

Pathophysiology of Gastric Ulcer Development and Healing: Molecular Mechanisms and Novel Therapeutic Options

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1. Introduction

The stomach plays a pivotal role in the digestion of foods that we eat. With the exception of rare cases, this organ can resist to a large variety of noxious factors, including hydrochloric acid, refluxed bile salts and alcohol, with a wide range of temperatures and osmolality. This high resistance to injuries depends on a number of physiological responses elicited by the mucosal lining against potentially harmful luminal agents, as well as to the ability of rapidly repairing the mucosal damage when it does occur (Laine et al., 2008). Nevertheless, when these protective mechanisms are overwhelmed by injurious factors, a gastric mucosal lesion may develop. Major detrimental effects on gastric mucosa are exerted by non-steroidal anti-inflammatory drugs (NSAIDs). These drugs are able not only to exert gastric injuring effects, but also to delay the healing of ulcer lesions through a variety of local and systemic mechanisms (Musumba et al., 2009).

Since the discovery that prostaglandin biosynthesis could be inhibited by NSAIDs through the blockade of cyclooxygenase enzymes, there has been a great interest in the contribution of prostaglandins to the mechanisms of gastric mucosal defense. Thus, it has been appreciated that these lipidic mediators are able to modulate virtually every factor involved in mucosal protection, and the importance of this contribution is made evident by the increased susceptibility of the stomach to injury following the intake of NSAIDs. Indeed, chronic treatments with these drugs can be associated with the development of ulcers in the stomach, and research over the past two decades has helped to identify some of the key events, triggered by cyclooxygenase blockade, which take part to ulcer formation and/or impairment of ulcer healing. Since many years, it has been recognized that NSAIDs can interfere with gastric mucosal physiology also through injuring mechanisms unrelated to the inhibition of prostaglandin biosynthesis, such as oxidative stress and changes in epithelial cell proliferation/apoptosis balance.

Following the discovery of two isoforms of cyclooxygenase (COX-1 and COX-2), and based on the assumption that COX-2 was an inducible enzyme responsible for inflammation, but devoid of gastroprotective functions (Vane et al., 1998), selective COX-2 inhibitors (coxibs, including celecoxib, rofecoxib, valdecoxib, parecoxib, etoricoxib and lumiracoxib) were

clinically developed as novel anti-inflammatory/analgesic drugs characterized by reduced gastric toxicity (Dubois et al., 2004). These advances have then fostered intensive preclinical and clinical research supporting the view that coxibs may confer advantages over conventional non-selective NSAIDs in terms of gastrointestinal risk reduction. Nevertheless, there are still a number of unresolved issues in this field, and the criteria for an appropriate use of coxibs in patients with various degrees of gastrointestinal risk, including ongoing gastric ulcerations, remain matter of discussion.

Another relevant topic, regarding the integrity of gastric mucosa, is represented by the use of proton pump inhibitors (PPIs). These drugs have been proven not only to prevent NSAID-induced upper gastrointestinal injury, but also to promote the healing process once the damage has occurred, even in the presence of a continued NSAID administration. The beneficial effects of PPIs can be largely ascribed to their ability to maintain a sustained inhibition of gastric acid secretion. However, there is also evidence to suggest that pharmacodynamic properties unrelated to acid inhibition may contribute to the therapeutic actions of these drugs (Blandizzi et al., 2008).

Recent research has highlighted the fact that, beside prostaglandins, gastric mucosal protective functions can be accomplished by other mediators, with particular regard for the gaseous mediators nitric oxide (NO) and hydrogen sulfide (H₂S). Moreover, anti-inflammatory drugs endowed with dual cyclooxygenase/5-lipoxygenase inhibitory effects, such as licofelone, could represent novel therapeutic strategies helping to drive the development of safer anti-inflammatory drugs and effective therapies to accelerate and improve the quality of ulcer healing (Blandizzi et al., 2009).

This chapter is focused on the available evidence on the molecular mechanisms underlying the pathophysiology of gastric injury development and healing, as well as on novel therapeutic options for prevention and treatment of gastric ulcers.

2. Mechanisms of gastric mucosal defense

The mechanisms of gastric mucosal defense include several local and neurohormonal protective factors, which allow the mucosa to resist against frequent exposures to damaging factors (Laine et al., 2008). In the following sections, a detailed description of the mucosal defense mechanisms is provided.

2.1 Local mechanisms of gastric mucosal defense

2.1.1 Mucus-bicarbonate-phospholipid barrier

The first line of gastric mucosal defense is represented by the mucus-bicarbonate-phospholipid barrier (Lichtenberger, 1999). The surface of gastric mucosa is covered by a layer formed by mucus gel, bicarbonate anions and surfactant phospholipids. This unstirred layer is capable of retaining the bicarbonate ions secreted by surface epithelial cells and maintaining a microenvironment with a pH near to 7 at the mucus-mucosa interface. The mucus layer is also able to prevent the penetration of pepsin, thus avoiding the proteolytic digestion of epithelium (Allen and Flemstrom, 2005). In addition, the luminal surface of mucus gel is covered by a film of surfactant phospholipids which confers hydrophobic properties to the mucus layer (Lichtenberger, 1999).

The mucus gel is secreted by surface epithelial cells and is formed by a large amount of water (about 95%) and various kinds of mucin glycoproteins (i.e., MUC2, MUC5AC, MUC5B and MUC6), the production of which may vary in different regions of the gastric

mucosa (Allen and Flemstrom, 2005; Ho et al., 2004). Gel-forming mucin units polymerize into large mucin multimers, which are essential for gel formation. The mucus gel is secreted along with low-molecular weight trefoil factor (CRF) family (TFF) peptides, which play a relevant role in the formation of the mucus layer (Newton et al., 2000). For example, TFF2 is known to increase the viscosity of gastric mucin and stabilize the gel network (Thim et al., 2002). The secretion of gastric mucus is regulated also by various gastrointestinal hormones, including gastrin and secretin, as well as prostaglandins and acetylcholine (Allen and Flemstrom, 2005).

The secretion of bicarbonate into the mucus gel layer is essential to maintain a pH gradient at the epithelial surface, which represents a first line of defense against gastric acid (Allen and Flemstrom, 2005). Bicarbonate secretion from the apical membrane of surface epithelial cells is mediated by a $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger, and it is stimulated by various factors, including prostaglandins (via EP_1 receptors), luminal acid, corticotrophin-releasing factor, melatonin, uroguanylin and orexin A (Allen and Flemstrom, 2005; Montrose et al., 2006).

