JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1295–1304

Received February 20, 2006
Accepted March 21, 2006
DOI 10.1211/jpp.58.10.0001
ISSN 0022-3573

Review Article

Assessment and prevention of gastrointestinal toxicity of non-steroidal anti-inflammatory drugs

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for analgesic, anti-inflammatory and, in the case of aspirin, for anti-thrombotic actions. The serious gastrointestinal side-effects associated with these drugs are of concern and pose a significant obstacle to their use. This review discusses the pathogenic mechanisms by which the conventional acidic NSAIDs induce gastrointestinal toxicity, with particular emphasis on non-prostaglandin effects. Methods of assessment of NSAID-induced enteropathy are reviewed, with particular emphasis on the use of functional measurement of NSAID-induced changes in the gastrointestinal tract. The advances in our knowledge of the pathogenesis of these effects have resulted in the development of a range of novel NSAIDs. Where functional assessment of the effects of NSAIDs has been employed, it appears to be more useful as an indicator of early-stage changes rather than a predictor of the effects of long-term NSAID exposure. Successful pharmaceutical strategies now offer considerable promise for reducing the severity of NSAID damage to the gastrointestinal tract. The utility of intestinal permeability measurements for selection and assessment of these strategies is discussed.

Introduction

As with many therapeutic agents, the non-steroidal anti-inflammatory drugs (NSAIDs) have their origin in the historical recognition that certain plants were observed to produce therapeutic effects in disease conditions. Salicylate-containing plants, such as the bark of the willow, were used by ancient Egyptians and Romans to relieve pain in childbirth and gout, and in the Middle Ages there are written records of these plants being used to treat wounds, inflammation and pain. Pure salicylic acid was obtained from plants in the early 19th century. While working for Bayer in the 1890s, Felix Hoffman initiated the synthesis of commercial quantities of the acetylated form of salicylic acid: acetylsalicylic acid. Bayer introduced this new drug as "aspirin" in 1899 and that marked the beginning of the development of NSAIDs (Pierpoint 1997).

Aspirin was initially used in the treatment of headaches and fever associated with colds and influenza, and was eventually recognized as the standard for the treatment of pain and inflammation in rheumatoid arthritis up until the mid-1970s. In the decades following the discovery of aspirin there was very little development in the treatment of rheumatic diseases until the 1950s, partly because the mechanisms underlying the development of the disease were little understood. In the 1950–1960s, the drugs available for the treatment of pain and inflammation in rheumatic diseases included aspirin (and the other salicylates), aminophenols (phenacetin) and pyrazolones (discovered in the early 1900s) and phenylbutazone. The inhibition of the cyclooxygenase (COX) enzyme and subsequent reduction in prostaglandin synthesis was proposed as the major mechanism of action of NSAIDs in 1971 (Vane 1971).

By the beginning of the 1990s, a large range of chemically diverse NSAIDs were available, which shared a variety of pharmacological actions and adverse effects. The discovery of an inducible isoform of COX, COX-2, in addition to the constitutively expressed COX-1 isoform, required a refinement of the theory that non-specific inhibition of COX activity explained both therapeutic and side-effects of NSAIDs (Herschman 1994). Subsequent pharmacological results suggested that selective COX-2 inhibition

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provides the therapeutic or anti-inflammatory activity of NSAIDs, whereas inhibition of constitutive COX-1 is responsible for their gastric and renal side-effects, as well as for their anti-thrombotic activity. This resulted in the development of highly selective COX-2 inhibitors with little or no COX-1 inhibitory activity, in contrast to the non-specific actions of the classical NSAIDs on COX-1 and COX-2 isoforms. The COX-2 inhibitors were subsequently marketed on the presumption that the main mechanism by which non-selective NSAIDs cause gastrointestinal ulcers is inhibition of COX-1.

The prescription market for all NSAIDs is currently worth £212 million a year in the UK and the value of the pharmaceutical market for pain relief worldwide was almost $23 billion in 2004 (Pain Therapeutics 2005). Factors contributing to the ever increasing use of these drugs include the greater availability of over-the-counter preparations and the aging of the population with a concomitant increase in inflammatory conditions. With increased consumption of NSAIDs, there is potential for a greater incidence of adverse reactions. The most common NSAID-induced adverse reactions are associated with the upper gastrointestinal tract and the search for a NSAID without these gastrointestinal side-effects has been underway for many years. An appraisal of current strategies for delivery of the conventional acidic NSAIDs is timely in light of the side-effects and recent withdrawal of certain COX-2 specific drugs (Fitzgerald 2004). Representative members of the acidic NSAIDs currently in clinical use are illustrated in Figure 1.

