects of diclofenac (10 mg/kg) and an equimolar dose of its NO-releasing derivative, nitrofenac, on renal blood flow in healthy rats. ⋆ = $P < 0.05$ compared to the corresponding basal period.

NO-NSAIDS: EFFICACY

Several of the NO-NSAIDs have been compared to the parent drugs in models of acute and chronic inflammation [Wallace et al., 1994a,b; Cuzzolin et al., 1995; Davies et al., 1996]. In each case, these compounds were found to reduce inflammation at least as effectively as the parent NSAID. The NO-releasing diclofenac derivative has also been shown to have virtually identical anti-pyretic activity to diclofenac [Wallace et al., 1995b]. The NO-naproxen derivative has significantly enhanced analgesic activity over the parent drug [Davies et al., 1996], while an NO-aspirin derivative was found to have markedly increased anti-thrombotic properties [Wallace et al., 1995a].

The ability of NO-NSAIDs to suppress inflammation, pain, and fever is not surprising given the observation that these derivatives retain the ability of the parent drugs to inhibit prostaglandin synthesis (with the exception of the NO-aspirin derivatives; see below) [Wallace et al., 1994a,b; Mitchell et al., 1994]. The NO-NSAIDs themselves inhibit cyclo-oxygenase activity (types 1 and 2) without being metabolized, indicating that these derivatives are not just pro-drugs [Mitchell et al., 1994]. In the case of the NO-aspirin derivatives, however, the activity on cyclo-oxygenase activity is greatly reduced, perhaps contributing to the profoundly reduced gastric toxicity of these drugs [Wallace et al., 1995a]. Both NCX-4215 and NCX-4016 have been shown to have weak inhibitory activity on platelet thromboxane synthesis [Wallace et al., 1995a, 1997b]. With repeated dosing over several days, however, the level of inhibition of thromboxane synthesis increases to levels comparable to equimolar doses of aspirin [Wallace et al., 1997b]. However, this weak effect on thromboxane synthesis does not account for the anti-thrombotic properties of the two NO-aspirin derivatives. These compounds have been shown to generate NO when incubated with platelets, and to cause a significant increase in platelet cyclic GMP levels [Wallace et al., 1995a, 1997a]. Indeed, there is strong evidence that a significant component of the anti-platelet effects of NO-aspirin derivatives is due to NO generation [Wallace et al., 1995a]. These compounds also have the ability to suppress neutrophil adherence to the vascular endothelium [Wallace et al., 1997a], which might add to their anti-thrombotic effects. This raises the possibility that an NO-releasing aspirin derivative may have utility in long-term use for the prevention of myocardial infarction and stroke. Aspirin is presently used for these indications [SALT Collaborative Group, 1991; Meade et al., 1992; Patrono, 1994], but despite the fact that low doses of this drug are used, there is still a significant increase in the incidence of gastrointestinal bleeding and other hemostatic complications [SALT Collaborative Group, 1991; Meade et al., 1992; Cryer et al., 1995].

NITRATE VS. NITRITE: A CRITICAL DIFFERENCE

Several options existed with respect to the NO-releasing moiety to link to NSAIDs. For example, nitrites, nitrates, and S-thiol-glutathione are all well-characterized NO donors [Moncada et al., 1991]. The selection of a nitrate group was based on two key issues: stability and toxicity.

Stability of a flurbiprofen nitrite ester and a flurbiprofen nitrate ester were compared using nuclear magnetic resonance. When dissolved in dimethylsulfoxide and incubated at 20°C for 1 h, the nitrite-containing compound was found to undergo 25% hydrolysis, while no hydrolysis of the nitrate-containing compound occurred. Moreover, the native nitrate-containing compound was found to be stable at room temperature for more than a year.