The mucus-bicarbonate barrier is the only system which segregates the epithelium from the gastric lumen. Therefore, when this protective barrier breaks down during pathological events or upon detrimental actions by injuring agents, a second line of protective mechanisms comes into play. They include intracellular acid neutralization, rapid epithelial repair, and maintenance of mucosal blood flow.

2.1.2 Epithelial cells

The continuous layer of surface epithelial cells represents the next line of mucosal defense. This epithelial tissue is responsible for the production of mucus, bicarbonate and other components of the gastric mucosal barrier. These cells are hydrophobic in nature, being able to repel acid- and water-soluble injuring agents, owing to the presence of phospholipids on their surface (Lichtenberger, 1999). Surface epithelial cells are also closely interconnected by tight junctions, forming a continuous barrier, which prevents back diffusion of acid and pepsin (Allen and Flemstrom, 2005). Another relevant protective factor, available in the epithelial cells, is represented by heat shock proteins, which are activated in response to stress, including temperature increments, oxidative stress and cytotoxic agents (Tanaka et al., 2007). These proteins can prevent protein denaturation and protect cells against injury. Cathelicidin and beta-defensin are cationic peptides which play a relevant role in the innate defensive system at the mucosal surface, preventing bacterial colonization (Yang et al., 2006). In addition, TFFs secreted by epithelial cells regulate the re-epithelization process and exert mucosal protective actions (Taupin and Podolsky, 2003).

2.1.3 Mucosal cell renewal

The integrity of gastric epithelium is maintained by a continuous process of cell renewal ensured by mucosal progenitor cells. These cells are subjected to a continuous, well coordinated and controlled proliferation, which ensures the replacement of damaged or aged cells on the epithelial surface. The process of complete epithelial renewal takes about 3-7 days, while the overall glandular cell replacement requires months. However, the restitution of surface epithelium after damage occurs very quickly (i.e., few minutes) and results by migration of preserved cells located in the neck area of gastric glands (Laine et al., 2008).

The process of cell turnover is regulated by growth factors. In particular, a marked expression of epidermal growth factor receptor (EGF-R) has been detected in gastric progenitor cells. Such a receptor can be activated by mitogenic growth factors, such as

transforming growth factor- α (TGF- α) and insulin-like growth factor-1 (IGF-1) (Nguyen et al., 2007). In addition, PGE₂ and gastrin are able to transactivate the EGF-R and promote the activation of mitogen-activated protein kinase (MAPK) pathway, with consequent stimulation of cell proliferation (Pai et al., 2002). Notably, the presence of EGF has not been detected in the normal mucosa, although it is contained in the gastric juice, as a product of salivary and esophageal glands, and can stimulate mucosal cell proliferation in case of injury (Milani and Calabrò, 2001). In addition, mucosal progenitor cells do express survivin, an antiapoptotic factor, which inhibits apoptotic cell death (Chiou et al., 2005).

2.1.4 Mucosal blood flow

Mucosal blood flow is essential to deliver oxygen and nutrients and to remove toxic metabolites from gastric mucosa. Arteries embedded into the muscularis mucosae branch into capillaries, which then enter the lamina propria and travel toward the proximity of glandular epithelial cells. Endothelial cells, lining these microvessels, produce NO and prostacyclin (PGI₂), which act as potent vasodilators, thus protecting the gastric mucosa against damage and counteracting the detrimental effects of various vasoconstrictors, including leukotriene C₄, thromboxane A₂, and endothelin. In addition, NO and PGI₂ maintain the viability of endothelial cells and inhibit platelet and leukocyte adhesion to the microvasculature, thus preventing the occurrence of microischaemic phenomena (Laine et al., 2008).

When the gastric mucosa is exposed to irritants or acid back-diffusion, a massive and rapid increase in mucosal blood flow occurs. This process allows removal and dilution of back-diffusing acid or noxious agents. The increase in blood flow is regarded as a pivotal mechanism for preventing gastric mucosal cell injury, and its decrease results in the development of tissue necrosis. The increase in mucosal blood flow is mediated by NO release, and there is experimental evidence demonstrating that NO protects the gastric mucosa against injury induced by ethanol or endothelin 1, while the inhibition of NO synthase enhances mucosal injury (Holzer, 2006). It has been also observed that another endogenous compound, H₂S, can exert protective actions against gastric mucosal injury. In particular, this compound has been shown to reduce the expression of tumor necrosis factor α (TNF- α), to decrease leukocyte adhesion to vascular endothelium, and to prevent NSAID-induced gastric mucosal damage (Fiorucci et al., 2006).

2.1.5 Sensory innervation

The vasculature of gastric mucosa and submucosa is innervated by extrinsic primary afferent sensory neurons, which are arranged in a plexus at the base of the mucosal layer (Holzer, 2007). The nerve fibers stemming from this plexus run along with capillary vessels and reach the basal membrane of surface epithelial cells. These nerves can detect luminal acidity or back-diffusing acid through acid-sensing channels. The activation of such sensory nerves modulates the contractile tone of submucosal arterioles, thus regulating the mucosal blood flow. In particular, the stimulation of sensory nerves leads to the release of calcitonin gene-related peptide (CGRP) and substance P from nerve terminals surrounding large submucosal vessels (Holzer, 2007). CGRP then contributes to the maintenance of mucosal integrity through the vasodilation of submucosal vessels mediated by NO release. Sensory innervation plays a prominent role in the protection of gastric mucosa from injury, as demonstrated by studies where the ablation of sensory transmission (i.e., with capsaicin)

impaired the vasodilatory response and increased the sensitivity of gastric mucosa to injuring agents (Holzer, 2007).

