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Prostaglandins, NSAIDs, and Gastric Mucosal Protection: Why Doesn't the Stomach Digest Itself?

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Wallace JL. Prostaglandins, NSAIDs, and Gastric Mucosal Protection: Why Doesn't the Stomach Digest Itself? *Physiol Rev* 88: 1547–1565, 2008; doi:10.1152/physrev.00004.2008.—Except in rare cases, the stomach can withstand exposure to highly concentrated hydrochloric acid, refluxed bile salts, alcohol, and foodstuffs with a wide range of temperatures and osmolarity. This is attributed to a number of physiological responses by the mucosal lining to potentially harmful luminal agents, and to an ability to rapidly repair damage when it does occur. Since the discovery in 1971 that prostaglandin synthesis could be blocked by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), there has been great interest in the contribution of prostaglandins to gastric mucosal defense. Prostaglandins modulate virtually every aspect of mucosal defense, and the importance of this contribution is evident by the increased susceptibility of the stomach to injury following ingestion of an NSAID. With chronic ingestion of these drugs, the development of ulcers in the stomach is a significant clinical concern. Research over the past two decades has helped to identify some of the key events triggered by NSAIDs that contribute to ulcer formation and/or impair ulcer healing. Recent research has also highlighted the fact that the protective functions of prostaglandins in the stomach can be carried out by other mediators, in particular the gaseous mediators nitric oxide and hydrogen sulfide. Better understanding of the mechanisms through which the stomach is able to resist injury in the presence of luminal irritants is helping to drive the development of safer anti-inflammatory drugs, and therapies to accelerate and improve the quality of ulcer healing.

I. INTRODUCTION

The stomach is a remarkable organ. It secretes a juice that can digest the various foods that we eat, but it seldom digests itself (37). The reasons for this enigma have been pondered by scientists for centuries, and still remain incompletely understood. Where significant progress has been made in recent years is in the appreciation of the contribution of a number of autacoids in mediating the resistance of the stomach lining to injury.

These include such substances as the prostaglandins, gaseous mediators (nitric oxide and hydrogen sulfide), and neuropeptides (calcitonin gene-related peptide; CGRP). The focus of this article is the crucial role of prostaglandins as mediators of mucosal defense. These compounds, in particular, have been extensively investigated in this context, mainly because of the inhibitory effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on prostaglandin synthesis and the contribution of this effect to the gastrointestinal ulceration and bleeding associated with the use of this class of drugs.

The discovery of an association between colonization of the stomach by *Helicobacter pylori* infection and gastric ulcer disease (122) led to even greater focus on the mechanisms underlying mucosal defense. This was driven particularly by the fact that only a minority of patients (~15%) with this infection will develop an ulcer (214), so host factors are almost certainly a critical factor in determining whether an ulcer forms or not.

This review is focused on the stomach, but prostaglandins contribute to mucosal defense throughout the gastrointestinal (GI) tract. Much of what is known about prostaglandins and mucosal defense in the stomach also pertains to the rest of the GI tract. On the other hand, the mechanisms through which NSAIDs contribute to mucosal injury appear to differ from one region of the digestive system to the next (143, 189).

II. HISTORICAL PERSPECTIVE

Prostaglandins are a group of fatty acids that were first isolated from seminal fluid by von Euler (186). Indeed, they are so named because they were believed (incorrectly) to be a prostatic secretion. A crucial discovery, in terms of understanding the role of prostaglandins in the stomach, was the finding by Vane in 1971 that aspirin and other NSAIDs inhibited the synthesis of prostaglandins (185). Vane proposed that this was the mechanism underlying the anti-inflammatory effects of these drugs, since prostaglandins were known, at the time, to contribute to edema formation and to the pain associated with inflammation. Vane also suggested that inhibition of prostaglandin biosynthesis by NSAIDs may underlie the ability of this class of drugs to induce ulceration in the gastrointestinal tract.

In the decade that followed Vane's prediction, there was enormous interest in prostaglandins as physiological mediators in the GI tract and elsewhere. In the context of this review, the work of Andre Robert and colleagues is particularly important. Robert demonstrated that administration to rats of nanomolar doses of prostaglandins prior to oral administration of any of a selection of "necrotizing agents" resulted in an apparent protection of the stomach from damage induced by the necrotizing agent (144, 145). This was a remarkable finding given that the necrotizing agents that were tested included boiling water, absolute ethanol, 25% sodium chloride, 0.2 N sodium hydroxide, and 0.6 N hydrochloric acid (144). At the time, ulcer research was very much influenced by what was called "Schwarz' dictum": no acid, no ulcer (158). Thus ulcer disease was believed to be attributable largely to the erosive effects of gastric acid (normal or elevated levels), and it therefore followed that anything that reduced ulcer disease likely did so by reducing gastric acid secretion. In studies of rats, Robert et al. (145) noted that prostaglandins were capable of suppressing gastric acid secretion, but he found that doses well below those necessary for this antisecretory effect produced the protective effect against oral administration of necrotizing agents. At the suggestion of Dr. Eugene Jacobson, Robert adopted the term *cytoprotection* to describe the remarkable ability of prostaglandins, at sub-antisecretory doses, to reduce damage to the stomach induced by necrotizing agents.

Before examining the question of how prostaglandins may contribute to mucosal defense, it would be beneficial to review the major elements that contribute to the resistance of the gastric mucosa to damage.

III. MUCOSAL DEFENSE

"Mucosal defense" is a term used to describe the various factors and components that permit the mucosa to remain intact despite its frequent exposure to substances with a wide range of temperature, pH, and osmolarity, as well as to substances with detergent or cytotoxic actions, and bacterial products capable of causing local and systemic inflammatory reactions (191). It is important to realize that the gastric mucosa is not impervious to damage by these agents. The use of the term *gastric mucosal barrier* has often been misconstrued to suggest that this tissue is impenetrable. It was coined by Charles Code (36) to explain the relatively small amount of hydrochloric acid that "back-diffuses" into the mucosa. In fact, mucosal injury occurs regularly, but does not lead to clinically significant disruption of the function or even the "barrier" properties of the tissue. The reasons for this include the fact that there are several "layers" to mucosal defense, with secondary components becoming more important when more superficial components are breached, and because of a very rapid process of repair when damage to the epithelium occurs (191). Moreover, the various components of mucosal defense can be modulated by a number of endogenous substances, including prostaglandins. The net result is that the systemic circulation is protected from invasion by microbes, microbial products, and other toxins. On the other hand, there are certain circumstances in which mucosal defense is impaired, such as after administration of NSAIDs, thereby rendering the mucosa more susceptible to injury.

As alluded to above, mucosal defense is a dynamic process. In a healthy organism, the resistance of the gastric mucosa to injury is enhanced when irritants are present in the stomach, such as through an augmentation of mucosal blood flow and an efflux of mucus from surface epithelial cells. There are some conditions in which there is an impairment of these adaptive responses, rendering the gastric mucosa more susceptible to injury induced by luminal irritants. Examples of these will be discussed below.

The various levels of mucosal defense can be viewed in a structural sense, starting at the lumen and moving into deeper levels of the tissue. Which of these components will participate in a defensive response depends on the intensity of the challenge and the extent to which any toxin is able to diffuse into the mucosa.

