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Review Article

Nitric oxide-releasing NSAIDs: a new dimension in nonsteroidal antiinflammatory drugs

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Summary

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most commonly prescribed drugs in the world, but their use as antiinflammatory, antipyretic, antithrombotic and analgesic agents is limited by their adverse effects on the gastrointestinal tract. In recent years, two promising new strategies in the development of NSAIDs that spare the gastrointestinal tract have emerged. First, the development of selective inhibitors of cyclooxygenase-2 (COX-2), the inducible isoform of the prostaglandin G/H synthase enzyme. Since COX-1, the constitutively expressed isoform of COX, has been identified in many tissues, it has been suggested that a selective inhibitor of COX-2 would suppress prostaglandin synthesis at sites of inflammation but spare the constitutive COX-1 in other tissues, such as the gastrointestinal tract. Although the development of selective COX-2 inhibitors appears to be a rational approach in the evolution of NSAIDs with improved gastrointestinal tolerability, it was recently reported that COX-2 may be important for homeostasis in health and disease. Furthermore, it has been speculated that COX-2 specificity limits the therapeutic use of COX-2 inhibitors; for example, they lack the cardioprotective effects of aspirin which are mediated through COX-1. A more recent approach to reducing the gastrointestinal toxicity of conventional NSAIDs has been the development of nitric oxide (NO)-releasing NSAIDs. Nitric oxide (NO) has been reported to play a critical role in maintaining the integrity of the gastroduodenal mucosa and exerts many of the same effects as endogenous prostaglandins. The present article focuses on the NO-NSAIDs that have potential clinical applications, as well as some drug candidates now in development.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most commonly used drugs for the treatment of rheumatic and degenerative inflammatory joint disorders. Unfortunately, all NSAIDs are associated with some adverse effects (1). The most common adverse effects of NSAIDs are related to the gastrointestinal (GI) system and include abdominal pain or discomfort, heartburn, dyspepsia and perforations (2-4). Inhibition of cyclooxygenase (COX) and therefore prostaglandin (PG) production is the common mechanism of action of NSAIDs (5). It is now well established that COX exists in two isoforms: COX-1 which is constitutively expressed and present in the endothelium, stomach and kidney, and COX-2 which is induced by proinflammatory cytokines and endotoxins (6). Thus, the side effects of NSAIDs are associated with their ability to inhibit COX-1 (7) whereas their therapeutic antiinflammatory effects are attributable to their ability to inhibit COX-2 (8).

Several strategies have been used to reduce the GI toxicity of NSAIDs, including enteric coating to prevent absorption in the stomach, parenteral administration, formulation of prodrugs, complexing with divalent metal ions (9) and coadministration of either suppressors of acid secretion or exogenous PGs. However, in recent years two promising new approaches to the development of NSAIDs have emerged that spare the GI tract (10). The first is the development of selective inhibitors of COX-2 enzyme, the inducible isoform of the PG G/H synthase enzyme (11). Currently marketed conventional NSAIDs, such as diclofenac, naproxen, indomethacin and ketoprofen, are nonselective inhibitors of COX and have greater selectivity towards COX-1 than COX-2. As the constitutively expressed isoform COX-1 has been identified in

many tissues, it has been suggested that a selective inhibitor of COX-2 would suppress PG synthesis at sites of inflammation but spare COX-1 in other tissues such as the GI tract (12). While the development of selective COX-2 inhibitors seems to be a rational approach in the therapeutic evolution of NSAIDs with improved GI tolerability, it is recently reported that COX-2 may be important for homeostasis in health or disease (13). Thus, the use of COX-2 inhibitors has raised many questions as to their therapeutic application.

COX-2 induction has been demonstrated in *Helicobacter pylori* gastritis and other inflammatory bowl diseases or GI inflammation (14). Furthermore, it has been suggested that inhibition of COX-2 would delay ulcer healing (15-17). Whether specific inhibitors of COX-2 would become harmful in the presence of GI inflammation depends on whether COX-2 is induced to the extent that it becomes a predominant source of PGs. Recently, it was reported that COX-2 is expressed together with COX-1 in human gastric mucosa (18). Furthermore, COX-2 selective inhibitors suppress the formation of PGs from samples of human gastric and colonic tissue (19). Thus, a number of lines of evidence suggest that COX-2 is expressed throughout the human GI tract. Whether COX-2 activity in the gut acts as COX-1 in gastroprotection remains to be established, and the safety of COX-2 inhibitors in GI inflammation remains questionable.

