Mechanisms of Protection and Healing: Current Knowledge and Future Research

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The resistance of the gastric mucosa to injury is attributable to a series of factors collectively known as "mucosal defense." Many components of mucosal defense are regulated by prostaglandins and nitric oxide (NO). Thus, inhibition of the production of these mediators predisposes the stomach to injury. Administration of these agents, as synthetic prostaglandins or NO donors, can restore mucosal defense and thereby prevent damage induced by several irritants. Repair of gastric ulcers is also influenced by NO and prostaglandins. Furthermore, a variety of growth factors appear to play critical roles in stimulating the formation of granulation tissue (the "foundation" for repair), the formation of new blood vessels, and the proliferation of epithelial cells. Better understanding of the factors that regulate ulcer healing should provide clues for the development of drugs that can produce better "quality" healing of ulcers. *Am J Med.* 2001;110(1A):19S–23S.

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain among the most commonly used pharmacologic agents.[1] The ability of NSAIDs to cause gastrointestinal (GI) ulceration was first demonstrated more than 60 years ago,[2] and several attempts have been made to produce NSAIDs without these adverse effects. However, the use of NSAIDs for their anti-inflammatory and analgesic properties has remained limited by their toxicity in the GI tract and kidney.[3] In the past, one of the major problems with the attempts to produce GI-sparing NSAIDs has been that both the detrimental and desired effects of NSAIDs are linked to the ability of these agents to suppress prostaglandin synthesis. Reducing the ability of a compound to suppress prostaglandin synthesis also reduces its efficacy as an anti-inflammatory drug. Reducing the time of contact between the compound and the luminal surface of the GI tract—through enteric coating, formulation as a prodrug, or parenteral administration—does not influence the ability of the drug to suppress prostaglandin synthesis systemically and therefore may not significantly alter its ability to induce ulcers.[4–9] The recently developed selective inhibitors of cyclooxygenase (COX)-2 appear to have reduced ulcerogenic effects in the stomach but are not devoid of toxicity in the gut or elsewhere. In particular, these drugs appear to share at least some of the same toxicity in the kidney as is seen with conventional NSAIDs. Moreover, selective COX-2 inhibitors have been shown in animal models to delay the healing of gastric ulcers and exacerbate colitis to the same extent as conventional NSAIDs.[10–13] Thus, there remains a need for anti-inflammatory drugs that spare the stomach and intestine injury and do not interfere with healing.

MECHANISMS OF MUCOSAL PROTECTION

The ability of the gastric mucosa to resist injury by endogenous secretions (acid, pepsin, and bile) and by ingested irritants (e.g., alcohol, NSAIDs) can be attributed to a number of factors that have been collectively referred to as "mucosal defense"[14] (**Figure 1**). Acid itself can in some senses be viewed as the first line of mucosal defense, because it is important for reducing the possibility of bacterial colonization of the stomach and therefore the entry of bacteria into the systemic circulation when there is a breach in the gastric epithelium. Likewise, the mucus secreted onto the luminal surface plays an important role in

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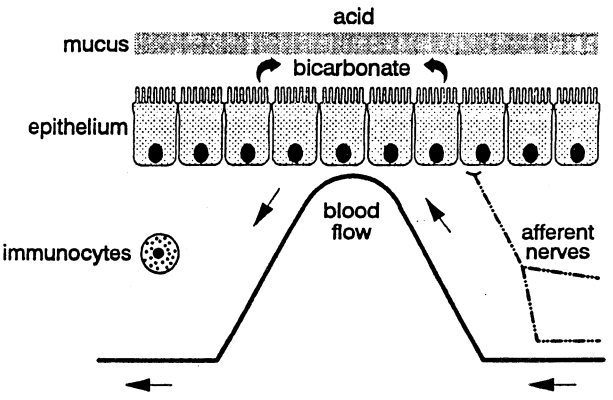

Figure 1. Schematic diagram illustrating some of the key components of mucosal defense. Extramucosal defense consists of acid, mucus, and bicarbonate, all of which are secreted by the mucosa. The epithelium is very resistant to damage by acid and pepsin, and can undergo very rapid and efficient repair when damage occurs. Sensory afferent nerves underlying the epithelium are sensitive to acid and can trigger a rapid increase in mucosal blood flow. Immunocytes within the mucosa release a variety of vasoactive and chemotactic factors, which can coordinate an acute inflammatory response to injury. These cells are also important sources of many growth factors that regulate ulcer healing.