2.1.6 Prostaglandins

The gastric mucosa represents a source of continuous prostaglandin production, such as PGE₂ and PGI₂, which are regarded as crucial factors for the maintenance of mucosal integrity and protection against injuring factors (Halter et al., 2001; Brzozowski et al., 2005a). It has been demonstrated that prostaglandins have the potential to stimulate almost all the mucosal defense mechanisms. In particular, they reduce acid output, stimulate mucus, bicarbonate and phospholipid production, increase mucosal blood flow, and accelerate epithelial restitution and mucosal healing (Brzozowski et al., 2005a). Prostaglandins are also known to inhibit mast cell activation as well as leukocyte and platelet adhesion to the vascular endothelium (Halter et al., 2001; Brzozowski et al., 2005a). The beneficial actions exerted by PGE₂ have been shown to be mediated by activation of specific EP receptor subtypes. In particular, the activation of EP₁ receptors mediates the most important protective effects of prostaglandins, through an increase in bicarbonate secretion and mucosal blood flow in the damaged mucosa and a decrease in gastric motility (Takeuchi et al., 2002). Other EP receptor subtypes are also involved in the protective actions of PGE₂. For example, EP₃ receptors inhibit the gastric acid secretion, while EP₄ receptors stimulate the secretion of mucus (Kato et al., 2005).

2.2 Neurohormonal mechanisms

Gastric mucosal defense is supported by mechanisms activated, at least in part, by the central nervous system and hormonal factors (Laine et al., 2008). Experimental studies have demonstrated that central vagal activation stimulates mucus secretion and increases intracellular pH in the surface epithelial cells of in the stomach. In addition, while the CRF pathway is involved in endocrine responses to stress (Chatzaki et al., 2006). In addition, peripheral CRF contributes significantly to the regulation of gastric defense mechanisms, in particular, the CRF2 receptor is known to mediate antiapoptotic effects in gastric epithelial cells as well as to inhibit gastric emptying and motility (Chatzaki et al., 2006).

Other hormone mediators, including gastrin-17, cholecystokinin, thyrotropin-releasing hormone, bombesin, EGF, peptide YY and neurokinin A, play significant roles in the regulation of gastric protective mechanisms, which can be blunted by afferent nerve ablation, CGRP receptor blockade, and inhibition of NO synthase (Peskar, 2001; Moszik et al., 2001). Ghrelin, a hormone peptide produced by gastric A-like cells in rodents and P/D1 cells in humans, is involved in the regulation of growth hormone secretion and appetite stimulation (Brzozowski et al., 2005b). Moreover, it is also able to exert significant protective effects at gastric level, including the enhancement of mucosal blood flow via stimulation of NO and CGRP release from sensory afferent nerves (Brzozowski et al., 2005b).

Glucocorticoids have been shown to support the mechanisms of protection at gastric level. These hormones are involved in the response to stress, and represent potent gastroprotective factors against injury (Filaretova et al., 1998). Consistently with this contention, glucocorticoid antagonists enhanced the severity of stress-induced erosions, further supporting a protective role of these hormones during stress (Filaretova et al., 2001). The mechanisms through which glucocorticoids exert their protective effects include the maintenance of glucose homeostasis, the increase in mucosal blood flow and mucus

secretion, and the attenuation of both enhanced gastric motility and microvascular permeability (Filaretova et al., 2007).

3. Mechanisms of gastric mucosal damage

Gastric mucosal injury may occur as a consequence of various conditions, including alcohol intake, refluxed bile salts, stress, aging and *Helicobacter pylori* infection, although the most important agents known to impair the mechanisms of gastric mucosal defense are represented by NSAIDs. For this reason, in the following sections a detailed description of NSAID-related mechanisms of gastric injury is provided.

3.1 Effects of NSAIDs on gastric mucosa

The pathophysiology of gastric injury associated with NSAID administration depends partly on cyclooxygenase inhibition and partly on cyclooxygenase-independent mechanisms, which result mainly from local direct actions (Scarpignato and Hunt, 2010). Cyclooxygenase blockade has been shown to increase the susceptibility of gastric mucosa to NSAID-induced injury by suppression of a number of prostaglandin-mediated protective functions. For instance, prostaglandins reduce the activation of neutrophils and the local release of reactive oxygen species (ROS). The production of prostacyclin by the endothelium of mucosal microcirculation is also highly relevant in ensuring a tonic inhibition of neutrophil adhesion. Therefore, NSAIDs can shift the mucosal balance toward the recruitment and endothelial adhesion of circulating neutrophils through the inhibition of prostaglandin biosynthesis (Whittle, 2002). Once adhered, neutrophils clog the microvasculature causing a local decrease in mucosal blood flow and a marked release of tissue damaging factors, including proteolytic enzymes and leukotrienes, which enhance the vascular tone, exacerbate tissue ischaemia, stimulate the production of ROS, and promote the destruction of intestinal matrix, leading to a severe degree of focal tissue necrosis, particularly in the presence of a low luminal pH (Whittle, 2002; Jimenez et al., 2004).

As anticipated above, cyclooxygenase-dependent inhibition of bicarbonate secretion contributes also to the gastric mucosal injury elicited by NSAIDs. Indeed, the secretion of bicarbonate ions in the mucus gel layer generates a pH gradient on the mucosal surface, thus providing a first line defense against luminal acid (Allen and Flemstrom, 2005). A number of studies have demonstrated the expression of bicarbonate/chloride ion exchangers in the apical membranes of gastric surface epithelial cells, and shown that cyclooxygenase-derived prostaglandins stimulate bicarbonate secretion via activation of EP₁ receptors (Takeuchi et al., 1997; Rossmann et al., 1999).

Most NSAIDs are weakly acidic in nature and this property accounts for their local cyclooxygenase-independent injuring actions on the gastric mucosa. In the presence of gastric acidity, the undissociated lipophilic form of acidic NSAIDs can impair the hydrophobic surface barrier of the stomach. This transformation of the gastric mucosal surface from a non-wettable to a wettable state appears to be linked with the ability of acidic NSAIDs to destabilize the extracellular lining of zwitterionic phospholipids, particularly phosphatidylcholine, which are present within and on surface of the mucus gel layer (Lichtenberger et al., 2007). Previous studies have demonstrated that such an effect contributes significantly to NSAID-induced gastric injury in experimental models, and that it can persist for prolonged periods after discontinuation of NSAID administration (Lichtenberger, 2001). There is also consistent evidence that the protonophore actions of

aspirin and other acidic NSAIDs take a significant part in the topical damage to gastric mucosa. In particular, upon exposure to the acidic environment of gastric lumen, the undissociated lipid-soluble form of aspirin is able to penetrate cell membranes and accumulate into epithelial cells, where the inner pH is at a physiological level of 7.4. At this pH value, aspirin dissociates and remains segregated within cells. This accumulation enhances the inhibition of prostaglandin biosynthesis, and it brings also into play other properties of aspirin, such as the uncoupling of mitochondrial oxidative phosphorylation. The consequences of such mitochondrial dysfunction are a decrease in ATP production and an increase in AMP and ADP levels, which are then responsible for increments of intracellular calcium concentration. These changes are followed by mitochondrial injury, increased generation of ROS and alterations in the Na^+/K^+ balance, which lead to weakening of the mucosal barrier and cellular necrosis (Wallace, 2001; Bjarnson et al., 2007).

