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Review

Nonsteroidal anti-inflammatory drug-induced gastrointestinal toxicity: New insights into an old problem

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Abstract: Nonsteroidal anti-inflammatory drugs are widely used for the treatment of chronic arthropathies, but their gastrointestinal damage remains a significant limitation to their use. In this review, the pathogenic mechanisms through which these drugs are believed to cause gastrointestinal damage are outlined. A better understanding of the pathogenesis of gastric and intestinal injury has resulted in novel strategies that are being employed to develop nonsteroidal anti-inflammatory drugs that do not have significant adverse effects on the gastrointestinal tract.

Key words: non-steroidal anti-inflammatory drugs, gastrointestinal, cyclooxygenase, nitric oxide, prostaglandins

Introduction

Salix derivatives have been used through the ages, from the times of Pliny and Hippocrates, until the active ingredient, salicylic acid, was discovered by Gerhardt at the end of the last century. Phenylbutazone was subsequently introduced, in 1952, followed by indomethacin in 1963. During the last 40 years, a plethora of nonsteroidal anti-inflammatory drugs (NSAIDs) have been introduced on the market, this being indicative of the commercial potential for such compounds and their efficacy as anti-inflammatory/analgesic agents. There are currently more than 35 NSAIDs available for clinical use worldwide,[1] with a market of over US$6 billion per year.

NSAIDs encompass a wide variety of structural chemical classes, the vast majority being weak acids. The anti-inflammatory and analgesic properties of NSAIDs make this therapeutic class of particular utility in the management of rheumatic diseases and musculoskeletal disorders. NSAIDs are also widely used as analgesics in the treatment of pain of varying origin, such as that due to dental extraction, trauma, surgery, dysmenorrhea, and postpartum episiotomy.

All NSAIDs, when given in equipotent doses, have comparable efficacy.[3–4] Given the apparent equivalent efficacy, the safety or tolerability profile of individual NSAIDs has become a principal criterion for therapeutic selection. NSAID prescription is currently commonplace, and with the increased introduction of over-the-counter NSAIDs, there is a potential for NSAID-associated gastrointestinal (GI) side effects to increase.

This review describes the current understanding of the pathogenesis of NSAID-induced GI toxicity, as well as two novel preventative approaches to reduce the severity of damage to the GI tract.

NSAID-Induced GI side effects

The clinical use of NSAIDs has been associated with numerous side effects, the most important in frequency and clinical impact being GI disturbances. The GI side effects of salicin, a drug that would now be classified as an NSAID, were first recognized more than a century ago.[5] NSAID-induced GI pathology accounts for more than 70000 hospitalizations and 7000 deaths annually in the United States.[6] Adverse effects in the GI tract have contributed to the termination of clinical studies and the withdrawal from the market of at least 17 individual NSAIDs.[7] The most apparent side effect of NSAID use is irritation of the upper GI mucosa, which may manifest itself as gastric pain, heartburn, nausea, vomiting, bleeding, dyspepsia, ulceration, perforation, and in severe cases, hemorrhage and death.[8]

Numerous articles examining the gastric and duodenal damage caused by NSAIDs have been published;

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however, there is often a lack of correlation between gastric symptoms and endoscopic evidence of mucosal damage, with as many as one-third of patients being completely asymptomatic.[9] Moreover, among patients with upper GI lesions and blood loss, healing of the lesions is not always accompanied by an improvement of anemia.[10] This may suggest the occurrence of GI afflictions in more distal sites of the GI tract.