The aims of this review are three-fold: (i) to outline our current understanding of the COX-independent mechanisms underlying gastrointestinal side-effects induced by non-specific acidic NSAIDs; (ii) to place in context the measurement of intestinal permeability as a method of assessing NSAID-induced enteropathy; and (iii) to review the utility of intestinal permeability measurements for selection and assessment of promising formulations and strategies

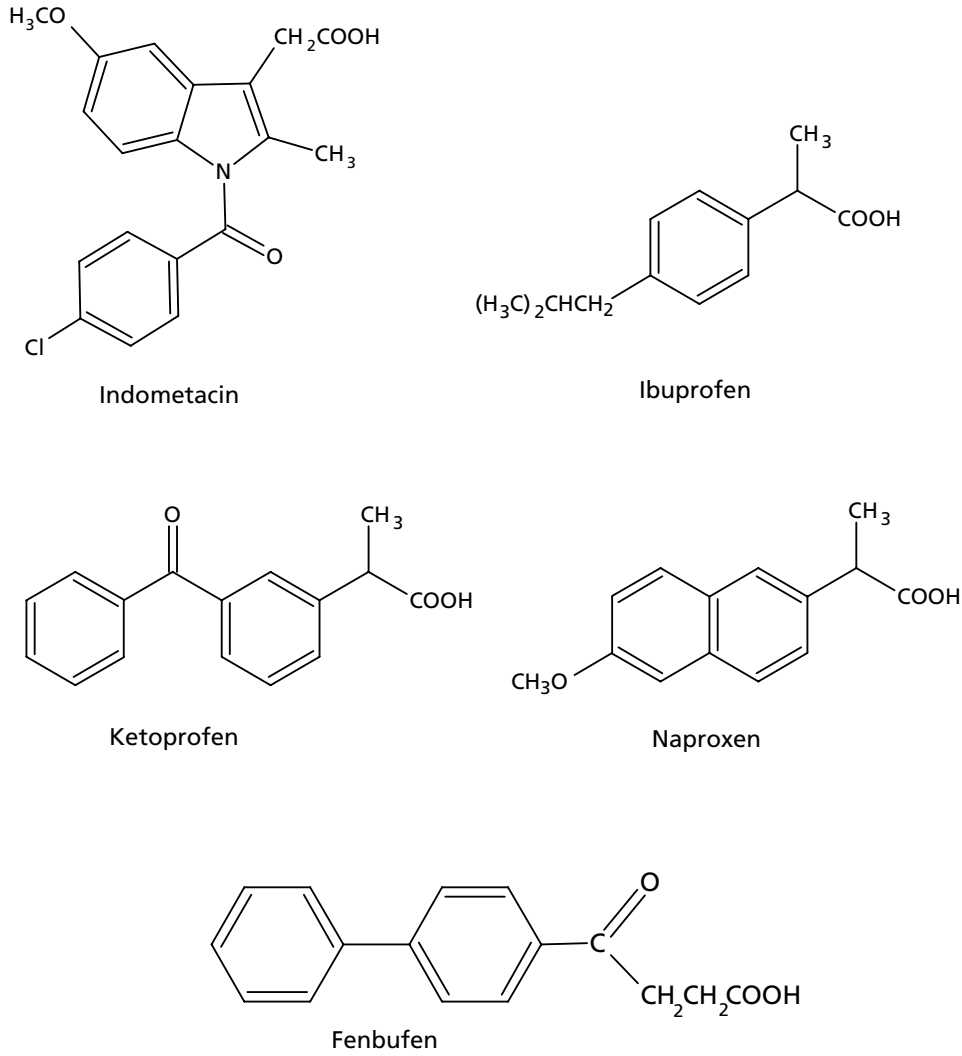

Figure 1 Representative examples of arylalkanoic acid non-steroidal anti-inflammatory drugs.

designed to spare the gastrointestinal tract from NSAID side-effects.