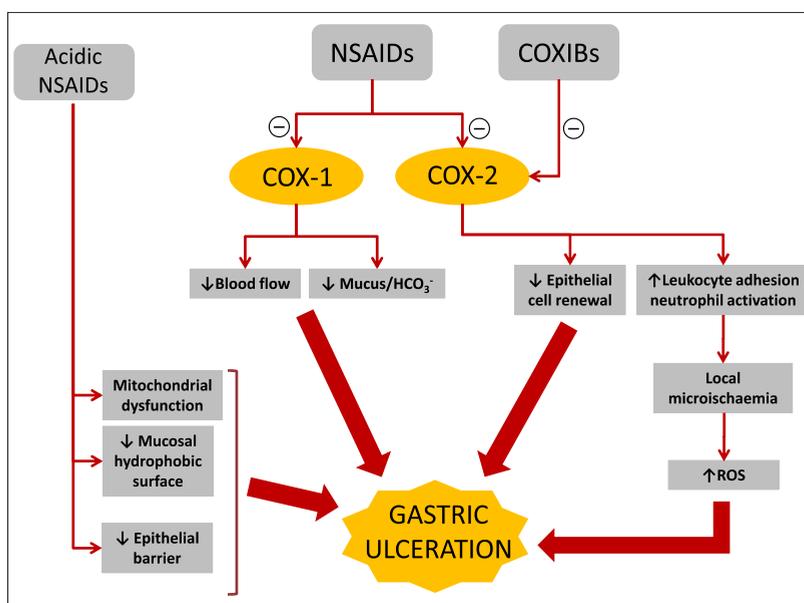


Fig. 1. Pathophysiology of gastric injury induced by non-selective NSAIDs. These anti-inflammatory drugs exert their detrimental effects on the gastric mucosa through two key mechanisms: simultaneous inhibition of COX-1 and COX-2, and direct topic cytotoxic effects. The topic injuring actions depend on the acidic chemical structure of NSAIDs. Coxibs do not harm the gastric mucosa owing to their ability to selectively inhibit COX-2, while not affecting the protective functions of COX-1. ROS: reactive oxygen species.

An additional mechanism, involved in the injurious effects of NSAIDs on gastrointestinal mucosa, is related to the detrimental actions of these drugs on the integrity of epithelial tight junctions, which are known to segregate the apical from basolateral cell surface domains, in order to establish cell polarity and provide a barrier function against the back diffusion of acid and other solutes through the paracellular space (Schneeberger and Lynch, 2004). It has been suggested that cyclooxygenase inhibition may be implicated in NSAID-induced

alterations of intercellular epithelial permeability (Joh et al., 2003). However, recent evidence indicates that aspirin can elicit gastric epithelial barrier dysfunction through down-regulation of claudin-7, a member of the claudin protein family, which play important roles in the formation of tight junctions (Oshima et al., 2008).

Coxibs do not alter the integrity of normal gastric mucosa in preclinical models, and their clinical development was based on the assumption that COX-2 is not expressed in the gastric mucosa (Laine et al., 2008). However, this initial hypothesis has not been supported by subsequent observations, demonstrating the constitutive presence of both COX-1 and COX-2 in human and rodent gastric mucosa (Zimmermann et al., 1998). In addition, studies on COX-1-knockout mice have provided no evidence of spontaneous gastric injury and demonstrated the ability of NSAIDs to damage the gastric mucosa via COX-2-dependent mechanisms (Langenbach et al., 1995). Wallace et al. (2000) investigated further the functional roles of COX isoforms in the gastric mucosa, showing that COX-1-dependent prostaglandins are involved in the maintenance of mucus/bicarbonate secretion and blood flow, while COX-2 protects the mucosa from leucocyte endothelial adhesion and supports epithelial renewal. In addition, these Authors observed that selective COX-1 or COX-2 inhibitors did not damage the stomach when tested alone, while NSAIDs or the combined administration of COX-1 plus COX-2 selective inhibitors resulted in gastric erosions (Wallace et al., 2000). A schematic diagram illustrating the mechanisms of gastric mucosal injury exerted by cyclooxygenase inhibitors is provided in Figure 1. Overall, it is currently acknowledged that NSAIDs can impair gastric protection via a concomitant blockade of COX-1 and COX-2, while coxibs lack damaging actions on gastric mucosa by preserving COX-1-dependent prostaglandin production (Wallace, 2006).

4. Mechanisms of gastric ulcer healing

Gastric ulcer results from mucosal tissue necrosis triggered primarily by ischemia, with cessation of nutrient delivery and ROS formation. Tissue necrosis and subsequent release of arachidonic acid metabolites from injured cells, including leukotrienes B, attract leukocytes and macrophages, which then phagocitize the necrotic tissue and release pro-inflammatory cytokines, which in turn activate local fibroblasts, endothelial cells and epithelial cells to attempt a tissue restoration (Cotran et al., 1999; Tarnawski, 2005). Morphologically, gastric ulcer consists of two components: the margin, surrounded by adjacent non-necrotic mucosa, and the base, consisting of granulation tissue, which is a connective tissue rich in macrophages, fibroblasts and proliferating microvessels (Cotran et al., 1999). Ulcer healing is a complex process, in which the tissue repairs itself after injury, attempting a restitution towards integrity. It has been proposed that such a process can be distinguished in sequential, partly overlapping, phases: haemostasis, inflammation, proliferation and remodeling (Stadelmann et al., 1998). According to Schmassmann (1998), the phases and time course of ulcer healing can be described as follows: ulcer development phase (within 3 days from injury), characterized by tissue necrosis, inflammatory infiltration, formation of ulcer margin (de-differentiation) and development of granulation tissue; healing phase (after 3-10 days from injury), which includes an early healing (rapid migration of epithelial cells and contraction of ulcer base) followed by a late healing (angiogenesis in ulcer bed, remodeling of granulation tissue and complete re-epithelialization of ulcer crater); reconstruction phase (day 20-40 after ulceration) consisting of the reconstruction of glands, muscularis mucosae and muscularis propria; maturation phase (40-150 days after

ulceration), characterized by maturation and differentiation of specialized cells (Schmassmann, 1998).