In terms of toxicity, the primary concern in choosing an appropriate NO-releasing group was mutagenicity. The Ames test, which measures the potential of a test substance to induce point mutations in *Salmonella typhimurium*, was used to compare the effects of sodium nitrite, sodium nitrate, S-nitroso-glutathione, and four nitrate-containing NO-NSAIDs (derivatives of flurbiprofen, aspirin, naproxen, and diclofenac). In this assay, mutagenic substances induce reversion of a histidine-deficient strain of *S. typhimurium* such that it is then able to grow and form colonies in a histidine-limited medium. Concentrations up to 5000 µg per plate of each drug were tested. As illustrated in Figure 5, sodium nitrite caused a significant increase in the number of revertants (over that seen with vehicle) at a concentration as low as 312.5 µg/plate, while S-nitroso-glutathione induced a positive response at ≥1250 µg/plate. On the other hand, sodium nitrate and all four of the nitrate-containing NO-NSAIDs

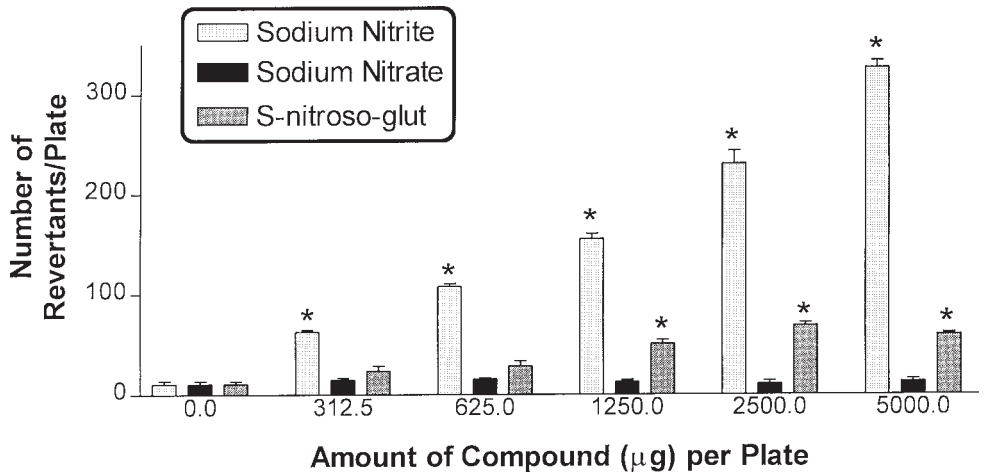

Fig. 5. Effects of sodium nitrate, sodium nitrite, and S-nitroso-glutathione on mutations of *S. typhimurium*. Results are shown as the number of revertants per plate following exposure to the test substances at a range of concentrations. * = $P < 0.05$ compared to the vehicle-treated group.

failed to induce a positive response at any of the concentrations tested.

The differences in mutagenicity of nitrate- vs. nitrite-containing compounds can also be considered in the context of the amounts of these substances likely to be ingested in comparison to World Health Organization recommendations [JECFA, 1996]. We calculated the amount of nitrate and nitrite that would be ingested per day based on maximum daily doses of the parent NSAID. These calculations were performed for nitrite- and nitrate-containing NO-releasing derivatives of diclofenac, aspirin, flurbiprofen, naproxen, and ketoprofen. In the case of nitrite-containing NO-NSAIDs, ingestion of any of the compounds would result in nitrite ingestion far in excess of WHO recommendations. For example, with NO-diclofenac, NO-aspirin, and NO-naproxen, the nitrite consumption would exceed WHO recommended maximal levels by threefold, tenfold, and 40-fold, respectively. On the other hand, in none of the five cases examined would the amount of nitrate ingested with nitrate-containing NO-NSAIDs exceed WHO recommendations. For example, the amount of nitrate ingested with maximal doses of NO-diclofenac and NO-flurbiprofen would be 1/25th and 1/7th of the WHO recommended maximal levels, respectively.

SUMMARY

NO-releasing NSAIDs were developed on the basis of observations that suggested that NO had the capacity to interfere with several key steps in the pathogenesis of NSAID-induced gastrointestinal ulceration. This novel class of compounds exhibits greatly reduced gastrointestinal and renal toxicity. NO-NSAIDs exhibit reduced toxicity not only in the normal gastrointestinal tract, but also in situations in which there was preexisting ulceration and inflammation. Moreover, these compounds have comparable anti-inflammatory and anti-pyretic activity to the parent drugs, but have enhanced analgesic and anti-thrombotic properties. Nitrate-containing NO-NSAIDs have superior stability to nitrite-containing NO-NSAIDs. Moreover, the nitrate-containing NO-NSAIDs do not exhibit the mutagenicity seen with nitrite-containing NO-NSAIDs. Clinical trials are presently under way to examine the safety and efficacy of several NO-NSAIDs in humans.

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