Major gastrointestinal side-effects

Numerous studies have established the association between the use of the non-specific NSAIDs and upper gastrointestinal haemorrhage, perforation, pain, nausea, vomiting, heartburn, dyspepsia, bleeding, ulceration, and death.

Using endoscopic studies, a prevalence rate of 14–25% of gastric and duodenal ulcers has been reported in users of conventional NSAIDs (Hudson & Hawkey 1993). Dyspeptic symptoms may occur in up to 20% of patients taking NSAIDs (Larkai et al 1997). Other side-effects of NSAIDs include inflammation of the small intestine including blood loss and protein loss and, occasionally, strictures (Bjarnason et al 1987; Bjarnason & Macpherson 1994). Attendant with blood and protein loss, patients may present with chronic iron deficiency anaemia or hypoaluminaemia (Morris et al 1991, 1992; Bjarnason et al 1993a).

Mechanisms of induction of gastrointestinal damage

There are two components to the gastrointestinal toxicity induced by conventional NSAIDs: first, the sequence of events resulting from COX inhibition and, second, a local irritancy effect. In this review, it is intended to focus on the latter rather than the former.

Evidence of the direct superficial damaging effect of a number of NSAIDs has been extensively reported in the literature (Lichtenberger et al 1994, 1995; Lugea et al 1997). Studies carried out by Lichtenberger and co-workers have suggested that the topical effects of NSAIDs may result from their ability to perturb the physicochemical properties of phospholipids. Because of their detergent-like properties (Gullikson et al 1977, 1981), NSAIDs share the capacity to insert themselves into lipid bilayers and alter the lipid orientation of both gastrointestinal phospholipid vesicles and artificial membranes. This perturbation may compromise the ability of gastric surface active phospholipids to form a protective bilayer (Giraud et al 1999).

A further component to the local effects associated with NSAIDs is the "ion trapping" hypothesis, where NSAIDs accumulate within cells during drug absorption and cause damage (Brune et al 1977; Brookes & Day 1991). This postulates that the extent of the accumulation of acidic NSAIDs in intestinal epithelial cells depends largely on the interaction of the acidity of the NSAID (pK_a) and lumenal pH. However, although the ion trapping hypothesis provides a basis for the local action of NSAIDs (along with molecular size, lipid solubility, contact time etc), it does not explain the underlying mechanism of the damage induced.

Changes in mitochondrial energy have been proposed as irrefutable evidence that inhibition of COX cannot be the sole mechanism of NSAID-induced gastrointestinal damage. In a study of 15 common acidic NSAIDs, all were found to uncouple oxidative phosphorylation at concentrations easily achievable within intestinal epithelium (Bjarnason & Hayllar 1996). These changes may be an important component of the topical phase of damage induction by NSAIDs. The relative importance and pathophysiological consequences of uncoupling versus inhibition of COX by administration of *R*- and *S*-flurbiprofen were also investigated. The *R*-isomer selectively uncouples, while the *S*-isomer is also an effective COX inhibitor. *R*-Flurbiprofen uncoupled in-vitro and in-vivo, increased intestinal permeability and caused mild intestinal inflammation, but had no significant effect on prostanoid levels and produced no ulcers. *S*-Flurbiprofen uncoupled and increased intestinal permeability equally, but was associated with significant decreases in intestinal prostanoid levels, more inflammation and numerous ulcers.

Uncoupling of oxidative phosphorylation and/or inhibition of the respiratory chain will lead to alterations in cellular ATP levels and oxidative stress, and, consequently, increased intestinal permeability (Vaananen et al 1991; Mahmud et al 1996; Somasundaram et al 1997b; Basivireddy et al 2002). Bjarnason and others have suggested that uncoupling may underlie the "topical" phase of NSAID damage, which leads to increased intestinal permeability and inflammation, but concomitant inhibition of COX is essential to drive the inflammation to ulcers (Figure 2).

For the conventional acidic NSAIDs, it has further been established that NSAID-induced increases in small intestinal permeability precede any intestinal inflammatory changes in man (Bjarnason et al 1984, 1986a, 1991; Jenkins et al 1987). Villus contraction and altered microvascular flow may occur as early pathogenic events in the damage, but it is not yet clear if COX inhibition is involved (Bjarnason & Thjodleifsson 1999).