A. Luminal Factors

While the epithelium is often viewed as being the physical manifestation of "the barrier," there are several components of mucosal defense on the luminal side of the epithelium. Gastric juice contains a number of elements capable of reducing bacterial colonization of the stomach, including acid, immunoglobulins, and lactoferrin. Few microbes can survive in the acid secreted by the stomach. The importance of acid as a defensive factor is evident from the observations that hypochlorhydria and achlorhydria increase the risk of and exacerbate the severity of bacterial and certain parasitic infections (57). Bacterial counts in the stomach and duodenum are inversely related to the level of gastric acid secretion (66). The mucus that is secreted onto the surface of much of the stomach acts both as a lubricant, to reduce physical damage to the epithelium by ingested materials, and as a trap for bacteria (19, 52). Thus mucus can diminish the ability of bacteria to gain access to the epithelium. Ironically, it is the mucus layer in the stomach (primarily in the antrum) that is the site of colonization by *H. pylori* (214). Mucus performs an important structural role in creating an unstirred layer on the mucosal surface which supports maintenance of a near-neutral pH at that surface as well as acting as a physical barrier against luminal pepsin (5). Different forms of mucus produced by mucous neck and surface epithelial cells may contribute to the creation of a stable layer of mucus on the epithelial surface (80, 131). Bicarbonate secreted by the epithelium can be concentrated within the surface mucus, creating a microenvironment with a pH closer to neutrality than that found in the luminal gastric juice (14, 33, 56). Mucus has been suggested to retard the diffusion of protons (157), which would further aid in maintaining a favorable pH at the apical surface of the epithelium. However, others have provided evidence that it is the carefully regulated secretion of alkali and the trapping of that alkali within the unstirred layer on the surface of the epithelium that is more important to mucosal defense than any impedance of the diffusion of protons by mucus (16). There is considerable controversy with respect to the importance of the mucus-bicarbonate "barrier" in mucosal defense which centers on three main aspects: *1*) whether or not the thickness of surface mucus is important for protecting the epithelium from the damaging effects of acid (22, 33), *2*) whether there is a continuous or discontinuous layer of mucus covering the mucosal surface (13, 33, 131), and *3*) if the mucus layer is continuous, how does the acid produced in the gastric glands traverse this layer so that it can gain access to the lumen? With respect to the latter, a model for acid movement through "channels" in mucus has been proposed (20) and is supported by some data (89), but other groups have challenged the existence of such channels based on confocal microscopic studies of the rat stomach (33).

A novel hypothesis for the resistance of the gastric epithelium to acid-induced injury was proposed by Lichtenberger and colleagues in 1983 (78). They demonstrated that the surface of the stomach is hydrophobic, and therefore a barrier to acid back-diffusion, because of the presence of a surfactant-like layer of surface-active phospholipids. This layer was localized either on the surface of the epithelium itself, or on the most luminal surface of mucus overlying the epithelium (62, 78). Disruption of this layer, using aspirin or bile salts, resulted in elevated diffusion of acid into the mucosa, and to mucosal necrosis (62). Interestingly, phospholipase enzymes and ammonium ions released by *H. pylori* can reduce the effectiveness of the hydrophobic lining of the stomach, consistent with the observations that there is diminished hydrophobicity of the gastric surface seen in individuals infected with *H. pylori* (110).

B. The Epithelium

Consistent with the notion that there are several "layers" of mucosal defense (with a degree of redundancy of function), experimentally reducing the effectiveness of the mucus-bicarbonate layer on the epithelial surface does not usually result in epithelial damage (188). This may be in part related to the inherent ability of gastric epithelial cells to remain intact and functional when continuously exposed to high concentrations of acid. Sanders et al. (151) demonstrated that the apical membrane of cultured chief cells was highly resistant to damage by acid. Exposure of the apical surface of these cells to a solution of pH 2 for more than 4 h did not damage the cells. However, the basolateral membrane of these cells was very sensitive to acid, being damaged when exposed to a solution with a pH of only 5.5. These observations

suggest that the apical membrane of gastric epithelial cells is highly resistant to high concentrations of acid. This hypothesis is supported by the findings of Boron et al. (24), who performed studies using rabbit gastric glands. They concluded that the apical membrane of parietal and chief cells was exceptionally resistant to diffusion of hydrogen ions. Takezono et al. (176) have similarly demonstrated resistance of cultured rat gastric epithelial cells to acid-induced damage, and dynamic regulation of paracellular permeability in response to exposure to acid.

A further feature that makes the gastric epithelium resilient to injury is its relative "youth"; that is, the human gastric epithelium is renewed every 2–4 days (222). The ability to replace older cells on a continuous and rapid basis without there being a significant break in epithelial continuity and barrier function can be attributed to the process of extrusion of cells as they undergo apoptosis. The cells surrounding the apoptotic cell gradually pinch in at the base of that cell until the apoptotic cell is no longer attached to the basement membrane (70).

C. Mucosal Blood Flow

With adequate vascular perfusion, epithelial damage does not generally progress to necrosis of deeper layers of the mucosa. Indeed, the entire luminal epithelium can be destroyed in the rat, and there is little macroscopic evidence of the injury, other than extensive mucus release (193, 196). Microscopically, there is clear evidence of destruction of the epithelium, but remarkably, reestablishment of epithelial continuity can be seen within minutes to hours of induction of the damage. This rapid repair has been termed "restitution," and it involves the migration of healthy epithelial cells from the gastric pits over the denuded basement membrane (102, 130, 210). This is another element of mucosal defense for which there is good evidence of modulation by prostaglandins. While this process can be observed in vitro, it is clear that in an in vivo setting, vascular perfusion is crucial in providing a "back-up" level of mucosal defense during the critical period after injury has occurred and the basement membrane is exposed to luminal contents. The mucus released from damaged epithelial cells and plasma exuding from the mucosal vasculature coalesces to form a protective layer over the denuded region that has been termed the "mucoid cap" (193, 196). Even in the presence of very high levels of hydrochloric acid in the stomach (i.e., pH <1), the pH within the mucoid cap can be maintained at close to neutrality. As the basement membrane is highly sensitive to damage by acid, this protection is crucial to permit restitution to occur. The maintenance of the relatively high pH microenvironment is dependent on undisturbed mucosal blood flow. If blood flow to the stomach is interrupted, then the pH within the mucoid cap drops precipitously and hemorrhagic lesions form. This is the case either with mechanical occlusion of the gastric arterial supply or by administration of a vasoconstrictor such as endothelin (193). Acid is permitted to diffuse deeper in to the mucosa, causing extensive necrosis and hemorrhage. Importantly in the context of this review, prostaglandins appear to play a significant role in the maintenance of mucosal blood flow during this critical period of epithelial repair, since these events can be prevented by luminal application of a prostaglandin (193).

The gastric mucosa can be exposed to high concentrations of acid without significant epithelial injury occurring. Part of the reason for this is that the mucosal vasculature responds very quickly to the presence of acid in the superficial mucosa, so as to buffer, dilute, and remove the acid (27). This is accomplished via a sensory afferent nerve-mediated reflex (84). Sensory afferent nerve endings in the superficial mucosa can detect the presence of acid, and they respond by releasing, in the vicinity of submucosal arterioles, the vasodilator CGRP (108). This results in relaxation of the smooth muscle surrounding the arterioles, resulting in an elevation of blood flow in the mucosa. The relaxant effects of CGRP on vascular smooth muscle are largely mediated via nitric oxide (acting on soluble guanylate cyclase) (115), but there is also evidence for participation of prostaglandins in this vasodilatory response (60). Interruption of the reactive hyperemic response, with CGRP antagonists, NSAIDs, nitric oxide synthase inhibitors, or through ablation of the sensory afferent neurons, results in a significant increase in the susceptibility of the mucosa to injury (83, 85, 134, 220). Moreover, there are some disease conditions in which the reactive hyperemic response is impaired, leading to greater susceptibility to gastric ulceration and bleeding. One such example is portal hypertension (18) (Fig. 1). Indeed, the underlying mechanism for the loss of this important component of mucosal defense in portal hypertension is a marked disruption of the prostaglandin- and nitric oxide-mediated reactive hyperemic response (18, 44).

D. Inflammation

Superficial injury to the gastric mucosa also triggers an acute inflammatory response, characterized by the above-mentioned increase in blood flow, as well as by plasma exudation and recruitment into the mucosa of leukocytes. The objective of this response is to minimize tissue injury, facilitate repair of damaged tissue, and prevent entry into the systemic circulation of foreign substances, including microbes and microbial products (191). This inflammatory response is coordinated via the release of an array of soluble mediators, from cells such as mucosal mast cells that act as "sentinels" within the mucosa.