preventing bacterial colonization and translocation. Mucus also plays an important role in prevention of mechanical injury to the epithelium and in providing a microenvironment over sites of superficial injury in which rapid repair (restitution) can occur.[15] Mucus, in combination with bicarbonate secreted by the surface epithelial cells, has long been thought to play a key role in protecting the gastric epithelium from damage induced by acid and pepsin,[16] but this remains controversial.[17] The secretion of both mucus and bicarbonate are to some extent regulated by means of prostaglandin synthesis (**Table 1**). Thus, NSAIDs can reduce the secretion of both of these factors and thereby increase the susceptibility of the mucosa to injury. The epithelium itself is adapted in ways not completely understood such that it is very resistant to damage induced by acid.[18] Moreover, the rapid turnover of the epithelium and the ability for repair of a damaged epithelium to occur very rapidly further contribute to the resistance of the mucosa to injury.

One of the most important components of mucosal defense is the mucosal blood flow response to irritants. When acid or other irritants enter the subepithelial compartment, sensory afferent neurons are able to trigger a rapid increase in mucosal blood flow that allows the buffering of acid and the rapid removal of toxic substances, thus limiting their penetration into deeper layers of the mucosa.[14] The sensory afferents, when appropriately stimulated, release calcitonin gene–related peptide (CGRP) in the vicinity of the submucosal arterioles.[19] CGRP is able to dilate these vessels, through a nitric oxide (NO)–mediated pathway, and thereby cause a rapid increase in mucosal blood flow. Ablation of the sensory afferent neurons (through administration of the neurotoxin capsaicin) results in the abolishment of the "reactive hyperemic" response to topical irritants and greatly increases the susceptibility of the gastric mucosa to injury.[20] Inhibition of NO synthesis also abolishes the reactive hyperemic response and greatly increases the susceptibility of the stomach to injury.[21] The hyperemic response is also very important in the restitution process. By trapping plasma over sites of damage[22] to create a microenvironment with a high pH, mucus also contributes to circumstances that are conducive to repair.[22] A constant delivery of plasma from the subepithelial blood vessels is crucial to the maintenance of a repair-conducive microenvironment. Even a very brief interruption of blood flow results in a rapid decrease in the pH at the site of injury, leading to disruption of the repair process and progression of damage to deeper layers of the mucosa.[22] In addition to NO, prostaglandins appear to be important in the maintenance of blood flow during the restitution process. Administration of NSAIDs has been shown to reduce mucosal blood flow[23–26] and thereby reduce the pH in the sites overlying epithelial damage, ultimately leading to inhibition of restitution and the development of hemorrhagic lesions.[22] On the other hand, administration of prostaglandins can prevent these detrimental effects of NSAIDs.[22]

When the superficial levels of mucosal defense fail or are overwhelmed by a luminal insult, the next level of mucosal defense that is called into play is the acute inflammatory response. Neutrophils are recruited from the circulation to the sites of injury to facilitate repair and to reduce the entry of microbes into the systemic circulation. The process of neutrophil recruitment has been described in detail elsewhere.[27] Briefly, the release of chemotactic factors (e.g., leukotriene B_4, platelet-activating factor) from mucosal immunocytes, such as mast cells and macrophages, is the key signal that leads to the extravasation of neutrophils with migration to the site of

Table 1. Effects of Prostaglandins and Nitric Oxide (NO) on Gastric Mucosal Protection and Healing

Effect	Prostaglandins	Nitric Oxide	References
Increase and/or maintain mucosal blood flow	Yes	Yes	21, 61
Stimulate mucus secretion	Yes	Yes	62, 63
Inhibit neutrophil adherence and activation	Yes	Yes	29, 31
Able to protect the stomach against ulcerogenic agents	Yes	Yes	42–44
Suppression of synthesis increases mucosal susceptibility to damage	Yes (suppression of synthesis by NSAIDs)	Yes (suppression of synthesis by NO synthase inhibitors)	21, 64

NSAIDs = nonsteroidal anti-inflammatory drugs.

injury. In addition to removing damaged cells, foreign matter, and microbes, neutrophils also participate in the formation of granulation tissue, which is critical to the repair process (discussed in more detail below). Interestingly, both prostaglandins and NO exert inhibitory effects on neutrophil adherence, whereas NSAIDs and inhibitors of NO synthesis increase neutrophil adherence to the vascular endothelium.[28–32] Moreover, prostaglandins and NO can downregulate the release of inflammatory mediators from mast cells and macrophages.[33–41] Nevertheless, the net effect of prostaglandins and NO to mucosal defense is protective, and exogenously applied NO or prostaglandins have potent protective effects.[42–44] These mediators are also important in promoting the repair of established ulcers (see below).