In general, following the ulcerative injury, a set of complex biochemical events takes place to provide support for cellular migration from ulcer margin and attachment to the ulcer base, with subsequent cellular proliferation and restoration of the epithelial layer. Ulcer healing is initiated by formation of the 'healing zone', consisting of dilated glands, whose cells undergo de-differentiation, express epidermal growth factor receptor (EGF-R) and starts to actively proliferate. At this stage, inflammatory infiltration occurs closely to the necrotic tissue and ulcer crater. In response to growth factors, the ulcer margin is formed, cells adjacent to the margin de-differentiate, and granulation tissue develops at the ulcer base. During healing, the granulation tissue undergoes continuous remodeling, contraction and changes in cellular composition, whereby the inflammatory cells, appeared in the early phase of healing, are replaced by fibroblasts and microvessels in the late healing phase (Cotran et al., 1999). Wong et al. (2000) analyzed the sequential expression of various genes during ulcer healing and were able to distinguish the following arrays: *genes involved in early response* (EGF-R, c-fos, c-jun, egr-1, sp-1, trefoil factor-2/spasmodic peptide [TFF-2/SP]), which are all activated shortly after ulcer formation (i.e., within 30 minutes-2 hours); *intermediate response genes* (EGF, basic fibroblast growth factor [bFGF], platelet derived growth factor [PDGF] and vascular endothelial growth factor [VEGF]), which become activated within 6 hours-2 days; *late response genes* (hepatocyte growth factor [HGF], intestinal trefoil factor [ITF], c-met/hepatocyte growth factor receptor [HGF-R]), which are activated within 14 days (Wong et al., 2000). The subsequent proliferation step is initiated within 3 days from ulceration, and it is essential for the healing process, since it supplies the epithelial cells needed for re-epithelialization mucosal surface and gland reconstruction of (Cotran et al., 1999). There is evidence that mucosal ulceration leads to the development of a novel cell lineage designated as *ulcer associated-cell lineage*, which stems from the base of surviving crypts (Cotran et al., 1999). These cells, which express EGF-R and initiate the synthesis of EGF, HGF, trefoil peptides and other growth factors, promote epithelial tube formation, migration and invasion of granulation tissue, and ultimately drive gland reconstruction within the ulcer scar (Tarnawski, 2005). Time-sequence analysis has shown that trefoil peptides are expressed much earlier than EGF following the induction of tissue ulceration. Furthermore, receptor analysis, using radioligand binding assays and immunohistochemistry, has shown a rapid increase in EGF-R expression and a rapid decrease in somatostatin receptor density in the ulcer margin (Reubi et al., 1994).

The major stimuli for cell migration and ulcer re-epithelialization are mediated by growth factors which are produced by platelets, injured tissue and macrophages. Current evidence suggests also that the epithelium of ulcerated mucosa can be regenerated by bone marrow-derived adult stem cells, since biopsy specimens of gastric mucosa, obtained from female patients receiving bone marrow transplants from male donors, were found to contain cells equipped with chromosome Y (Okamoto et al., 2002). The migration of epithelial cells from the ulcer margin, to restore the continuity of epithelial lining, is essential for ulcer healing, and it is subjected to a fine regulation, since it generates a barrier protecting the granulation tissue from any mechanical and chemical damage. Notably, cell migration requires complex cytoskeletal rearrangements. In particular, it has been appreciated that cytoplasmic microfilaments, consisting of G-actin, polymerize into F-actin and the latter, together with myosin II, provides contractile bundles through which cell motility can take place (Chai et al., 2004). A schematic diagram showing the main factors involved in gastric ulcer healing is provided in Figure 2.

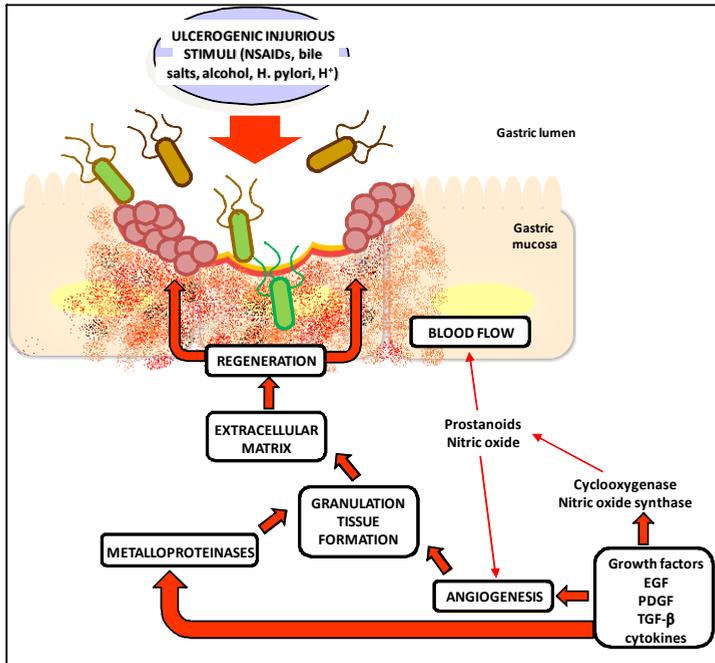


Fig. 2. Main mechanisms involved in gastric ulcer healing. EGF: epidermal growth factor; PDGF: platelet-derived growth factor; TGF- β : transforming growth factor- β .