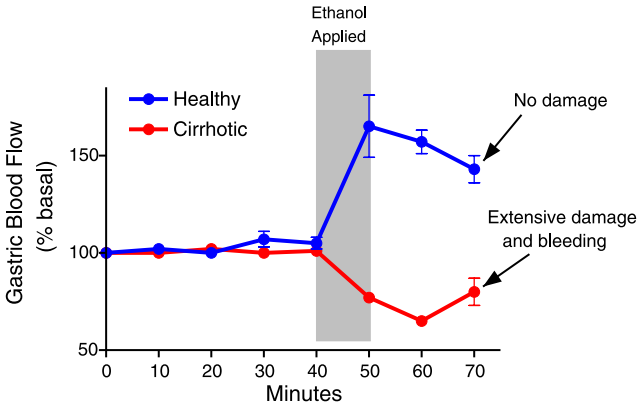

FIG. 1. Impaired mucosal defense in cirrhotic rats is in part due to the lack of a normal hyperemic response to topical irritants. While in healthy rats the topical application of 20% ethanol results in a marked and rapid increase in mucosal blood flow (measured by laser-Doppler flowmetry), a decrease in blood flow is observed during the same challenge in cirrhotic rats. The gastric mucosa of the healthy rats is resistant to damage induced by the ethanol (no hemorrhagic erosions form); however, in cirrhotic rats, extensive hemorrhagic damage develops after the exposure to ethanol. [Data from Beck et al. (18).]

This subject has been reviewed in more detail elsewhere (123). It is important to note that while the acute inflammatory response is aimed at reducing mucosal injury, there are circumstances in which this response can be dysregulated and can contribute to mucosal injury. Interestingly, NSAIDs can trigger some of the elements of an acute inflammatory response, and this contributes to their ability to cause mucosal injury (discussed in more detail below).

E. Ulcer Healing

When the above-mentioned components of mucosal defense are insufficient to limit injury to the mucosa, an ulcer forms. An ulcer is a lesion that penetrates the muscularis mucosa. Repair of ulcers is a highly regulated and complicated process that involves inflammation, cell proliferation (particularly at the ulcer margin), formation of granulation tissue at the base of the ulcer, and angiogenesis (new blood vessel growth). In response to ulceration, a new type of cell appears in the ulcer margin which secretes large amounts of epithelial growth factor (EGF) (223), acting as a potent stimulus for reepithelialization. Glandular structure is gradually reestablished, along with the mucosal microcirculation. Platelets contribute significantly to ulcer healing, at least in part through the delivery of numerous growth factors that can promote angiogenesis and epithelial cell proliferation (118, 202). Of course, platelets are also an important element in hemostasis, and bleeding of ulcers is a very important clinical concern. Some of the clinical benefit of drugs that suppress gastric acid secretion may be related to a facilitation of platelet aggregation; thus platelet aggregation will not occur at a pH <5.4 (67). The process of ulcer healing has been reviewed in detail elsewhere (178).

IV. HOW DO PROSTAGLANDINS CONTRIBUTE TO MUCOSAL DEFENSE?

The major prostaglandins produced by the human and rodent gastric mucosa are PGE_2 and PGI_2, with lesser amounts of $PGF_{2\alpha}$ and PGD_2 also being detectable (94, 138, 139). Thromboxane has also been detected in the gastric mucosa, but much of what can be measured is actually from platelets within the gastric microcirculation (194). The prostaglandin receptors that mediate many of the effects of prostanoids on mucosal defense have been characterized through the use of pharmacological probes and knock-out mice (167) (Table 1).

A. Luminal Factors

As mentioned above, prostaglandins can inhibit gastric acid secretion. Studies in rodents suggest that such effects are produced via EP3 and IP receptors (92, 133) (Table 1). In rodents, inhibition of acid secretion by prostaglandins is only observed with doses well above those required to elicit protective effects against various noxious substances (ethanol, aspirin, etc.). More pertinent to

TABLE 1. *Prostaglandin receptor and COX isoforms in gastric mucosal defense*

Effect (Species)	Receptor(s)	COX Isoform	Reference Nos.
Inhibition of gastric acid secretion (rat)	EP3	COX-1	15, 92
Inhibition of gastric acid secretion (mouse)	EP3, IP	COX-1	133
Stimulation of gastric acid secretion (rat)	EP4		92
Mucus secretion in stomach (rabbit)	EP4		171
Bicarbonate secretion/juxtamucosal pH gradient in stomach (mouse)	EP1	COX-1	17, 173
Maintenance of mucosal surface hydrophobicity (mouse)		COX-1	35
Decreased epithelial permeability to acid (rat)		COX-1	176
Mucosal blood flow (rat)	EP2, EP4	COX-1	9, 209
Ulcer healing (rat)	EP4	COX-2	117, 175
Ulcer healing (mouse)	EP4	COX-2	72, 129
Enhancement of histamine-induced vascular permeability (rat)	EP1		71
Damage-induced gastric hyperemia (mouse)	EP1		95
Resistance to ischemia/reperfusion-induced gastric damage (mouse)	IP	COX-2	97
Prostaglandin (E_2) protection against gastric injury induced by ethanol or indomethacin (rat)	EP1		9, 168

COX, cyclooxygenase.

the enhancement of mucosal defense, prostaglandins stimulate mucus and bicarbonate secretion in the stomach (and elsewhere in the GI tract) (33, 56). At least in rodents, these effects appear to be mediated via EP4 and EP1 receptors, respectively (171, 175), and COX-1-derived prostaglandins contribute significantly to the maintenance of a pH gradient at the mucosal surface (17) (Table 1). Prostaglandins may also enhance the effectiveness of the layer of surface-active phospholipids on the mucosal surface. Kao and Lichtenberger (91) demonstrated that an analog of PGE_2 increased the volume of certain subcellular organelles within gastric surface epithelial cells that are thought to be storage sites for gastric surfactant.

While protective and antisecretory effects of prostaglandins have been clearly distinguished in animal studies, the beneficial effects of prostaglandin analogs in humans have been seen only at doses that produce significant inhibition of gastric acid secretion (21, 64). Indeed, the beneficial effects of these prostaglandins in terms of preventing NSAID-induced gastric damage are likely due, in large part, to the suppression of gastric acid secretion (21, 64).

B. The Epithelium

In addition to stimulating epithelial cells to release more bicarbonate and mucus, prostaglandins can reduce the permeability of the epithelium and thus reduce acid back-diffusion (176). Prostaglandins applied directly to epithelial cells in culture can also increase the resistance of those cells to damage induced by exposure to an NSAID or to ethanol (179). The underlying mechanism for this effect has not yet been identified.

C. Mucosal Blood Flow

Prostaglandins of the E and I series are potent vasodilators, producing this effect in the stomach through EP2/EP4 and IP receptors, respectively (9, 97) (Table 1). As such they can increase mucosal blood flow, and this increases the resistance of the gastric mucosa to injury. Moreover, vasodilatory effects of prostaglandins facilitate epithelial restitution by contributing to the creation of a relatively high pH microenvironment within the mucoid cap that forms over sites of epithelial damage (193). The prostaglandins that contribute to basal mucosal blood flow are derived principally from COX-1 (209), while in circumstances in which mucosal integrity is challenged, such as during ischemia-reperfusion injury, COX-2-derived prostaglandins are of increased importance for the maintenance of blood flow (97). The decrease in gastric blood flow in a healthy stomach that can be observed following administration of a selective COX-1 inhibitor is acid dependent (54). In the absence of luminal acid, inhibition of COX-1 does not alter mucosal blood flow. Thus COX-1-derived prostaglandins may not play an important role in modulating basal gastric vascular tone so much as they protect the vasculature from acid-induced injury (54). This may be related to the above-mentioned ability of prostaglandins to reduce the permeability of the gastric epithelium (directly, or via enhancement of the effectiveness of surface-active phospholipids) (176), thereby reducing acid back-diffusion.