Evidence that NSAIDs induce small-intestinal manifestations comes from several case reports.[11–13] Recently, the more distal intestinal disturbances caused by NSAIDs have been well characterized.[14] It has also been reported, in a study population of 70, that 41% of patients with rheumatoid arthritis taking NSAIDs who had iron deficiency anemia and undiagnosed gastrointestinal blood loss had evidence of small-intestinal lesions, erosions, and ulcers iatrogenically attributed to NSAIDs upon small-bowel enteroscopy.[15] Additionally, vitamin B_{12} and bile acid absorption may be impaired, contributing to anemia and increasing morbidity.[15] Studies have shown that up to 60% of patients chronically taking NSAIDs develop intestinal inflammation associated with blood and protein loss, and on discontinuation of NSAIDs this intestinal inflammation may persist for up to 16 months.[16] Further studies have shown that long-term NSAID treatment leads to enhanced migration of ^{111}indium-labelled leucocytes to the ileum, indicating a chronic inflammatory process involving the small bowel. This, together with evidence of increased fecal ^{111}indium excretion, provides further evidence that NSAIDs induce intestinal inflammation in a number of patients receiving these drugs. It has therefore been suggested that the prevalence of side effects in the lower GI tract may exceed that detected in the upper GI tract and may be of major toxicological significance.[17] In an epidemiological study, the expected incidence of lower bowel perforations and bleeding was determined to be 10 and 7 per 100000, respectively.[18] Considering the magnitude of worldwide NSAID use, these clinical manifestations undoubtedly contribute to significant morbidity in many patients.[19] While the clinical presentation of NSAID-induced gastropathy, namely bleeding, may be more dramatic than enteropathy, the long-term impact of enteropathy has not been fully characterized.

An increasing number of reports have also described deleterious effects of NSAIDs in the large intestine, with findings ranging from asymptomatic mucosal inflammation, to bloody diarrhea, diaphragmatic strictures, perforations, and hemorrhage.[20] NSAIDs can also exacerbate inflammatory bowel disease.[21] Hence, it is becoming increasingly apparent that NSAID-induced toxicity is not confined to the gastroduodenum, but can also extend into the small and large intestine.

Pathogenesis of NSAID-Induced gastroduodenal damage

Role of cyclooxygenase

Traditionally, the therapeutic effects and major toxic side effects of NSAIDs have been attributed to the ability of these drugs to inhibit the synthesis of prostaglandins (PGs), through a direct action on prostaglandin H synthetase, which serves both as a cyclooxygenase (COX) and as a peroxidase.[22] Inhibition of the synthesis of "cytoprotective" PGs is regarded as a major factor accounting for the development of GI ulceration and hemorrhage.[23] COX metabolizes arachidonic acid to PGH_2, which, in turn, is metabolized to various other PGs, prostacyclin, and thromboxanes. NSAIDs inhibit synthesis of PGs via inhibition of COX, resulting in anti-inflammatory, antipyretic, analgesic, anti-thrombotic, and cytotoxic effects.

It has recently been demonstrated that there are at least two isoforms of COX: a house-keeping isozyme that is consitutively expressed in many tissues, including the upper GI tract, called COX-1, and an inducible isozyme called COX-2.[24] It is postulated that COX-1 produces prostaglandins that exert cytoprotective effects, while COX-2 produces prostaglandins that contribute to inflammation. Hence, NSAIDs with weaker activity against COX-2 than COX-1 are expected to cause more GI cytotoxicity, since the concentration to elicit beneficial effects exceeds that which yields GI toxicity. GI toxicity and the highly individualistic response often seen with NSAID therapy may be due, in part, to the nature of the isoenzymes involved in a specific patient's inflammatory condition and the differential efficacy of various NSAIDs in inhibiting that isoenzyme form. Obviously, the results of in vitro experiments should be cautiously related to the in vivo situation, due to the different pharmacokinetic patterns of NSAIDs. In addition, in vitro experiments cannot take into account the impact of the type of formulation administered, the route of administration, or the site of the damage induced in the GI tract. Furthermore, a prediction of toxicity based solely on relative in vitro activities against COX-2/COX-1 attributes the GI toxicity of NSAIDs entirely to the inhibition of COX-1, whereas many other pathogenic mechanisms are clearly involved.

Role of neutrophils

It is unclear how suppression of gastric prostaglandin synthesis leads to ulcer formation; however, there is now considerable evidence for the role of neutrophils in the pathogenesis of NSAID-gastropathy, at least in experimental models. An early event after NSAID

administration is a significant leukocyte adherence to the vascular endothelium in the gastric and mesenteric microcirculation.[25–27] NSAIDs also up-regulate adhesion molecule expression on the vascular endothelium of gastric blood vessels following their administration.[28] Neutropenic rats exhibit significantly increased resistance to the gastric damaging actions of NSAIDs.[29,30] Further, the prevention of leukocyte adherence to the vascular endothelium with monoclonal antibodies that prevent leukocyte adherence results in a marked attenuation of NSAID-induced gastric damage.[31–32]