MECHANISMS AND REGULATION OF ULCER HEALING

By definition, an ulcer penetrates through the muscularis mucosae into the underlying submucosa and sometimes to the muscularis externae. Repair of such damage requires the complete reestablishment of a connective tissue "foundation," the re-formation of glandular architecture, and the growth of new blood vessels (see Jones et al[45] for a recent review). Infiltration of the ulcer bed by granulocytes is important for minimizing the translocation of bacteria from the lumen, but these cells also play a key role in forming the "granulation tissue" in which new blood vessel growth occurs. This is a poorly understood process, but it is clear that administration of agents that interfere with the infiltration of granulocytes (e.g., corticosteroids) results in inhibition of repair. Angiogenesis within the granulation tissue is essential for the creation of new glands and for providing blood flow to the reestablishing epithelium.[45] Angiogenesis is stimulated by means of a variety of growth factors, many of which are secreted by neutrophils, mast cells, and fibroblasts within the granulation tissue. Some examples of key growth factors involved in ulcer repair are heparin, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF). Production of several of these growth factors can be found to be upregulated in the presence of an ulcer. For example, when an ulcer is present in the stomach, VEGF is upregulated in granulocytes, fibroblasts, and the regenerated epithelium.[46] Upregulation of receptors for some of these growth factors, such as the EGF receptor, has been demonstrated to occur in the cells lining the ulcer margin.[47] The ulcer repair process can be accelerated through administration of these growth factors. For example, heparin has recently been shown to accelerate gastric ulcer healing in the rat, independently of its anticoagulant activity.[48] Similarly, EGF and bFGF have been shown to accelerate ulcer healing.[47,49,50] Rapid proliferation of the epithelial cells occurs at the margin of the ulcer, and these cells gradually occupy the space over the granulation tissue. A new cell lineage can be observed at the ulcer margin that secretes EGF.[51]

As is the case in rapid reepithelialization, blood flow at the ulcer margin is essential for the formation of a new epithelium over the denuded granulation tissue. Reduction of blood flow at the ulcer margin, as can be seen when animals are exposed to cigarette smoke or are given NSAIDs or inhibitors of NO synthesis, results in impaired ulcer healing.[52–54]

Ulcer healing can also be affected by luminal factors. In animal models, bacteria that are ingested with meals seem to be particularly important: ulcers in the rat stomach are rapidly colonized, particularly by gram-negative bacteria.[55] These bacteria retard ulcer repair through mechanisms not yet fully understood. Thus, treatment with antibiotics that significantly reduce the extent of colonization by bacteria resulted in acceleration of ulcer healing.[55] This raises the interesting possibility that bacteria other than *Helicobacter pylori* may influence the natural history of ulcers in humans.

As in the case of mucosal protection, prostaglandins and NO appear to play important roles in regulating ulcer healing. Inhibition of prostaglandin or NO synthesis impairs the healing of experimental ulcers in rats,[54,56] whereas administration of prostaglandins or NO donors can significantly accelerate ulcer healing.[54,57,58] NSAIDs have well-characterized inhibitory effects on ulcer healing in humans.[59,60] With respect to the role of prostaglan-

dins in ulcer healing, some recent reports suggest that COX-2 is particularly important in this process.[10–12] Selective inhibition of COX-2 leads to impaired ulcer healing.[10–12] Derivatives of NSAIDs that release NO have been suggested to be an alternative to selective COX-2 inhibitors for use in patients with preexisting ulcers, because these "NO–NSAIDs" do not interfere with ulcer healing and, in some cases, can accelerate ulcer healing.[12,57]

FUTURE DIRECTIONS

The key to developing better strategies for accelerating ulcer healing is the identification and characterization of the growth factors and mediators that play key roles in the ulcer repair process. Much progress in this effort has been made over the past two decades, but this has not yet been translated to a marketed agent for accelerating ulcer healing per se. Another important area for future research is to better understand how we can improve the quality of ulcer healing. In other words, can ulcers be healed in such a way that they are less likely to recur? Part of this latter approach will involve gaining a better understanding of the long-term consequences of ulcers on the gastric mucosa. For example, do ulcers alter mucosal defense in an "irreversible" manner such that the tissue is more prone to recurrent tissue injury? It is possible that in the future we will be able to use a range of agents to heal ulcers, irrespective of the factor(s) that caused the ulcer to form, such that ulcers no longer recur.

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