COX-1 inhibition also leads to significant release of endothelin-1 (54), a very potent vasoconstrictor which has been shown to induce mucosal injury when administered intravenously to rats (201). The releasing of endothelin-1 only occurred when the mucosa was exposed to acid (thus it was a response to back-diffusing acid, rather than to the suppression of COX-1 itself) (54). Thus acid back-diffusion, facilitated by changes in epithelial permeability as a result of inhibition of COX-1, leads to release of endothelin-1, which reduces mucosal blood flow.

D. Inflammation

Prostaglandins downregulate the release of a number of other inflammatory mediators that have been suggested to contribute to the generation of mucosal injury in certain circumstances (123). For example, PGE_2 has been shown to be a potent inhibitor of the release of histamine, tumor necrosis factor-α, and platelet-activating factor from mast cells (81), the release of tumor necrosis factor-α and interleukin-1 from macrophages (98–100), and the release of leukotriene B_4 and interleukin-8 from neutrophils (69, 73, 216). Several of these inflammatory mediators have been shown to increase the susceptibility of the stomach to damage induced by NSAIDs or other topical irritants, and/or to mediate the mucosal injury that occurs during hemorrhagic or endotoxic shock (8, 63, 146, 152, 182, 195, 197, 201, 204, 213). There is also evidence that the nuclear transcription factor, NFκB, which regulates the expression of genes for several proinflammatory cytokines and adhesion molecules that have been implicated in the pathogenesis of NSAID-induced gastric mucosal injury, is activated by NSAIDs, and inhibition of its activation can prevent NSAID-induced mucosal injury (26).

Prostaglandins are also potent inhibitors of leukocyte adherence to the vascular endothelium (25). Indeed, the leukocyte adherence that occurs within the gastrointestinal microcirculation following administration of an NSAID can be prevented by prostaglandin administration (10, 11), and this likely contributes to the protective effects of prostaglandins on the gastric mucosa (189).

The above-mentioned studies suggest that prostaglandins can increase the resistance of the gastric mucosa to injury via their ability to downregulate inflammatory responses. In recent years, substantial evidence has been generated to suggest that certain prostaglandins contrib-

ute significantly to the resolution of ongoing inflammatory responses, in the GI tract and elsewhere. For example, COX-2-derived PGD_2 has been shown to be an important inhibitor of leukocyte recruitment during acute colitis (3). PGD_2 metabolites, including 15-deoxyΔ^{12-14} PGJ_2, also derived from COX-2, have been implicated as key mediators of the resolution of inflammation in various tissues (58, 160).

E. Ulcer Healing

Prostaglandins accelerate ulcer healing in experimental models and in humans (75, 165). The mechanisms responsible for this effect are not fully understood, but the above-mentioned ability of prostaglandins to reduce gastric acid secretion would contribute to acceleration of ulcer healing (as is observed with other antisecretory drugs). Also, it has been suggested that blood flow to the ulcer margin is important (29), given that it is the ulcer margin where regeneration of epithelial cells primarily occurs. It is likely that the vasodilatory properties of prostaglandins of the E and I series accelerate ulcer healing, at least in part, via this mechanism. The ability of prostaglandins to stimulate mucus and bicarbonate secretion may also contribute significantly to the promotion of ulcer healing (119). Indeed, it has been demonstrated in rats that the ulcer bed has a pH substantially above that on the surface of nonulcerated tissue (41). Prostaglandins also trigger the release of vascular endothelial growth factor (VEGF) (128, 170), which has been shown to make an important contribution to ulcer healing (118, 169, 202), likely via stimulation of angiogenesis.

The endogenous prostaglandins that contribute to ulcer healing are derived principally from COX-2; that is, selective COX-2 inhibitors impair gastric ulcer healing, and mice deficient in COX-2 exhibit impaired ulcer healing (117, 129, 154). The beneficial effects of PGE_2 on gastric ulcer healing in rodents appear to be mediated via the EP4 receptor (72, 175).

V. MECHANISMS OF NSAID-INDUCED GASTRIC DAMAGE

NSAIDs induce clinically significant ulceration, bleeding, and/or obstruction in 1–4% of patients chronically taking these drugs (163). Even higher rates of ulceration are seen in patients with a history of peptic ulceration; in patients concurrently taking anticoagulants, low-dose aspirin, or glucocorticoids; and in the elderly (103, 163). In a recent clinical trial, "at-risk" patients (≥60 years of age and/or a history of ulcers) taking conventional NSAIDs or selective COX-2 inhibitors were studied over a 6-mo period; 17.1 and 16.5%, respectively, developed clinically significant ulcers (153). As well as documenting the high susceptibility of this group of patients to the ulcerogenic effects of NSAIDs, this study confirmed previous reports that selective COX-2 inhibitors offered little, if any, benefit in high-risk patients (104).

The mechanisms through which NSAIDs produce damage in the stomach can be subdivided into local (topical) actions and systemic actions (Fig. 2). The topical actions of NSAIDs on the gastric epithelium may involve several mechanisms. Some NSAIDs, particularly those of acidic nature, can directly kill epithelial cells (4, 179). Various mechanisms have been proposed for this cytotoxic action, including the induction of osmotic lysis subsequent to trapping of charged NSAIDs with the epithelial cells (156), and death of the epithelial cell subsequent to uncoupling of oxidative phosphorylation (164). NSAIDs can also reduce mucus and bicarbonate secretion, thereby decreasing the effectiveness of the juxtamucosal pH gradient in protecting the epithelium (4, 17, 88, 140). NSAIDs can also disrupt the layer of surface-active phospholipids on the mucosal surface, independent of effects on prostaglandin synthesis (35, 59, 61, 113). Such an action would render the mucosa less able to resist damage induced by luminal acid.

NSAIDs can also diminish the ability of EGF to promote epithelial repair. Thus inhibition of epithelial proliferation has been observed when the cells are exposed to NSAIDs, and this appears to involve a reduction of EGF binding to its receptor (53) and inhibition of EGF signaling pathways (90, 135).

While the above-mentioned mechanisms likely contribute to the toxicity of NSAIDs in the stomach (and even more so in the small intestine, where enterohepatic recirculation of some NSAIDs leads to repeated exposure of

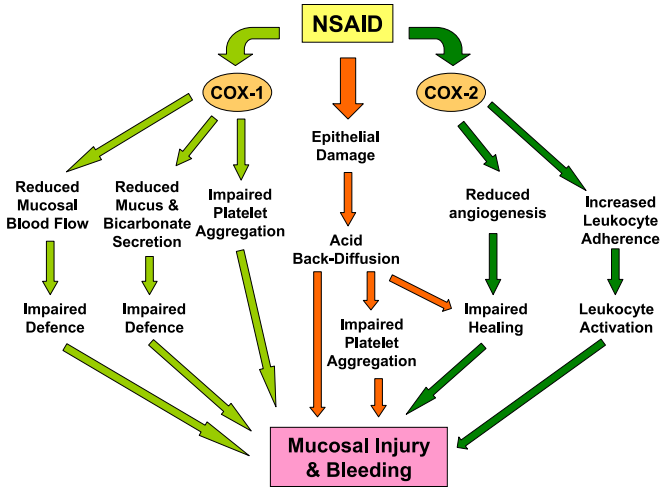

FIG. 2. Pathogenesis of NSAID-induced gastric injury and bleeding. NSAIDs induce injury/bleeding via three key pathways: inhibition of cyclooxygenase (COX)-1 activity, inhibition of COX-2 activity, and direct cytotoxic effects on the epithelium. Effects produced via only one of these pathways (e.g., selective inhibition of COX-1 or of COX-2) are unlikely to produce significant damage.

the epithelial cells to these drugs) (143), they are unlikely to be the sole mechanism for ulcer formation. For example, gastric ulcers occur when NSAIDs are administered parenterally (42, 77, 192). It is possible that an NSAID that is excreted in bile may reflux into the stomach and then cause damage to the epithelium. However, it has been demonstrated that aspirin, which is not excreted in bile (28), can induce gastric ulcers in cats when administered intravenously (218). Further supporting an important contribution of nontopical actions of NSAIDs to ulcer formation is the observation that the incidence of significant gastric ulceration and bleeding is not appreciably reduced when the NSAIDs are enteric-coated to prevent direct contact of the NSAID molecule with the gastric mucosa or when the NSAIDs are formulated as a prodrug that is inactive until metabolized in the liver (30, 65, 76).