Prostaglandins can also down-regulate several of the neutrophil functions that might contribute to GI injury. For example, administration of prostaglandins at doses that are gastroprotective has been shown to prevent NSAID-induced leukocyte adherence.[26] Adherence of leukocytes to the vascular endothelium is likely accompanied by activation of these cells, which would lead to the liberation of oxygen-derived free radicals and proteases.[33] There is good evidence for the role for reactive oxygen metabolites in experimental NSAID-induced gastropathy.[34] Prostaglandins can also suppress the generation of superoxide anions.[35] Moreover, adherence of neutrophils to the vascular endothelium could lead to capillary obstruction, resulting in a reduction in gastric mucosal blood flow, which results in further mucosal injury.[33]

Role of cytokines

There is growing evidence that prostaglandins, and therefore NSAIDs, can modulate the synthesis and release of a number of cytokines, while several cytokines can influence prostaglandin synthesis through modulation of COX activity. For example, prostaglandins can suppress the release of tumor necrosis factor (TNFα) from macrophages and mast cells,[36,37] while NSAIDs have been shown to increase the release of this cytokine.[38] TNFα is an important signal for the expression of the adhesion molecule ICAM-1 (intracellular adhesion molecule-1) on vascular endothelial cells.[39] It has been suggested that TNFα is responsible for increased leukocyte adherence within the gastric microcirculation following NSAID administration.[38] However, more recent studies in our laboratory suggest that the role of TNFα in the pathogenesis of NSAID-induced gastric damage may be unrelated to effects on neutrophil adherence.[40]

In contrast to the role of TNFα in NSAID-gastropathy, interleukin-1 has been shown to reduce the severity of gastric damage induced by these agents.[41] Interleukin-1β significantly reduced leukocyte margination within the gastric microcirculation following NSAID administration, and depressed the responsiveness of circulating neutrophils to stimulation with chemotactic factors.[42]

Role of nitric oxide

Nitric oxide (NO) is a potent vasodilator and an inhibitor of leukocyte activation and adherence.[43] NO may also scavenge free radicals induced by neutrophils and thereby prevent GI damage induced by NSAIDs.[44] NO donors have been shown to reduce GI damage in various animal models,[45–47] while suppression of NO synthesis leads to leukocyte adherence,[43] reminiscent of that seen following NSAID adminstration,[26,48] and a marked increase in susceptibility of the stomach to injury induced by a variety of agents, including NSAIDs.[49] As discussed in further detail below, the ability of NO to protect the gastric mucosa from injury induced by NSAIDs has been exploited in the design of a series of GI-sparing NSAID derivatives.

Role of oxidative phosphorylation

NSAIDs have also been suggested to uncouple oxidative phosphorylation, resulting in depletion of cellular adenosine triphosphate (ATP).[50,51] NSAID-induced side effects to the GI mucosa have, therefore, been suggested to result from the uncoupling of oxidative phosphorylation and the competitive inhibition of specific enzymatic steps in the anaerobic glycolytic pathway and the tricarboxylic acid cycle, thereby reducing ATP production in epithelial cells, leading to cell death.[52] The effect of indomethacin on the GI tract in both humans[53] and rats[54,55] can be reversed by coadministration of a glucose/citrate mixture. However, more recent experimental evidence has questioned this approach and the mechanisms by which the observed protection occurs.[56] This strategy was found to be ineffective with many other NSAIDs tested in the rat and was not reproducible when indomethacin was administered subcutaneously. The observed cytoprotective effect when indomethacin and glucose/citrate are concomitantly administered orally may be explained, at least in part, by a reduced solubility of indomethacin, and a dramatically impaired bioavailability of the indomethacin and glucose/citrate formulation through a physico-chemical interaction between glucose/citrate and indomethacin in the GI tract.[56]

Pathogenesis of NSAID-Induced small-intestinal damage

Role of cyclooxygenase

It has previously been suggested that, unlike the damage in the stomach, intestinal injury induced by

NSAIDs may not be related to suppression of prostaglandin synthesis.[57] Experimental evidence has indicated a lack of a temporal relationship between prostaglandin synthesis inhibition and intestinal damage.[58] Some COX inhibitors, such as aspirin and nabumetone, produce little if any small intestinal damage. Furthermore, there is a report that misoprostol failed to reduce indomethacin-induced intestinal permeability in humans, suggesting that this "damage" was not attributable to a deficiency of prostaglandins in the intestinal tissue.[59]