COX-2 inhibitors cause fluid retention and induce renal failure or exacerbate hypertension. COX-2 is also expressed constitutively in the kidney, particularly in the macula densa, where it is inducible in response to salt restriction and appears to be important in the control of renin release (20). It is also suggested that mice genetically engineered to be COX-2 deficient develop severe nephropathy (21). Therefore, it is important to determine the extent to which COX-2 inhibitors share the effects of nonselective NSAIDs on renal function.

Cyclical hormonal induction of COX-2 has an important role in ovulation. COX-2 is induced at the end of pregnancy where it is involved in the onset of labor (22). Specific COX-2 inhibitors are, therefore, likely to exhibit effects on ovulation and parturition.

Alternatives to COX-2 inhibitors: NO-releasing NSAIDs

A more recent approach to reducing the GI toxicity of conventional NSAIDs is to link a NO-releasing moiety to conventional NSAIDs (23, 24). This approach has opened up a new avenue for development of safe and gastroprotective NSAIDs.

NO, PGs and gastric mucosal integrity

NO is a crucial mediator of GI mucosal defense (25-28). PGs and NO are thought to play a major role in gastric cytoprotection and it has been suggested that both may act synergistically to mediate cytoprotection (29). In 1995, Elliott also reported that NO participated in ulcer healing (30).

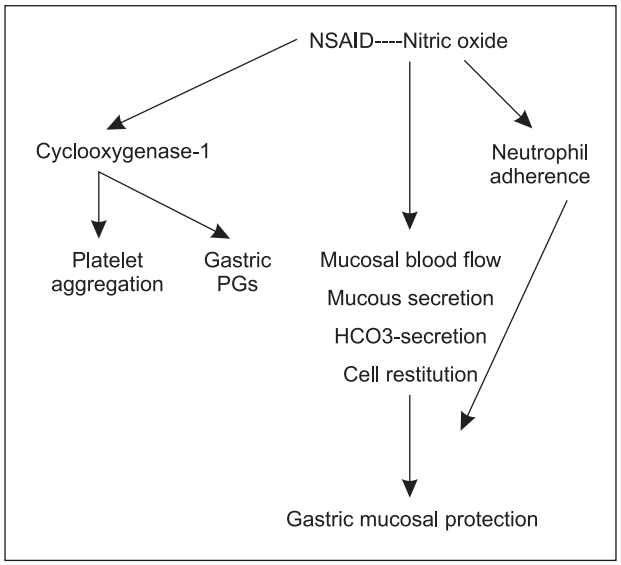

Fig. 1. Postulated mechanism by which nitric oxide-releasing NSAIDs maintain the ability to protect the gastroduodenal mucosa while suppressing mucosal prostaglandins.

In the stomach, NO exhibits many of the same actions as PGs (*i.e.*, stimulation of the mucous secretion and maintenance of blood flow) (31). Moreover, NO is an important signaling molecule and cytotoxic effector molecule of nonspecific immune responses (32). Thus, local delivery of NO could substitute PGs in restoring the balance between aggressive and defensive factors in the GI tract that would be shifted by COX-1 inhibition. The hypothesized mechanism by which NO-releasing NSAIDs maintain the ability to protect the GI mucosa is shown in Figure 1.

Analgesic and antiinflammatory effects of NO-NSAIDs

The rationale behind this class of drugs is that NO, by maintaining gastric mucosal blood flow and preventing leukocyte adherence within the gastric microcirculation, may counteract the detrimental effects of COX suppression (33). These compounds include flurbiprofen nitroxybutylester (HCT-1026) (34), ketoprofen nitroxybutylester (HCT-2035), NO-aspirin (NCX-4016) (35), NO-naproxen (HCT-3012) (36) and NO-paracetamol (NCX-701) (Fig. 2). These compounds were reported to have antiinflammatory and antipyretic activity comparable to the parent NSAIDs in models of acute and chronic inflammation in rats (37), with GI sparing effects, and they may have analgesic effects that are superior to those of the parent compounds (38). However, one study recently reported