The most important of the systemic effects of NSAIDs, in terms of inducing gastric ulceration, is their ability to suppress prostaglandin synthesis. The evidence that this is the primary mechanism underlying the ulcerogenic effects of NSAIDs includes the following: *1*) the aforementioned observations that prostaglandins modulate many components of mucosal defense; *2*) a good correlation between the degree of suppression of gastric prostaglandin synthesis by various NSAIDs at various doses, and their ability to induce injury in the stomach (142, 207); and *3*) a good temporal correlation between the first manifestation of damage following NSAID administration and the suppression of mucosal prostaglandin synthesis (142, 206, 217). On the other hand, gastric prostaglandin synthesis can be markedly suppressed without ulceration ensuing (114, 209) (see Fig. 3). In all likelihood, therefore, it is the case that suppression of gastric prostaglandin synthesis renders the mucosa more susceptible to the damaging effects of luminal agents (acid, pepsin, ethanol, etc.) including, in some cases, the NSAID itself. This notion is perhaps best supported by the observations that extensive epithelial damage, created by a topical irritant (hypertonic saline), would normally heal without any deeper mucosal injury or hemorrhage. However, administration of an NSAID, or even very brief interruption of mucosal blood flow, results in a rapid transformation of the superficial injury into hemorrhagic, erosive damage penetrating through the full thickness of the mucosa (193). This effect of NSAIDs can be prevented by administration of a prostaglandin (193).

One of the earliest recognized effects of NSAIDs on the gastric mucosa was their ability to reduce mucosal blood flow (12, 55). As mentioned above, this effect is now recognized as being due primarily to suppression of COX-1 activity (209) and to be dependent on the presence of acid in the lumen (54). The magnitude of the reduction of gastric blood flow is usually not, in itself, sufficient to result in significant mucosal injury. However, reduced mucosal blood flow will render the mucosa more susceptible to damage induced by luminal irritants, and as outlined above, will impair the epithelial restitution process (193).

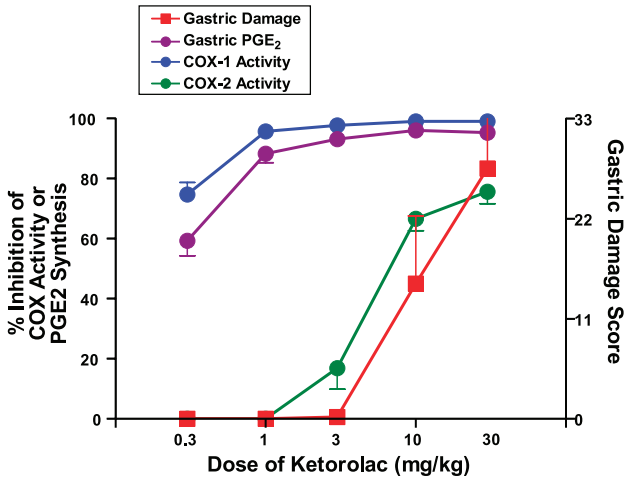

FIG. 3. Dose-related inhibitory effects of ketorolac on gastric prostaglandin synthesis and systemic COX-1 and COX-2 activity, and the relation to the formation of hemorrhagic erosions in the stomach. Note that the lower doses of ketorolac cause near-complete suppression of COX-1 and of gastric prostaglandin synthesis, but no gastric damage. Gastric damage was only observed with doses of ketorolac that produced significant inhibition of COX-2 (concomitantly with suppression of COX-1). These data support the hypothesis that NSAID-induced gastric damage requires the inhibition of both COX-1 and COX-2. [Data from Wallace et al. (209).]

In a series of studies aimed at better understanding the mechanisms responsible for NSAID-induced impairment of mucosal blood flow, Kitahora and Guth (93) made an important observation. They noted that "white thrombi" were apparent along the vessels walls in the gastric microcirculation shortly after exposure of the stomach to aspirin. Subsequently, mucosal blood flow decreased in the regions where the thrombi were observed, and later, the same areas became hemorrhagic. Based on the assumption that the white thrombi observed by Kitahora and Guth (93) were neutrophils, we investigated the possibility that neutrophils made an important contribution to the pathogenesis of NSAID-induced gastric injury. We observed that rats made neutropenic through treatment with an anti-neutrophil antibody did not develop hemorrhagic erosions when they were given NSAIDs (206). We also observed that prevention of neutrophil adherence to the vascular endothelium by treatment of rabbits with monoclonal antibodies directed against various endothelial or leukocyte adhesion molecules protected the stomach of these animals from the damaging effects of NSAIDs (198, 208). Moreover, administration of NSAIDs was found to trigger the adherence of leukocytes (primarily neutrophils) to the vascular endothelium (10, 11, 208). The time course of leukocyte adherence following NSAID administration was consistent with the time course of inhibition of prostaglandin

synthesis, and the NSAID-triggered leukocyte adherence could be prevented by administration of a prostaglandin (10, 11). NSAID-induced leukocyte adherence appears to be mediated via intercellular adhesion molecule (ICAM)-1 expression on the vascular endothelium and CD11b/CD18 expression on the leukocytes (7, 208). This process is mediated, at least in part, via leukotriene B_4 (10, 225), which also appears to contribute to the development of mucosal injury (86, 182). There is convincing evidence that tumor necrosis factor-α also contributes to the pathogenesis of NSAID-induced gastric damage (152), but this does not appear to be through effects of this cytokine on leukocyte adherence (8).

Neutrophils may trigger the endothelial injury that occurs very soon after administration of an NSAID (141, 206). Rats rendered neutropenic by treatment with an anti-neutrophil antibody did not exhibit endothelial damage following NSAID administration. Neutrophils are capable of inducing cell injury via release of a variety of reactive oxygen metabolites and proteases. Indeed, there is evidence consistent with a role of reactive oxygen metabolites in the pathogenesis of NSAID-induced gastropathy (39, 183).

As mentioned earlier, the beneficial effects of prostaglandins that have been demonstrated in humans are largely attributable to their ability to inhibit gastric acid secretion (21, 64). This underscores the importance of gastric acid in the pathogenesis of NSAID-induced gastric ulcers. Indeed, several studies have demonstrated that NSAIDs can elevate gastric acid secretion (43, 82, 107), although this appears to be an effect that is very much context dependent. For example, indomethacin increased basal but not pentagastrin-stimulated acid secretion in humans (43), while the same NSAID increased basal and stimulated acid secretion in rats, but only when the stomach was inflamed (15) (Fig. 4). Barnett et al. (15) demonstrated that it was the suppression of COX-1 by NSAIDs that resulted in elevated gastric acid secretion in the rat; that is, a nonselective COX inhibitor increased gastric acid secretion in rats with gastritis, but a selective COX-2 inhibitor had no effect (15) (Fig. 4).

The most compelling evidence in support of a role of acid in the pathogenesis of NSAID-induced ulceration comes from clinical trials of proton pump inhibitors (2, 31, 75, 153). For example, one recent study examined the effects of cotreatment for 6 mo with a proton pump inhibitor (esomeprazole) and an NSAID or selective COX-2 inhibitor in patients at high risk for ulceration (153). The incidence of ulceration in patients receiving placebo plus an NSAID or selective COX-2 inhibitor was 20.5%, while in the group receiving esomeprazole (40 mg), the ulcer incidence was only 4.7% ($P < 0.01$).

For many years it has been suggested that the strong contractions of the stomach that can be observed following administration of an NSAID play a role in the development of hemorrhagic lesions (126, 181). This effect occurred secondary to suppression of COX-1 by the NSAID (174). While it has been demonstrated that physical or pharmacological prevention of these contractions lessens the severity of mucosal injury (126, 181), the exact mechanism through which they might contribute to NSAID-induced gastropathy has not been clearly identified.