Assessment of NSAID damage in the gastrointestinal tract

The severity of NSAID damage in the small intestine is difficult to evaluate and there is no fully accepted algorithm to assess potential toxicity in the small intestine. The main approaches currently used for measurement of NSAID-induced enteropathy include imaging techniques and functional investigations.

Endoscopy and radiology

The principal imaging techniques are endoscopy and radiology. Endoscopy involves the use of a hollow, thin, flexible tube with a lens or miniature camera to view various areas of the gastrointestinal tract. Endoscopic observation of the small intestine is technically difficult because of its long length and multiple complex looped configurations. Newer endoscopic techniques that appear to address these shortcomings for measurement of NSAID-induced lesions have recently been developed, including double balloon endoscopy and capsule endoscopy (Appleyard et al 2000; Iddan et al 2000; Yamamoto et al 2004; Hayashi et al 2005). Radiology has been a useful tool to image the intestine and to screen and assess NSAID damage (Bjarnason et al 1988; Levi 1990; Zalev et al 1998).

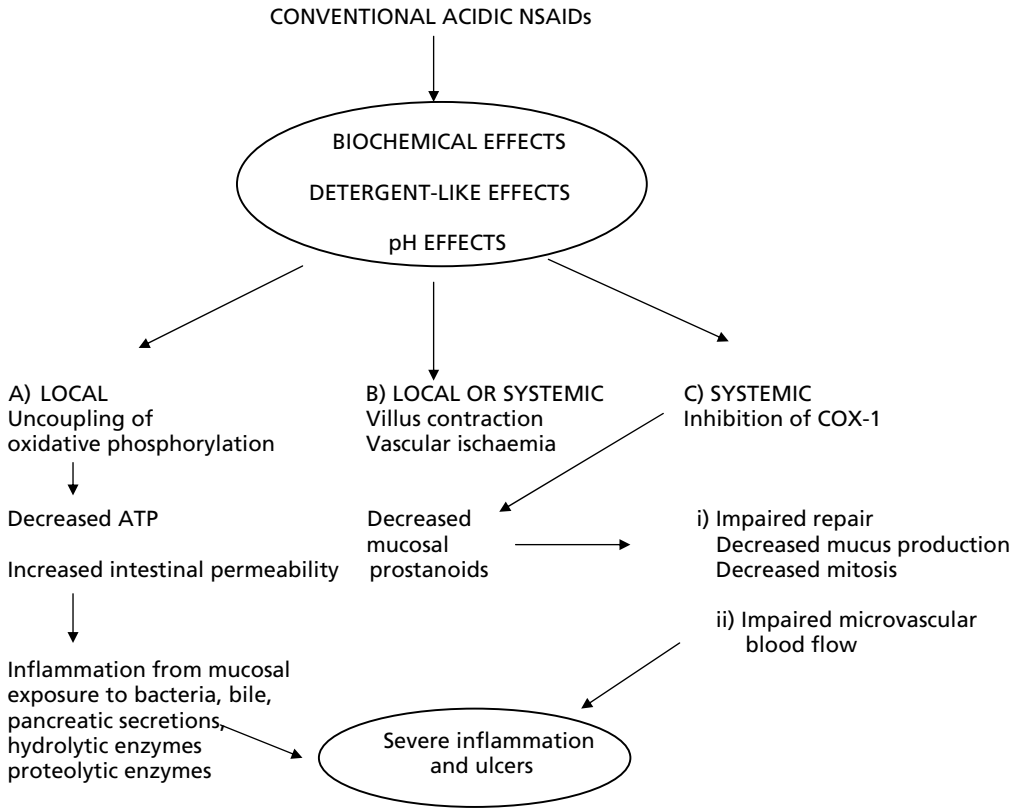

Figure 2 Mechanisms of induction of non-steroidal anti-inflammatory drug-induced gastrointestinal damage (adapted from Bjarnason & Thjodleifsson 1999).

Intestinal function tests

Functional investigations may be broadly divided into scintigraphy with the radioisotope indium (indium^{111}), use of faecal markers or assessment of changes in intestinal permeability.

Indium^{111} white cell scans and faecal excretion Long-term NSAID treatment is associated with enhanced migration of leucocytes to the ileum, indicating small bowel inflammation. When a patient's own radiolabelled neutrophils are re-injected, they migrate to sites of acute inflammation as well as to the liver, spleen and bone marrow (Saverymuttu et al 1983a, b). This allows the visualization of inflamed segments of the gut and the quantitation of the degree of inflammatory activity. As a refinement, the technique may be combined with actual measurement of the 4-day faecal excretion of labelled white cells (Bjarnason et al 1993b).