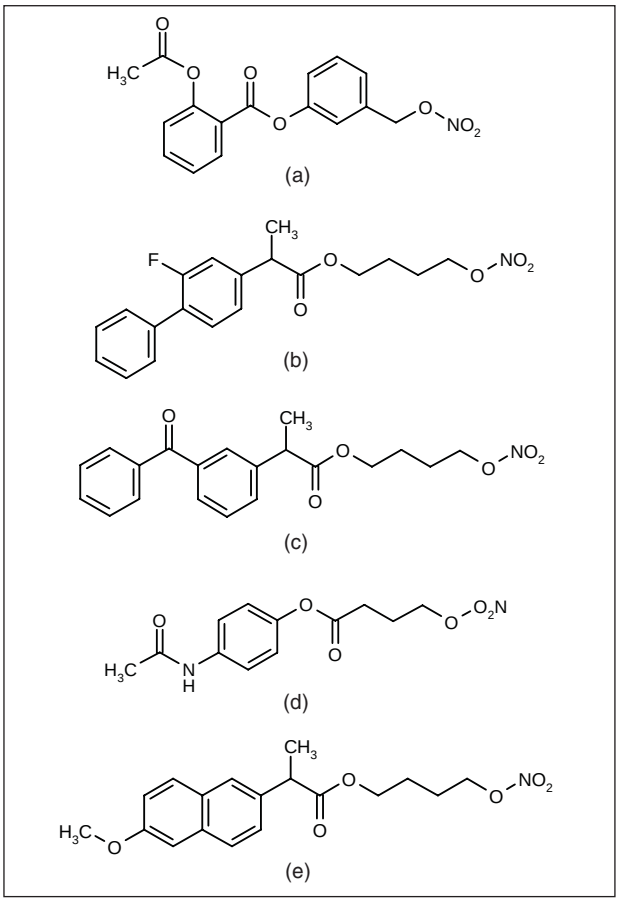

Fig. 2. Structures of (a) NCX-4016, (b) HCT-1026, (c) HCT-2035, (d) NCX-701 and (e) HCT-3012.

enhanced antiinflammatory activity of NO derivatives of mesalamine in the rat (39).

The biological activity of NCX-4016 has been evaluated in various animal models, where the compound was found to have similar antiinflammatory activity but marked analgesic activity in comparison to aspirin (32). Others have also reported enhanced antiinflammatory effect of NCX-4016 as compared to aspirin (40). In a recent clinical trial in healthy volunteers, HCT-1026 was found to be less ulcerogenic than the parent drug while maintaining the same inhibitory effects on gastric mucosal PG synthesis and serum thromboxane levels (41).

In a study in rats, HCT-3012 (14.5 mg/kg) was shown to significantly increase collagen deposition while celecoxib (10 mg/kg) had no effect (42). Both naproxen and HCT-3012 suppressed PGE_2 levels at the site of the wound and whole blood thromboxane synthesis to a similar degree but celecoxib had no effect on wound site PG levels. In animal studies, NCX-701, a NO derivative of paracetamol, not only exhibited increased antinociceptive activity in both rats and mice but, interestingly, was also antiinflammatory over a similar dose range in comparison with paracetamol (43).

Taken together, these data indicate that NO-NSAIDs may represent a safer alternative to standard NSAIDs for use as analgesic and antiinflammatory agents for post-surgical patients.

Antiplatelet activity of NO-NSAIDs

The antiplatelet activity of NO-NSAIDs has been studied *in vitro*. It was reported that these compounds have enhanced antiplatelet activity (44-48) due to the inhibitory effects of NO on platelet adhesion and aggregation (49). While NCX-4016 produced maximal inhibition of arachidonic acid-stimulated platelet aggregation at a concentration of 100 μM, aspirin induced the same effect at 10 μM. NCX-4016 was found to be more efficient than aspirin in inhibiting thrombin-induced platelet activation (aggregation and adhesion) (50). These results indicate that NO-NSAIDs may be useful as antithrombotic agents.

Conclusions

NO-NSAIDs are a new class of compounds that combine NO-releasing activity with COX inhibition. It was reported that NO-NSAIDs have good GI tolerability without compromising their therapeutic activity. NO-NSAIDs could replace COX-2 inhibitors because the benefits of COX-1 would be retained and these agents are not reported to delay or enhance healing of established ulcers in animal studies.

Moreover NO-aspirin, unlike COX-2 inhibitors, has the potential to be a safer agent for cardiovascular prophylaxis. This is important since aspirin causes more ulcer bleeding than non-aspirin NSAIDs. The NO-NSAIDs are currently under clinical investigation and it is too soon to predict the practical advantage of these newer drugs over COX-2 inhibitors.

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