NSAID-induced ulcer bleeding is likely to be due, at least in part, to effects of these drugs on platelets. Thromboxane produced by the platelet is a potent stimulus for platelet aggregation and a potent vasoconstrictor. Thromboxane release is triggered during the clotting process (such as by collagen), and its synthesis occurs via COX-1. NSAIDs that suppress COX-1 can therefore suppress

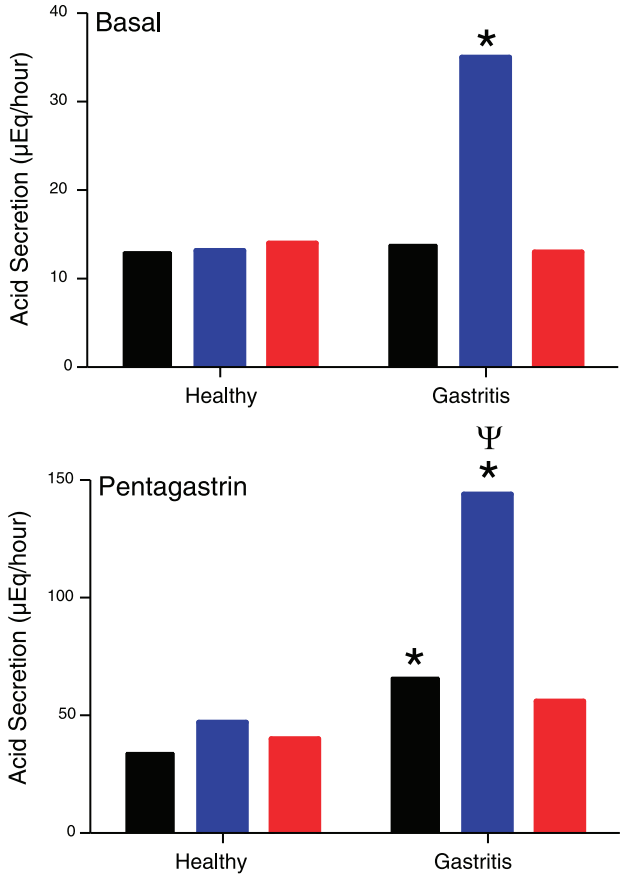

FIG. 4. Effects of inhibition of prostaglandin synthesis on basal and pentagastrin-stimulated acid secretion in healthy rats and rats with gastritis induced by iodoacetamide. Indomethacin (blue bars) suppresses both COX-1 and COX-2. It resulted in a significant increase in basal and pentagastrin-stimulated gastric acid secretion in rats with gastritis, but not in healthy rats. DuP-697 (red bars) is a selective inhibitor of COX-2. It had no effect on gastric secretion. Black bars represent the acid secretion in rats treated with vehicle. These results therefore suggest that prostaglandins derived from COX-1 have inhibitory effects on gastric acid secretion in the inflamed stomach, but no effect in the healthy stomach. *$P < 0.05$ versus corresponding healthy group. Ψ$P < 0.05$ versus corresponding vehicle-treated group. [Data from Barnett et al. (15).]

platelet thromboxane synthesis and thereby reduce the ability of platelets to aggregate. Thus the reduced gastric toxicity of selective COX-2 inhibitors can be attributed in part to their lack of inhibitory effect on platelet aggregation, and the resulting reduction in gastric (and intestinal) bleeding. Indeed, it has been argued that bleeding that was reduced in patients taking rofecoxib (23) and celecoxib (163) in large clinical trials was occurring distal to the ligament of Treitz, rather than from ulcers in the stomach or duodenum (125). Thus the absence of thrombocytopathy was suggested to be the primary benefit of selective COX-2 inhibitors in those studies (125). As outlined in more detail below, this conclusion is consistent with the observation that the benefits conferred by taking a selective COX-2 inhibitor versus a nonselective COX inhibitor are essentially lost with concurrent administration of low-dose aspirin. Somewhat ironically, the lack of inhibition of platelet thromboxane synthesis by selective COX-2 inhibitors is likely to have contributed significantly to the development of cardiovascular complications in patients taking those drugs (50).

The impairment of ulcer healing by NSAIDs is due in part to effects on platelets. Platelets make an important contribution to ulcer healing. Thrombocytopenic rats exhibit impaired ulcer healing, which can be restored by a transfusion of platelets from a healthy donor rat (118) (Fig. 5*A*). The beneficial effects of platelets on ulcer healing are likely related to the release of VEGF, which is a potent stimulus of new blood vessel growth (angiogenesis), an essential element in the ulcer healing process. Even oral administration of a suspension of platelets can accelerate ulcer healing in the rat, and this effect can be reversed by preincubation of the platelet suspension with an antibody directed against VEGF (202).

Treatment of rats with an NSAID results in a significant shift in the balance between serum pro- and antiangiogenic factors (specifically, decreased VEGF and increased endostatin) (118) (Fig. 5*B*). The same effect can be seen when rats are treated with a selective COX-2 inhibitor, consistent with aforementioned studies showing impaired ulcer healing in COX-2-deficient mice and in rodents treated with selective COX-2 inhibitors. The shift in the angiogenic balance was evident in experiments in which cultured human endothelial cells were exposed to the serum from rats treated with NSAIDs or with selective COX-2 inhibitors. In both cases, a decrease in endothelial cell proliferation and an increase in apoptosis were observed (117). These in vitro effects are consistent with in vivo observations of significantly reduced angiogenesis in the ulcer bed of rats treated with NSAIDs or selective COX-2 inhibitors (117, 118). They are also consistent with observations that COX-2-derived prostaglandins stimulate VEGF release from gastric fibroblasts (128).

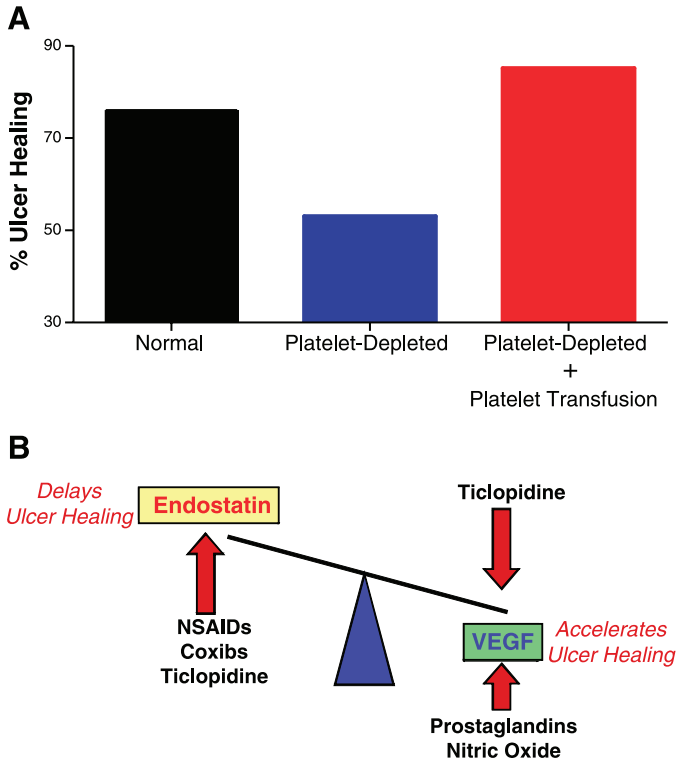

FIG. 5. Contribution of platelets to ulcer healing and angiogenesis in the rat. *A*: the importance of platelets to ulcer healing in the rat is shown by the reduced healing observed in rats immunodepleted of platelets, and the restoration of normal healing when thrombocytopenic rats are transfused with platelets from a healthy donor. [Data from Ma et al. (118).] *B*: effects of NSAIDs, coxibs, and ticlopidine (an ADP receptor antagonist) on platelet and serum levels of vascular endothelial growth factor (VEGF; proangiogenic) and endostatin (antiangiogenic). These drugs shift the balance of VEGF and endostatin such that the latter predominates, resulting in impaired angiogenesis and ulcer healing (117, 118).