Faecal markers The most commonly used faecal marker for assessment of NSAID-induced damage is calprotectin. Calprotectin is a calcium and zinc binding protein derived predominantly from neutrophils and monocytes. The influx of white cells into the intestinal mucosa when the gut is inflamed results in increased calprotectin levels of the protein in the faeces and hence its suitability as a marker of gut damage. Preclinical and clinical studies have demonstrated that increased faecal calprotectin is an effective and sensitive marker for diagnosing NSAID enteropathy (Meling et al 1996; Tibble et al 1999).

Assessment of intestinal permeability Permeability changes have most commonly been measured in-vivo by the administration of probes or marker molecules with or without urinary recovery. The markers used for in-vivo studies of permeability include three groups: sugars (Menzies et al 1984), polyethyleneglycols (Chadwick et al 1977) and radiolabelled molecules such as ^{51}Cr-EDTA (Bjarnason et al 1983).

The radioisotope ^{51}Cr-EDTA is the most common probe molecule reported in the literature for the measurement of NSAID damage (Bjarnason et al 1986b; Aabakken & Osnes 1990; Davies et al 1993). However, combinations of the three types of markers are frequently used in assessing NSAID permeability effects (Bjarnason et al 1989b; Khazaeinia & Jamali 2004).

In-situ animal models with an intact blood supply offer a useful model to study NSAID-induced permeability changes (Komiya et al 1980; Lane et al 1996). Using a perfused rat gut model, ibuprofen (4.8 mM), ketoprofen (2.2–8.6 mM) and naproxen (4.3 mM) were observed to significantly enhance the intestinal permeability of the polyethylene glycol marker, PEG 4000 (Lane et al 2006). NSAID damage was also scored by assessing morphological changes in the intestine; scores were assigned to the level of damage in tissue samples fixed and examined by light microscopy. When the acidic carboxylic

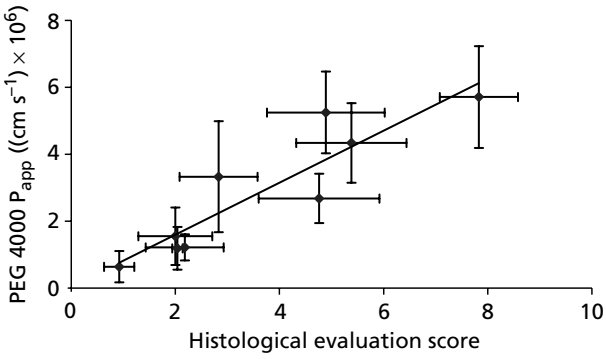

Figure 3 Histological evaluation scores plotted against PEG 4000 P_{app} (adapted from Lane et al 2006).

group of ibuprofen was conjugated to amino acids, the histological damage and PEG 4000 permeability decreased, both measures being proposed as indices of NSAID-induced damage (Figure 3; Lane et al 2006). These data support the hypothesis that the carboxylic acid group of such NSAIDs exerts a topical "irritant" effect on the intestine. Conjugation of the carboxylic acid group means that it is no longer available to exert such effects.

There are limited reports of in-vitro models for assessment of NSAID permeability changes. Legen & Kristl (2002) have reported the use of the Ussing-chamber model to study ketoprofen-induced changes in rat intestinal permeability of the marker molecule, fluorescein. These workers suggested that increased intestinal permeability of fluorescein at lower ketoprofen concentrations (<5 mM) is most probably a consequence of reduced prostaglandin tight junction control, whereas at higher concentrations, increased intestinal permeability may be because of ATP depletion. However, the permeability changes were evaluated at a pH of 7.51, whereas the pH in the upper part of the small intestine is slightly lower than in the distal regions (around pH 5.0–6.5 vs pH 6.9–7.6; Washington et al 2001). There are other methodological aspects that may preclude the use of such in-vitro models for assessment of NSAID-induced permeability changes. When compared with the perfused rat model, lower permeability values were obtained for high permeability drugs, possibly reflecting the lack of blood supply in the maintenance of the membrane characteristics, no unstirred water layer and an absence of the necessary sink conditions (Berggren et al 2003).