4.1 Early primary response genes: protooncogenes

Ulcer healing depends on a long-term array of responses, which requires de novo mRNA and protein synthesis as well as cell replication. Changes in gene regulation, in response to wounding or ulceration, result in an increase in cell proliferation to replace lost cells. To accomplish this task, the damaged tissue induces early primary response genes, belonging to the family of protooncogenes, which code for sequence-specific DNA-binding nuclear proteins, having the potential of directly influencing the expression of specific genes at the transcriptional level. Although a low basal expression of the nuclear protooncogenes *c-fos*, *c-jun* and *c-myc* is usually observed in most cells, their expression can be rapidly and transiently up-regulated following tissue wounding (Wang and Johnson, 1994). In the rat stress ulcer model, it has been demonstrated that exposure to stress resulted in a rapid increase in *c-fos* and *c-myc* mRNA levels, up to 3-4-fold the basal value. The change in the expression of these protooncogenes was found to precede an increased rate of DNA synthesis (Wang and Johnson, 1994). In another study, based on in situ hybridization, Ito et al. (1990) examined the changes in protooncogenes expression during gastric regeneration after stress injury. In this setting, cells expressing *c-myc* mRNA were identified as mucous neck, parietal, chief and enterochromaffin-like cells, and the distribution of cells in S-phase coincided with that of protooncogene expressing cells (Ito et al., 1990). The exact signal transduction pathways, leading to protooncogene up-regulation following tissue injury, are still unclear, but they are thought to result from modulation of gene transcription by the polyamines, spermine, spermidine and putrescine. These low-molecular-weight organic

cations are ubiquitous in eukariotic cells and able to bind negatively charged macromolecules, such as DNA, RNA and proteins, thus influencing the chromatin structure and sequence-specific DNA-protein interactions, with consequent changes in the regulation of initiation, elongation and termination of gene transcription (Li et al., 2001).

4.2 Angiogenesis and angiogenic growth factors

Following gastric ulcerative insults, all mucosal components, including microvessels, undergo destruction within the necrotic area. The healing of such deep mucosal lesions requires the reconstruction of surface epithelium and glandular epithelial structures, the restoration of lamina propria and the reconstruction of mucosal microvascular network, which is essential for delivery of oxygen and nutrients to the healing site (Tarnawski, 2005). The latter goal is achieved through angiogenesis, a finely regulated process, in which microvascular endothelial cells migrate from preserved microvessels at the wound edge, proliferate and attempt to re-establish a microvascular network through de novo vessel formation (Folkman and D'Amore, 1996). Angiogenesis occurs via a series of sequential steps, which include: degradation of capillary basement membranes by activation of matrix metalloproteinases (MMPs); endothelial cell migration into the perivascular space and proliferation; formation of microvascular tubes followed by anastomoses; establishment of lamina propria and basement membranes and, ultimately, formation of a novel capillary network (Folkman and D'Amore, 1996). The growth of granulation tissue and generation of new microvessels through angiogenesis is stimulated by bFGF, VEGF, PDGF, angiopoietins, other growth factors and cytokines, including IL-1 and TNF- α (Risau, 1997). Gastric mucosal angiogenesis is strongly stimulated by prostacyclin and human recombinant bFGF. Furthermore, the induction of mucosal injury triggers the activation of bFGF and its receptors, and enhances bFGF protein expression in the mucosa-bordering necrosis (Tarnawski, 2005).

VEGF is a pivotal regulator of angiogenesis. It binds at least two specific receptors, VEGF-R1 or flt-1 and VEGF-R2 or flk/KDR, which are expressed mainly on endothelial cells and initiate the phosphorylation of cytosolic proteins involved in signal transduction promoting endothelial cell proliferation, migration and microvascular formation (Ferrara, 2004). VEGF production is stimulated by PDGF, TGF- α , cytokines, NO and prostaglandin E₂. Hypoxia is one of the best characterized stimuli for the induction of VEGF expression, acting via a hypoxia-inducible factor (HIF)-1 binding site located on the VEGF gene promoter (Ferrara, 2004). Jones et al. (1999) demonstrated a 4-6 fold increase in VEGF mRNA and protein in the mucosa-bordering necrosis after 24 hours from the induction of ulcer by intragastric ethanol instillation. In this study, the quantitative assessment of angiogenesis demonstrated that almost 10% of microvessels in the mucosa-bordering necrosis displayed endothelial sprouting, reflecting the ongoing angiogenesis. Moreover, treatment with anti-VEGF neutralizing antibody reduced the angiogenic response and delayed ulcer healing (Jones et al., 1999). The activation of MAPK (Erk1 and Erk2) signal transduction pathway is crucial for VEGF-induced stimulation of angiogenesis in ulcer healing, and NSAIDs have been found to interfere with the angiogenic process in part by inhibiting the MAPK/Erk pathway (Jones et al., 1999). In normal gastric microvascular cells it has been demonstrated that prostaglandins can induce VEGF mRNA through transactivation of JNK by Erk2 (Pai et al., 2001). Moreover, this stimulant effect of prostaglandins is likely to be amplified via a positive feedback mechanism, since VEGF, once induced, activates COX-2 expression via an autocrine and paracrine action (Tamura et al., 2002).

4.3 Platelets

It is becoming increasingly appreciated that the platelet has the potential of performing a large array of functions, in addition to its role in haemostasis. Tissue repair is initiated with the aggregation of platelets, formation of fibrin clot and release of growth factors from platelets, injured cells and extracellular matrix. Platelets represent one of the largest source of growth factors in the body, and it is through the release of these growth factors that, at least in part, platelets are capable of markedly influencing the processes of tissue healing. Several potent angiogenic stimulators are stored in platelets, including VEGF, platelet derived endothelial growth factor (PDEGF), EGF and PDGF (Perini et al., 2005). These factors account for the ability of platelets to stimulate endothelial cell proliferation and capillary-like formation. Factors that influence the platelet content of pro- versus antiangiogenic factors, or their release from platelets, have the potential to markedly affect angiogenesis and ulcer healing. For example, treatment of rats for 1 week with ticlopidine, an antiplatelet drug acting as adenosine diphosphate receptor antagonist, resulted in a marked increase in platelets and serum levels of endostatin, without affecting platelet VEGF levels. Moreover, this treatment resulted in a marked delay in gastric ulcer healing (Ma et al., 2001). Notably, among a number of receptors, that are important in regulating platelet adhesion, aggregation and secretion, platelet membranes have been found to express proteinase-activated receptors (PARs), which are G protein-coupled receptors, activated by proteinase cleavage at a specific site in their extracellular NH₂-terminus. Four PARs have been cloned to date. The activation of PAR₁ by thrombin stimulates the release of VEGF, while inhibiting the release of endostatin. By contrast, the activation of PAR₄ mediates opposite effects on VEGF release (Ma et al., 2005). The balance in platelet and serum levels of pro- and antiangiogenic factors may influence the healing processes of gastric ulcer and raises the possibility that a selective modulation of PARs could be a viable pharmacological strategy for modulating ulcer healing.