VI. BOTH COX-1 AND COX-2 CONTRIBUTE TO GASTRIC MUCOSAL DEFENSE

The existence of multiple forms of prostaglandin synthase (cyclooxygenase) was suggested as early as 1972, with several studies thereafter providing supportive pharmacological evidence (51, 219). In 1991, the existence of a second isoform of COX (COX-2) was confirmed (224). COX-2 was subsequently found to be expressed in particularly high levels at sites of inflammation (184), while generally at low levels in healthy tissues, including the stomach. Selective inhibitors of COX-2 were expeditiously developed with the notion that they would inhibit inflammatory PG synthesis (thus reducing edema and pain), but not gastric PG synthesis (thus not causing ulceration).

While an attractive theory, particularly to pharmaceutical marketers, the expression of COX-1 and COX-2 turned out not to be quite so clearly divided as originally proposed. Selective COX-2 inhibitors, while in many stud-

ies have been found to produce less gastrointestinal injury than conventional NSAIDs (23, 79, 163), nevertheless do cause significant gastrointestinal injury (105). Moreover, their cardiovascular and renal toxicity was found to be comparable to (perhaps worse than) conventional NSAIDs (1, 50). This has contributed to several selective COX-2 inhibitors being withdrawn from the marketplace in recent years. Nevertheless, the development of the selective COX-1 and COX-2 inhibitors, as well as COX-1- and COX-2-deficient mice, has provided researchers with a set of useful tools to determine the contribution of these two enzymes to mucosal defense and repair.

While COX-1 is the predominant isoform expressed in the healthy gastric mucosa, COX-2 expression can be upregulated very rapidly (Fig. 5). For example, substantially increased COX-2 expression can be seen following exposure of the mucosa to an irritant (68), induction of ischemia (121) or when COX-1 activity is suppressed with aspirin (38). This upregulation of COX-2 appears to be a defensive and anti-inflammatory response aimed at enhancing mucosal defense (increased blood flow, reduction of leukocyte adherence, and activation). Thus, when COX-2 activity is inhibited in the face of one of the abovementioned challenges, the formation of mucosal erosions can be observed. For example, administration of a low dose of aspirin to rats does not cause hemorrhagic damage in the stomach, but does result in a rapid increase in COX-2 expression (47). If a selective COX-2 inhibitor is coadministered with the aspirin, extensive hemorrhagic damage develops. This phenomenon has also been observed in healthy human volunteers (49). Aspirin is a more potent inhibitor of COX-1 than of COX-2, so it is possible that the upregulation of COX-2 that is observed following aspirin administration results in elevated prostaglandin synthesis, via that isoform, which increases the resistance of the mucosa to injury. When a COX-2 inhibitor is administered together with aspirin, the "supplemental" prostaglandin synthesis from the induced COX-2 is removed, leading to diminished mucosal resistance to damage. Alternatively, it is possible that another mediator is produced via COX-2 which contributes to mucosal defense. This latter possibility is discussed below.

Further evidence for the importance of COX-2 in mucosal defense comes from studies utilizing selective COX-1 and COX-2 inhibitors. As outlined above, the development of selective COX-2 inhibitors was based in part on the notion that it is the inhibition of COX-1 by NSAIDs that accounts for gastric ulceration induced by these drugs. Indeed, this remains a commonly held belief, despite strong evidence to the contrary. Administration of selective COX-2 inhibitors to rats or mice does not result in mucosal injury. However, several animal studies have demonstrated that administration of selective COX-1 inhibitors, resulting in substantial suppression of gastric prostaglandin synthesis, does not result in mucosal injury (68, 177, 209). As was the case with low-dose aspirin, coadministration of a selective COX-2 inhibitor with a selective COX-1 inhibitor consistently results in the formation of hemorrhagic erosions in the stomach (209). Thus NSAID-induced gastric damage requires the inhibition of *both* COX-1 and COX-2. This is illustrated in Figure 3, which shows the dose-dependent effects of ketorolac on COX-1 and COX-2 activity, and on gastric prostaglandin synthesis, in parallel with the ability of this NSAID to cause gastric damage in the rat (209). Ketorolac is considered a COX-1 selective inhibitor (215). At the lower doses tested, this drug substantially inhibited systemic COX-1 activity (platelet thromboxane synthesis) and gastric prostaglandin synthesis, but did not affect COX-2 activity and did not induce gastric damage. It was only when doses of ketorolac were used that significantly inhibited COX-2 activity that gastric damage was elicited.

The notion that both COX-1 and COX-2 make important contributions to gastric mucosal defense is further supported by studies utilizing mice in which the gene for one of these isoforms has been disrupted. Even though COX-1-deficient mice have very low levels of gastric mucosal prostaglandin synthesis (106), they do not spontaneously develop gastric erosions, and actually show less susceptibility to NSAID-induced gastric injury than normal mice (162). On the other hand, COX-2-deficient mice develop erosions following administration of an NSAID, and actually demonstrate enhanced susceptibility to this damage compared with normal mice (106).

Interestingly, COX-2-deficient mice also exhibit an impaired capacity for inflammation to resolve, suggesting that COX-2 is an important source of anti-inflammatory mediators (199). One such group of anti-inflammatory substances that can be produced via COX-2 is the lipoxins (34, 159). Recent studies suggest that lipoxins also make an important contribution to gastric mucosal defense. As mentioned above, one of the possible explanations for the observation that coadministration of aspirin and a selective COX-2 inhibitor resulted in extensive gastric damage (47, 49) is that a COX-2-derived factor was contributing to the resistance of the mucosa to injury. Aspirin can covalently acetylate a serine residue of COX-1 leading to its permanent inactivation, in terms of metabolizing arachidonic acid to prostaglandins. However, the interaction of aspirin with COX-2 differs from that with COX-1 in a very important way. While aspirin still covalently acetylates a serine residue in COX-2, and still blocks the formation of prostaglandins, the acetylated COX-2 remains able to metabolize arachidonic acid to 15-*R*-hydroxyepitetraenoic acid. This substance can then undergo conversion via 5-lipoxygenase to form 15-epi-(*R*)-lipoxin A_4, also referred to as "aspirin-triggered lipoxin" (34). This lipoxin, like its epimer (lipoxin A_4; LXA_4), exerts many anti-inflammatory actions (172). Interestingly, it is also a very potent endog-

enous gastroprotective substance (47). Intraperitoneal administration of nanomolar doses of LXA_4 significantly reduced the severity of aspirin-induced damage in the rat stomach (47). LXA_4 is therefore very similar, in terms of potency as a gastroprotective substance, to prostaglandins of the E and I series (145). The rapid increase in COX-2 expression that can be observed in the stomach after administration of aspirin is accompanied by significant production of LXA_4 by the stomach. Coadministration of a selective COX-2 inhibitor with aspirin prevents the increase in gastric synthesis of LXA_4. The ability of LXA_4 to reduce gastric damage induced by NSAIDs may be related to the ability of this substance to suppress NSAID-induced leukocyte adherence (47), which has been shown to be a critical event in the pathogenesis of NSAID-induced gastric injury (198, 206, 208). The gastroprotective effects of lipoxins are produced via the "FPRL-1" receptor (137). Blockade of this receptor results in a significant augmentation of the gastric-damaging effects of aspirin (47). When the gastric mucosa is inflamed, there is greater expression of COX-2 and a greater contribution of COX-2-derived products to mucosal defense (166). Interestingly, annexin-1, a protein that participates in the resolution of inflammation and can activate the same receptor as LXA_4, has also been shown to exhibit significant protective effects in the stomach (227), and to contribute to the healing of experimental gastric ulcers and NSAID-induced mucosal erosions (124).