While cell culture models are generally used to study changes at the molecular level, it is difficult to study conventional NSAIDs at clinically relevant concentrations in such models, probably because of the surfactant action of the compounds (Tang et al 1993).

Protective strategies

As the conventional NSAIDs are definitively linked to the development of serious gastrointestinal side-effects, numerous strategies have been used to prevent or reduce this damage. The main strategies used have included co-administration of protective substances such as sucralfate, metronidazole, proton pump inhibitors or prostaglandin analogues.

Prostaglandin analogues

The rationale for co-administration of prostaglandin analogues stems from the decreased prostaglandin synthesis in intestinal cells induced by NSAIDs. The administration of the prostaglandin analogue misoprostol has shown a reduction in NSAID-induced ulceration compared with placebo ranging from 50% to 90% over 3–12 months; the dose initially used was 200 μg four times a day and subsequently 400–600 μg per day (Graham et al 1988, 1993). Co-administration of rioprostil significantly decreased the intestinal permeability of ^{51}Cr-EDTA (Bjarnason et al 1989a). Misoprostol has been combined with diclofenac (Arthrotec) and this has proved a successful therapy for certain patients.

H_2-antagonists

The use of H_2-receptor antagonists such as famotidine has been shown to significantly reduce the cumulative incidence of both gastric and duodenal ulcers in patients with rheumatoid arthritis on long-term NSAID therapy. However, neither cimetidine nor famotidine appear to possess any protective or antagonizing effect on naproxen-induced intestinal permeability (Aabakken et al 1989a, 1990).

Sucralfate

Sucralfate is a complex of sucrose, sulfate and aluminium. In acidic solutions (e.g. gastric acid), it forms a thick paste that has a strong negative charge. It binds to exposed positively charged proteins at the base of ulcers, thus coating the ulcer and forming a physical protective barrier. Sucralfate gel reduced the incidence of acute gastroduodenal mucosal lesions and symptoms in patients receiving short-term NSAIDs (Miglioli et al 1996); however, it did not provide protection from naproxen-induced permeability changes in the distal intestine (Aabakken et al 1989b).

Metronidazole

Bjarnason and co-workers reported that for patients on NSAIDs, metronidazole significantly reduced intestinal inflammation and blood loss. However, intestinal permeability did not change significantly and there were no significant changes in the endoscopic or microscopic appearances of the gastroduodenal mucosa (Bjarnason et al 1992a). Davies & Jamali (1997) reported a reduction in indometacin and flurbiprofen-induced intestinal permeability when these NSAIDs were co-administered with metronidazole. The earlier study was conducted with metronidazole administration over 12 weeks, while the latter involved a single dose study. This suggests that the improvements in intestinal permeability in acute studies may not translate into long-term improved benefits.

Glutamine

Since it has been suggested that increased permeability might be related to cell damage resulting from energy depletion, it was hypothesized that glutamine, which is the major energy

source of the intestinal mucosal cell, might prevent permeability changes. Basivireddy et al (2004) reported that glutamine administered for 7 days prior to indometacin dosage significantly reduced the oxidative stress in intestinal tissue in rats. However, these authors did not investigate any permeability changes. Ann et al (2004) administered glutamine in rats for 4 days prior to diclofenac administration. The changes in intestinal permeability induced by diclofenac were significantly less for glutamine-fed animals versus controls and, in addition, animals fed with glutamine did not exhibit the same extent of protein shedding in the gut. In humans, the timing of the dosage of glutamine appears to be more important; subjects fed glutamine for 1 week prior to NSAID administration did not show any reduction in permeability changes. However, multiple doses of glutamine administered close in time to NSAID-dosing resulted in significantly lower permeability compared with the NSAID without glutamine (Hond et al 1999).

Sulfasalazine

Sulfasalazine is a mainstay in the treatment of inflammatory bowel disease and appears to act via inhibition of adhesion molecule expression, thus limiting leukocyte recruitment to sites of intestinal inflammation (Cronstein et al 1993; Pooley et al 1995). The administration of 1 g twice daily for 3 days has been shown to prevent acute indometacin-induced increased intestinal permeability where 75 mg of indometacin was dosed orally twice daily for 1 day (Banerjee et al 1986). Data on possible benefits for long-term studies is lacking.