4.4 Heat shock proteins

In response to environmental or physical stress, such as heat or ethanol, eukaryotic cells induce the synthesis of intracellular proteins designated as heat shock proteins (HSPs) or stress proteins (Tsukimi and Okabe, 2001). These proteins function as molecular chaperones, which participate in the folding and assembly of nascent proteins, the refolding of partial damaged functional proteins, and the delivery of precursor proteins to mitochondria (Hightower, 1991). HSPs are classified into four major families according to their biological activities and apparent molecular weights: HSP90, HSP70, HSP60, which are constitutively expressed, and small HSPs, including HSP27 and HSP10, which are inducible by various conditions, including oxidative stress (Hightower, 1991). Tsukimi and Okabe (2001) found that the level of HSP70 in normal mucosa was quite low, while it was significantly higher in the ulcer base at the time of ulcer development. HSP70 is expressed in proliferating cells during re-epithelialization (Soncin and Calderwood, 1996), and thus it is likely to be involved in the regeneration of ulcerated mucosa. The induction of HSP70 in the ulcer base might either contribute to the de novo synthesis of proteins or regulate the activity of key enzymes involved in ulcer healing through a molecular chaperone activity. Of note, Ethridge et al. (1998) reported that the overexpression of COX-2 by transfected cDNA inhibited the expression of HSP70 and the activation of heat shock factor-1 (HSF-1) in response to heat shock in rat intestinal epithelial cells. Such inhibition was antagonized by

the COX-2 inhibitor NS-398. Accordingly, Ethridge et al. (1998) proposed that prostaglandins derived from COX-2 might be associated with HSP70 induced by heat shock, suggesting an inverse relationship between COX-2 expression and HSP70 induction. HSP47 is a 47 kDa stress protein that specifically binds collagen (Nagata et al., 1988). Collagen biosynthesis represents an essential step for granulation tissue formation. In this regard, HSP47 was found to be expressed in the ulcer base at the time of ulcer development, and its expression decreased with the progress of ulcer healing. Based on these results, it has been suggested that HSP47 might be involved in ulcer healing by playing a role in collagen biosynthesis (Tsukimi and Okabe, 2001).

4.5 Annexin-1

Annexin-1 is a 37-kDa member of the annexin family of proteins, which bind and activate 'formyl-peptide' receptors (FPR), known to mediate immune and anti-inflammatory responses. These receptors are expressed on the surface on a variety of cells, including subepithelial myofibroblasts, smooth muscle cells, leukocytes, mast cells and T cells (Chiang et al., 2006). Annexin-1 can also exert its anti-inflammatory actions after proteolytic removal of its NH₂ terminus (Martin et al., 2008). The expression of annexin-1, designated also as lipocortin, can be induced by glucocorticoids and it has been shown to contribute to their anti-inflammatory effects (Hannon et al., 2003). In mice, annexin-1 is expressed in the healthy gastric mucosa, and it is markedly up-regulated following ulcer induction by acetic acid. In this setting, treatment of mice with an annexin-1 mimetic peptide improved gastric ulcer healing. Furthermore, although annexin-1 deficient mice did not exhibit any difference from wild-type mice in terms of susceptibility to indomethacin-induced gastric damage, the healing of such lesions was impaired in annexin-1-deficient mice (Martin et al., 2008). These data are consistent with the hypothesis that annexin-1 contributes to ulcer repair through mechanisms depending on its anti-inflammatory actions. Consistently with this view, Martin et al. (2008) observed an increased expression of the 33-kDa cleavage product of annexin-1 in concomitance with the up-regulation of annexin-1 in the gastric ulcer of mice. The expression of this cleavage product was not observed in healthy stomachs, and therefore it is likely that annexin-1 cleavage, probably due to elevated protease levels, occurred as a consequence of factors induced during the inflammatory or repair process to generate peptide retaining anti-inflammatory properties (Martin et al., 2008).

4.6 Extracellular matrix and tissue remodeling

The replacement of granulation tissue with a connective tissue scar, as well as the reconstruction of mucosal architecture, involves tissue remodeling and changes in the composition of extracellular matrix (ECM). ECM consists of fibrous structural proteins, such as collagens and elastins, adhesive glycoproteins, including fibronectin and laminin, and an amorphous gel composed by proteoglycan and hyaluronan. ECM provides the supporting structure for epithelial, endothelial and smooth muscle cells and it is an essential component of connective tissue (Cotran et al., 1999). In the acetic acid-induced gastric ulcer model, Shahin et al. (2001) demonstrated a marked increase in procollagen I 3 days after ulcer induction. Procollagen gene expression remained elevated up to day 15, while returning to the initial levels on day 30. The highest procollagen transcript levels were found in the intact submucosa surrounding the ulcer margins, followed by the muscularis propria and serosa, with the lamina propria displaying the lowest transcript levels (Shahin et al., 1997). Beside

collagens, other important components of ECM are spatially and temporally regulated during ulcer healing. MMPs include collagenases, which cleave the fibrillar collagens. These enzymes are produced by several cell types, such as fibroblast, macrophages, neutrophils, endothelial cells and some epithelial cells, and their secretion is induced by growth factors, cytokines or steroids (Cotran et al., 1999). Activated MMPs are rapidly inhibited by specific tissue inhibitors, designated as tissue inhibitors of metalloproteinase (TIMP), to prevent uncontrolled actions by proteinases (Cotran et al., 1999). It has been reported that MMP-2 RNA expression can be detected as early as 24 hours after ulcer induction, a time point that coincides with the clearance of necrotic tissue (Shahin et al., 2001). Its further enhancement at the ulcer margin, after 48 hours, parallels the increment of ulcer diameter observed after the sloughing of necrotic tissue. TIMP-1 expression has been found to be enhanced at 72 hours, suggesting that MMP-2 may promote the ulceration process through local degradation of matrix and tissue proteolysis (Shahin et al., 2001).