Role of neutrophils and bacteria

As in the case of NSAID-induced gastropathy, there is some evidence consistent with the hypothesis that NSAID-induced intestinal damage is mediated in part by neutrophils. Significant infiltration of neutrophils into the intestinal mucosa of rats following indomethacin administration has been demonstrated.[60] The mechanisms responsible for the cytoprotective effect of metronidazole against NSAID-induced damage in the small intestine is not clear. In addition to its antibacterial effects, metronidazole has been shown to be capable of scavenging reactive oxygen species generated by neutrophils at sites of inflammation.[61] A clinical study has also suggested that neutrophils were the primary effector cells responsible for NSAID-induced enteropathy and that recruitment of neutrophils into the GI mucosa occurred in response to chemotactic factors produced by enteric bacteria.[62] On the other hand, Yamada et al.[63] demonstrated that the epithelial permeability changes induced by NSAIDs in rats were unaffected by prior induction of neutropenia, suggesting that neutrophils did not play an important role in the pathogenesis of this injury.

A recent report examining NSAID-induced ulcers of the small bowel in the rat has independently demonstrated the ability of metronidazole to prevent this damage.[64] There is considerable experimental evidence for a contribution of luminal bacteria to the development of NSAID-induced intestinal injury. Studies in laboratory animals have shown that indomethacin induces much less damage in germ-free or antibiotic-treated rats than in normal rats.[65–69]

Role of enterohepatic recirculation

The enterohepatic recirculation of the glucuronide conjugates of NSAIDs regenerated to the active drug by the β-glucuronidase in intestinal flora is also thought to be a major factor in the intestinal ulcerogenicity of NSAIDs. Experimental evidence for the role of enterohepatic recirculation has been demonstrated through studies in which the bile duct of the rat is ligated prior to NSAID administration, resulting in attenuation of the severity of small-intestinal damage.[69]

As mentioned previously, aspirin produces minimal intestinal damage in both humans and rats.[70,71] The lack of intestinal damage with aspirin may be a consequence of the absence of enterohepatic recirculation of this drug, and its very rapid hydrolysis to salicylic acid. Salicylic acid, which is a very weak inhibitor of COX-1, has also been shown to be ineffective in inducing leukocyte adherence to the vascular endothelium.[48]

Prevention and treatment of NSAID-Induced GI damage

As NSAIDs have been linked to the development of serious GI side effects, numerous strategies have been employed to prevent this mucosal damage. Various approaches have been taken, including the preparation of enteric-coated and modified release formulations, the development of prodrugs, and once-daily dosing (long half-life NSAIDs). An alternative approach has been the concomitant treatment with protective substances to circumvent NSAID-induced GI side effects. Preventative measures evaluated to date have included a wide variety of pharmacological approaches, including antisecretory agents (H_2-receptor antagonists, proton pump blockers, and anti-cholinergic agents) and antacids, as well as attempts to increase mucosal defense (sucralfate, and prostaglandin analogues). Each of these approaches has had some measurable success in achieving GI protection from NSAIDs, but none have proven to be highly effective and some are costly and associated with additional adverse effects.

There has also been a considerable effort in recent years to develop new NSAIDs that lack GI toxicity. However, at present, although clinically significant differences between the various drugs on the market exist,[4,6,72,73] all NSAIDs induce some type of GI manifestation and none has proved to be convincingly superior in reducing GI toxicity.

Selective COX_2 inhibitors

The important discovery of the COX isozymes has prompted many investigators to search for molecules that are effective as inhibitors of COX-2 but that exert little or no effect on COX-1. It is assumed that these agents will inhibit prostaglandin synthesis at sites of inflammation, but will not inhibit prostaglandin synthesis in tissues where COX-1 is constitutively expressed. Preliminary studies of highly selective COX-2 inhibitors in animal models suggest that these agents have reduced GI toxicity.[74,75]

Recent work using genetically manipulated "knock-out" mice deficient in the isozymes for COX-1 and COX-2 has shed light on the specific signaling roles of the two prostaglandin biosynthetic pathways defined by these enzymes.[76–78] COX-2 deficient mice developed severe nephropathy, showed an altered inflammatory response, and were susceptible to peritonitis.[76,77] Interestingly, COX-1 deficient mice exhibited no overt GI abnormalities, but exhibited decreased platelet aggregation, and a decreased inflammatory response to arachidonic acid.[78] It is entirely possible that some of the adverse effects of NSAIDs may be related to their ability to suppress COX-2, and that prostanoids derived via COX-1 contribute to inflammation, pain, and fever.