Phospholipids

Studies carried out by Lichtenberger and others have provided evidence that the gastrointestinal mucosa may be protected from damaging agents and microorganisms in the lumen by a hydrophobic extracellular lining comprised of zwitterionic phospholipids (Butler et al 1983; Hills et al 1983; Lichtenberger et al 1983, 1995). This stimulated the development of a family of NSAIDs chemically associated with either synthetic or purified phosphatidyl choline (Lichtenberger et al 1996). The effects of a diclofenac–dipalmitoyl phosphatidyl choline complex on upper and lower gastrointestinal permeability compared with diclofenac acid and its sodium salts have been investigated in rats (Khazaeinia & Jamali 2003). At 1 h post-dose, only diclofenac sodium induced a significantly increased upper gastrointestinal permeability. At 3 h post-dose, all formulations significantly increased upper gastrointestinal permeability, although the diclofenac acid had the least effect. In the lower gastrointestinal tract, the induced increase in permeability was significant at 1 and 3 h post-dose for all formulations, with no significant differences between them. Clinical trials are currently underway with a soy lecithin based derivative of ibuprofen; however, the effects of the derivative on human or animal permeability have not been reported (US Patent No. 6,943, 155, 2001).

Glucose/citrate

NSAIDs may inhibit glycolysis and the tricarboxylic acid cycle, resulting in the inhibition of oxidative phosphorylation with consequent cellular damage and loss of integrity of the intercellular junctions (Bjarnason et al 1993). In a single-dose study, administering indometacin with 15 mg glucose and 15 mg citrate for each milligram of indometacin prevented an increase in intestinal permeability above baseline values (Bjarnason et al 1992b). It was suggested that the sugars may modify the reaction to indometacin in the lumen or that citrate might be cytoprotective against the free radical damage caused by NSAIDs. No other studies have confirmed the protective effects of glucose or citrate and the compounds proved to be ineffective following long-term repeated administration of indometacin (Bjarnason et al 1993a).

Prodrugs

A number of NSAIDs have been formulated as prodrugs. Prodrugs are inactive pharmacological derivatives of a parent drug that must undergo enzymatic or spontaneous transformation resulting in release of the free drug (Albert 1958). Nabumetone is a non-steroidal anti-inflammatory prodrug, which exerts its pharmacological effects via the metabolite 6-methoxy-2-naphthylacetic acid. Nabumetone itself is non-acidic and, following absorption, it undergoes extensive first-pass metabolism to form the main circulating active metabolite 6-methoxy-2-naphthylacetic acid (Figure 4). In patients with rheumatoid arthritis, the intestinal permeability measured using ^{51}Cr-EDTA was unchanged with treatment of nabumetone 1 g per day compared with a significant increase in permeability using indometacin 150 mg per day (Bjarnason et al 1991). A more recent study in rats confirmed that indometacin pre-treated rats, but not nabumetone, demonstrated increased intestinal permeability of ^{51}Cr-EDTA (Somasundaram et al 2002). These differences between the two drugs suggest that the traditional NSAIDs induce local injury during the absorption phase with systemic effects being less important.

COX inhibiting nitric oxide donators

The recognition that nitric oxide can replicate many of the gastroprotective actions of the prostaglandins led to the development of a novel class of drugs, the COX inhibiting nitric oxide donators (Wallace & Miller 2000). These so called "NO-NSAIDs" may be generated by adding a nitroxybutyl or a nitrosothiol moiety to the parent NSAID via a short-chain ester. A study that compared the effects of diclofenac and nitrofenac (Figure 5A) in rats showed similar increases in intestinal permeability of ^{51}Cr-EDTA for both compounds. While diclofenac caused a progressive increase in epithelial permeability, nitrofenac caused similar changes in intestinal

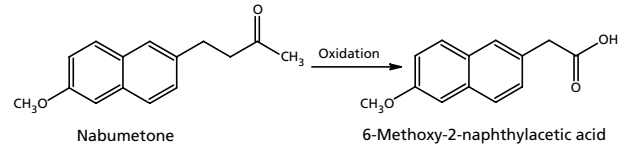

Figure 4 Chemical structures of nabumetone and its active metabolite, 6-methoxy-2-naphthylacetic acid.