VII. FUTURE SOLUTIONS TO NSAID GASTROPATHY

The failure of selective COX-2 inhibitors to provide a solution to the problem of GI ulceration induced by anti-inflammatory agents has resulted in a renewed search for other strategies. This has included investigations into the efficacy of cotreatment with proton pump inhibitors, which offers a cost-effective and safe alternative for reducing NSAID gastropathy (2, 31, 75, 153). There remains strong interest in developing novel drugs that produce the desired anti-inflammatory and analgesic effects of NSAIDs without untoward effects on the GI tract as well as renal and cardiovascular systems.

A. Phosphatidylcholine-Conjugated NSAIDs

Surface-active phospholipids contribute to the mucosal "barrier" to acid back-diffusion, and as outlined above, NSAIDs have been shown to disrupt this barrier. Lichtenberger et al. (112) demonstrated that preassociation of NSAIDs with zwitterionic phospholipids did not disrupt the barrier properties of the surface-active phospholipid layer and did not cause gastric damage. Moreover, absorption of the NSAID was enhanced, contributing to improved antipyretic and anti-inflammatory activities (112). For example, in studies in rats, ibuprofen preassociated with phosphatidylcholine (PC) was found to be better than ibuprofen at reducing pain and inflammation and more effective at suppressing COX-2 activity and prostaglandin synthesis (111). The PC-associated aspirin also significantly accelerated the healing of ulcers in rats (101). In a human study, aspirin-PC produced significantly less gastric erosions after 3 days of administration compared with an equimolar dose of aspirin, but still suppressed gastric prostaglandin synthesis (6).

B. Terminal Prostaglandin Synthase Inhibitors

The cardiovascular toxicity of selective COX-2 inhibitors and possibly other NSAIDs has been suggested to be a consequence of the inhibition of the synthesis of prostacyclin (PGI_2), which has antithrombotic properties, to an extent greater than inhibition of thromboxane (TxA_2), which has prothrombotic properties (50). As PGE_2 is the prostaglandin primarily associated with inflammation, it has been suggested that the selective inhibition of PGE_2 synthesis could be a rational approach to reducing inflammation without producing the cardiovascular and GI toxicity associated with NSAIDs (150). COX-1 and COX-2 metabolize arachidonic acid to PGH_2, which can then be converted via the action of several terminal prostaglandin synthases into the various species of prostanoids (e.g., PGE_2, PGI_2, TxA_2). Selective inhibition of PGE_2 synthesis at sites of inflammation may be achievable through the inhibition of one of the PGE synthases, namely, mPGES-1. This enzyme, like COX-2, is regarded as inducible, and it preferentially metabolizes PGH_2 derived from COX-2 (136, 161). Selective inhibitors of mPGES-1 are in development by several pharmaceutical companies, but their effects have not been extensively reported as yet. However, studies of mice genetically deficient of this isomerase provide some interesting insights. Cheng et al. (32) reported that the deletion of mPGES-1 did not affect thrombogenesis or blood pressure, consistent with the notion of sparing of prostacyclin (PGI_2) synthesis. As expected, they observed decreased PGE_2 synthesis, but interestingly, an increase in prostacyclin synthesis was observed in these mice. This suggests that a shunting of PGH_2 down at least one other terminal synthase pathway occurred. This could have therapeutic implications if it occurs when a selective inhibitor of mPGES-1 is used on a chronic basis; for example, prostacyclin has been implicated as a mediator of pain (132). On the other hand, elevated production of prostacyclin could provide benefit through its potent antithrombotic effects.

Shinji et al. (161) reported that a nonselective inhibitor of mPGES-1 (MK-886, which also inhibits leukotriene synthesis) inhibited interleukin-1β-stimulated mPGES-1

activity in vitro and suppressed VEGF production by gastric fibroblasts. This latter finding is potentially important given the very important role of VEGF in the healing of gastric ulcers (118, 202). VEGF release is stimulated by prostaglandins produced via COX-2 and mPGES-1 (128). Thus there is a strong possibility that selective inhibition of mPGES-1 would produce the same delay in ulcer healing that has been observed with selective COX-2 inhibitors.

C. Gaseous Mediator-Releasing NSAIDs

Attempts were made in the 1980s to develop stable prostaglandin analogs for the prophylaxis of NSAID-induced GI injury, but these drugs did not enjoy a great deal of success for this indication because of a high rate of adverse effects (most notably diarrhea). The approach of supplementing endogenous protective mediators to counteract the detrimental effects of NSAIDs remains an attractive strategy. Nitric oxide and hydrogen sulfide are endogenous gaseous mediators that produce many of the same effects as prostaglandins in the GI tract (46, 127). For example, they are both vasodilators and potent inhibitors of leukocyte adherence to the vascular endothelium (45, 226). Inhibition of mucosal synthesis of nitric oxide or H_2S renders the stomach more susceptible to the damaging effects of NSAIDs and impairs the healing of preexisting damage (40, 45, 96, 116, 200, 220). Administration of nitric oxide or H_2S donors increases the resistance of the gastric mucosa to injury induced by NSAIDs and other noxious substances (45, 120, 220) and can accelerate healing of ulcers in rodents (40, 96). At least in the case of nitric oxide, the latter effect may be due to a stimulatory effect of nitric oxide on release of VEGF (117), which can promote angiogenesis and ulcer healing (Fig. 5*B*).

These properties of nitric oxide and H_2S make them attractive candidates for "coupling" to NSAIDs so as to reduce GI toxicity. As nitric oxide and H_2S are also potent anti-inflammatory agents (127, 187), there is the possibility of enhancing anti-inflammatory activity of NSAIDs (and other drugs) when coupling them to gaseous mediator-releasing moieties. In the case of nitric oxide-releasing NSAIDs (also called CINODs; COX-inhibiting nitric oxide donors), there is evidence that these drugs produce significantly less gastrointestinal injury than the parent NSAIDs, both in animal studies (190, 205, 211, 212) and in human clinical trials (48, 74, 221). One of the CINODs, naproxcinod (155), is now in advanced phase 3 clinical trials. These trials are focused on confirming the increased cardiovascular safety of this drug compared with conventional NSAIDs and coxibs.

Less information is available on H_2S-releasing NSAIDs, but the data that are available suggest that these compounds are promising. An H_2S-releasing derivative of diclofenac was found not to cause gastric damage, and to cause >90% less intestinal damage than equimolar doses of diclofenac. Similar results have been reported for an H_2S-releasing indomethacin derivative (187). Interestingly, an H_2S-releasing derivative of diclofenac exhibited significantly greater anti-inflammatory effects than the parent drug, including the ability to significantly inhibit inflammatory cytokine expression/synthesis (109, 200). This may be related to the reported ability of H_2S-releasing drugs to inhibit the activation of NFκB (109), which has been implicated in the pathogenesis of NSAID-induced gastropathy (26). An H_2S-releasing salicylate derivative significantly improved the healing of established gastric ulcers in the rat (203).

In the aftermath of the withdrawal of several selective COX-2 inhibitors (rofecoxib, lumiracoxib, etoricoxib, paracoxib), regulatory agencies will be increasingly examining the cardiovascular safety of new anti-inflammatory agents, perhaps even more so than their gastrointestinal safety. It is noteworthy, in this regard, that both nitric oxide- and H_2S-releasing NSAIDs have been shown to protect the heart from ischemia/reperfusion injury in animal models (147, 148), in sharp contrast to the detrimental effects of selective COX-2 inhibitors (149).

VIII. SUMMARY

Significant advances have been made over the past two decades in understanding the pathogenesis of NSAID-induced injury and bleeding in the gastrointestinal tract. While still not completely understood, the identification of key events in the development of ulcers after NSAID administration has provided important clues as to how to design new anti-inflammatory drugs with greater margins of safety. Of course, the gastrointestinal tract is not the only organ that can be adversely affected by NSAIDs. The withdrawal of rofecoxib from the market led to increased attention on the cardiovascular risks associated with the use of the entire NSAID class. With any drug, one must look beyond the most obvious adverse effects and always consider the entire scope of risks associated their use, viewed in the context of the benefits that the drug delivers.

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