It has also been questioned whether selective inhibition of COX-2 would be advantageous in all situations.[79] For example, in situations where the mucosa is inflamed, such as in *Helicobacter pylori*-associated gastritis and inflammatory bowel disease, COX-2 will be expressed, and prostaglandins produced via this isozyme may be beneficial in terms of promoting mucosal defense and repair.[57] The clinical use of selective COX-2 inhibitors in situations of pre-exisiting inflammation may exacerbate mucosal damage. Indeed, we have recently observed that highly selective COX-2 inhibitors exacerbate colitis in the rat (unpublished observations).

Furthermore, differentiation between the gastric and the intestinal toxicological manifestations of NSAIDs has been largely ignored. Hence, it is not known if this suggested scheme for gastroduodenal protection will alleviate lesions of both the small and the large intestine. As outlined above, the pathogenesis of NSAID-induced distal intestinal damage may be mediated through prostaglandin-independent mechanisms.[58] Hence, the development of selective COX-2 inhibitors may not necessarily result in reduced toxicity in more distal sites of the GI tract.

NO-Releasing NSAIDs

Another novel strategy to reduce the GI ulcerogenicity of NSAIDs is the incorporation of an NO-releasing moiety into the NSAID molecule.[80–84] NO may counteract the detrimental effects of COX suppression, such as maintaining blood flow, and may prevent leukocyte adherence such that mucosal damage does not occur.[80–83]

Endogenous NO also appears to be involved in regulating the alkaline response of the stomach to mild irritants.[85] It has been speculated that irritation of the gastric mucosa might release NO, which in turn inhibits acid secretion and enhances the gastric alkaline response, promoting an alkaline microenvironment that favors epithelial restitution. In addition, NO-releasing compounds increase mucus gel thickness in the rat stomach.[86] It has been demonstrated that NO-releasing agents induce mucus secretion from isolated gastric mucosal cells, possibly via a direct effect of NO on the epithelial cells.[87]

NO-NSAIDs cause little, if any, small-intestinal injury after chronic dosing in rats.[80] The mechanisms by which NO-NSAIDs suppress small-intestinal toxicity have not been determined, but such suppression may be due to NO release, which prevents leukocyte adherence within the gastric microcirculation.[80–83] Additionally, NO moieties have been shown to be cytotoxic for invasive micro-organisms.[88] Preliminary data in our laboratory suggest that administration of NO-NSAIDs does not result in increases in luminal bacteria numbers in the small intestine, as is observed with the parent NSAIDs. The lack of bacterial proliferation may reduce the recruitment of neutrophils into the intestinal mucosa in response to chemotactic factors produced by enteric bacteria.

In addition, in models of pre-existing GI inflammation, NO-NSAIDs did not exacerbate pre-existing colitis, while standard NSAIDs caused detrimental effects.[80] Furthermore, administration of a NO-NSAID to rats with pre-existing ulcers resulted in significant acceleration of ulcer healing.[89]

Interestingly, a NO-aspirin derivative (NCX-4215) has been shown to exhibit enhanced anti-platelet activity relative to aspirin, which has been attributed to the inhibitory effects of NO on platelet adhesion and aggregation.[84] NCX-4215 does not cause gastric injury or alter systemic blood pressure and may prove useful as a novel GI-safe anti-thrombotic agent for the prophylaxis of stroke and myocardial infarction.

Summary

NSAIDs can induce mucosal injury throughout the GI tract. While inhibition of prostaglandin synthesis appears to be a key factor in the pathogenesis of gastric injury induced by these agents, the damage induced in the small intestine appears to occur through a prostaglandin-independent pathway. The pathogenesis of the more distal intestinal disturbances induced by NSAIDs appears to involve interactions between enteric bacteria, enterohepatic recirculation, and infiltrating neutrophils. The identification of at least two isoforms of the cyclooxygenase enzyme has led to the design of highly selective inhibitors of the inducible form, in the belief that these will spare the GI tract of injury. The addition of an NO-releasing moiety to standard NSAIDs, creating "NO-NSAIDs" is another approach that has been taken to develop GI-sparing anti-inflammatory drugs.

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