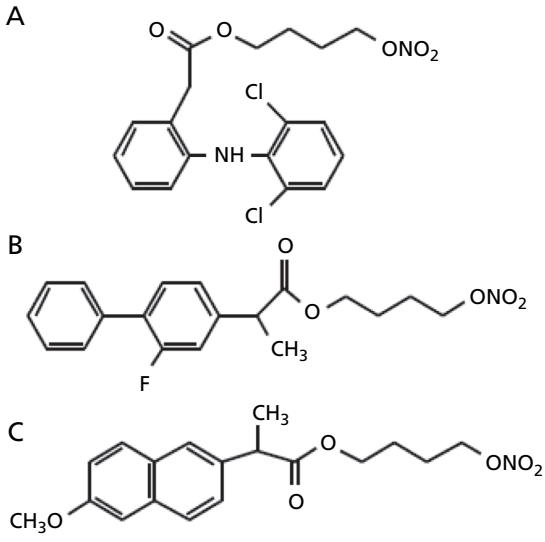

Figure 5 A. Nitrofenac. B. NO-flurbiprofen. C. NO-naproxen.

permeability after a single dose but no further increase with repeated administration (Reuter et al 1997).

Administration of equimolar doses of flurbiprofen and its nitroxybutyl derivative NO-flurbiprofen (Figure 5B) resulted in increased intestinal permeability of ^{51}Cr-EDTA in rats, with no significant differences in the permeabilities observed (Somasundaram et al 1997a). In addition, mild to severe uncoupling of oxidative phosphorylation was induced to similar extents by both compounds, and NO-flurbiprofen was associated with significantly less macroscopic damage in the small intestine than flurbiprofen. The permeability data and effects on mitochondria suggest that any beneficial effect of the nitroxybutyl compound is on the later pathogenic stages of gut damage. However, it should be noted that dosing on an equimolar basis may have biased the study against NO-flurbiprofen as the compounds are known to have comparable efficacy on a weight by weight basis (Wallace et al 1994). In the case of NO-naproxen (Figure 5C), reduced bioavailability of the NSAID moiety has been considered as a contributory factor to the decreased toxicity of NO-NSAIDs in rats (Davies et al 1997). Other names for this compound are HCT 3012 and AZD 3582. In one human study, subjects received equimolar doses of naproxen or its nitroxybutyl derivative for 12 days in a double-blind three-period cross-over volunteer study. Intestinal permeability as measured by differential urinary excretion of lactulose and L-rhamnose was increased by naproxen but not by NO-naproxen (Hawkey et al 2003). The available clinical and pharmacokinetic data support the theory that the more advantageous profile observed for the COX inhibiting nitric oxide donators in acute experiments rather than in repeated dose studies may reflect masking of the carboxyl group or an effect on drug dissolution rate (Fagerholm & Bjarnsson 2005).

Conclusions

The pathogenesis of NSAID enteropathy is a multistage process involving specific biochemical and subcellular organelle damage, followed by a relatively non-specific tissue reaction. An overwhelming body of research has demonstrated that COX-1 inhibition by itself is not the only factor in the development of the gastrointestinal damage of NSAIDs. The local effect of NSAIDs is an important initiating factor in their toxicity. Functional methods such as measurement of intestinal permeability changes have been widely used to assess the effects of NSAIDs in acute studies. However, while intestinal permeability tests compare favourably with other measures of NSAID-induced enteropathy, they should be regarded as indices of early intestinal permeability changes rather than a replacement for measurements of long-term NSAID exposure. Measurement of NSAID-induced intestinal permeation enhancement should distinguish between changes in permeability caused by abnormal cell function and changes caused by gross disturbance of the structure of the mucosal surface. For screening of NSAIDs and their novel formulations, the application of intestinal permeability measurement is a useful measure of acute intestinal permeability changes. However, such measurements cannot replace chronic indices of NSAID-induced damage such as gastrointestinal ulceration. To date, there does not appear to be any synthetic model capable of predicting NSAID absorption. While cultured cells, for example Caco-2, provide more realistic physiological representation of a real membrane, doses in-vivo cannot be evaluated in such systems because of their cellular toxicity to such fragile models. There may be improvements and refinements to these models in the future, but until then animal models such as those described here provide the most realistic attempt to assess NSAID absorption and NSAID effects on intestinal permeability.

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