5. Effects of NSAIDs and coxibs on gastric ulcer healing

The pharmacological modulation of cellular and molecular targets involved in the healing process can alter ulcer repair. Cell renewal in the ulcer margin and angiogenesis in the ulcer base have been found to be significantly impaired during NSAID treatment, with significant delay in ulcer healing (Levi et al., 1990). In the acetic acid-induced ulcer rat model, Sanchez-Fidalgo et al., (2004) found that the ulcerated area was characterized by increased bFGF expression and microvessel density in the granulation tissue at the ulcer base, in concomitance with increments of both apoptotic cell death and expression of proliferation cellular nuclear antigen (PCNA), a marker of cell proliferation. In this setting, both rofecoxib (a selective COX-2 inhibitor) and ibuprofen (a non selective NSAID) delayed ulcer healing, but only rofecoxib was found to reduce all the above mentioned parameters. More recently, indomethacin was tested for its effects on ulcer healing, PCNA and activated caspase-3 expression in acetic acid-induced gastric ulcers. In this study, indomethacin was found to delay ulcer healing, and to up-regulate caspase-3 but not PCNA in ulcerated tissues, suggesting that apoptotic cell death represents a relevant mechanism whereby NSAIDs can impair ulcer repair (Colucci et al., 2009).

Prostaglandins are known to stimulate angiogenesis *in vivo* and *in vitro* (Mehrabani et al., 2001; Cheng et al., 1998). Therefore, it is likely that drugs acting as cyclooxygenase blockers, such as NSAIDs, can interfere with angiogenesis in the setting of gastric ulcer healing. Tsujii et al. (1998) showed that aspirin, a non selective NSAID, and NS398, a selective COX-2 inhibitor, blocked angiogenesis in cultured human umbilical vein endothelial cells. Some clinical and experimental data support the view that both non-selective NSAIDs and COX-2 selective inhibitors can delay gastric ulcer healing, partly by inhibiting angiogenesis in the granulation tissue at the ulcer base (Tarnawski and Jones, 2003). In particular, indomethacin significantly reduced (by >37%) the number of microvessels in the ulcer granulation tissue, and the selective COX-2 inhibitors L-745,337, celecoxib and NS398 were found to exert similar effects (Tarnawski and Jones, 2003). The mechanisms by which NSAIDs inhibit angiogenesis appear to include a local change in angiogenic growth factor expression, alterations in key regulators of VEGF, increased endothelial cell apoptosis, inhibition of endothelial cell migration and recruitment of inflammatory cells and platelets (Tarnawski and Jones, 2003). In rat primary aortic endothelial cells, indomethacin and NS398 markedly

inhibited the tube formation and Erk2 nuclear translocation. Incubation with prostaglandins partly prevented the NS398-induced effects, but not those exerted by indomethacin, suggesting that both COX-1 and COX-2 are important for the regulation of ulcer angiogenesis, and that the inhibitory action of NSAIDs on angiogenesis depends on both prostaglandin-dependent and prostaglandin-independent mechanisms (Tarnawski and Jones, 2003). Pai et al. (2001) have proposed that NSAIDs can arrest endothelial cell proliferation by suppressing cell cycle proteins, since indomethacin was found to significantly inhibit bFGF-stimulated endothelial cell proliferation by reducing cyclin D1 and increasing p21 protein expression. Furthermore, in a study carried on microvascular endothelial cells, indomethacin and NS398 were found to be able to inhibit VEGF-induced early growth response factor (Egr) 1 gene activation, which is a transcription factor activated by hypoxia in angiogenesis (Szabo et al., 2001). Ma et al. (2002) examined the effects of cyclooxygenase inhibitors on the healing of gastric ulcer in rats, angiogenesis in granulation tissue, and serum levels of VEGF and endostatin. In this study, both celecoxib, a selective COX-2 inhibitor, and flurbiprofen, a non-selective NSAID, significantly impaired angiogenesis, delayed ulcer healing and increased serum endostatin levels (Ma et al., 2002). There is also evidence that NSAIDs can interfere with ulcer healing by both acid-dependent and acid-independent mechanisms (Schmassmann, 1998). In this respect, an experimental study has shown that: the thick granulation tissue below the ulcer crater was transformed into a thinner mature scar within 2 weeks from ulceration; in the presence of NSAIDs, the thickness of granulation tissue progressively increased, indicating an inhibition of its maturation process, and the ulcer healing was delayed; such detrimental effect of NSAIDs on the remodeling of granulation tissue could be reversed by omeprazole, suggesting the involvement of acid-dependent mechanisms (Schmassmann et al., 1995).

As anticipated above, preclinical studies have shown that the impairing actions of NSAIDs on ulcer healing can be shared by COX-2 selective inhibitors, suggesting a role for COX-2 in the process of ulcer repair. However, there is also evidence supporting the view that factors other than COX-2 could be important in the detrimental effects of NSAIDs and selective COX-2 inhibitors on ulcer healing (Blandizzi et al., 2009). First of all, data regarding COX-2 expression in gastric ulcer tissue are conflicting. Furthermore, Schmassmann et al. (2006) observed that treatment with selective COX-1 inhibitors did not delay ulcer healing in COX-1 knockout mice and wild type animals. However, in the same study, the combination of selective COX-1 and COX-2 inhibitors impaired ulcer healing to a higher extent than selective COX-2 inhibitors alone, suggesting that COX-1 could also contribute to ulcer healing process under a condition of COX-2 inhibition. It has been suggested also that the detrimental action of aspirin in combination with celecoxib on ulcer healing could result from the ability of aspirin to alter surface phospholipids, without significant involvement of the cyclooxygenase pathways (Lichtenberg et al., 2007). More recently, we have obtained preliminary evidence that the ulcer healing impairing effects exerted by treatment with indomethacin (COX-1/COX-2 inhibitor) or DFU (selective COX-2 inhibitor) could depend on the ability of these drugs to induce the expression of NSAID activated gene-1 (NAG-1), which is known to promote apoptosis (Colucci et al., 2008).

6. Effects of PPIs on gastric mucosal protection and ulcer healing

Several preclinical and clinical lines of evidence have demonstrated that PPIs are highly effective in promoting the healing of gastric damage induced by NSAIDs, even in the

presence of a continued NSAID administration, through the activation of both acid-dependent and -independent mechanisms (Blandizzi et al., 2008).

PPIs are substituted benzimidazole derivatives (Figure 3) endowed with potent inhibitory effects on gastric acid secretion.

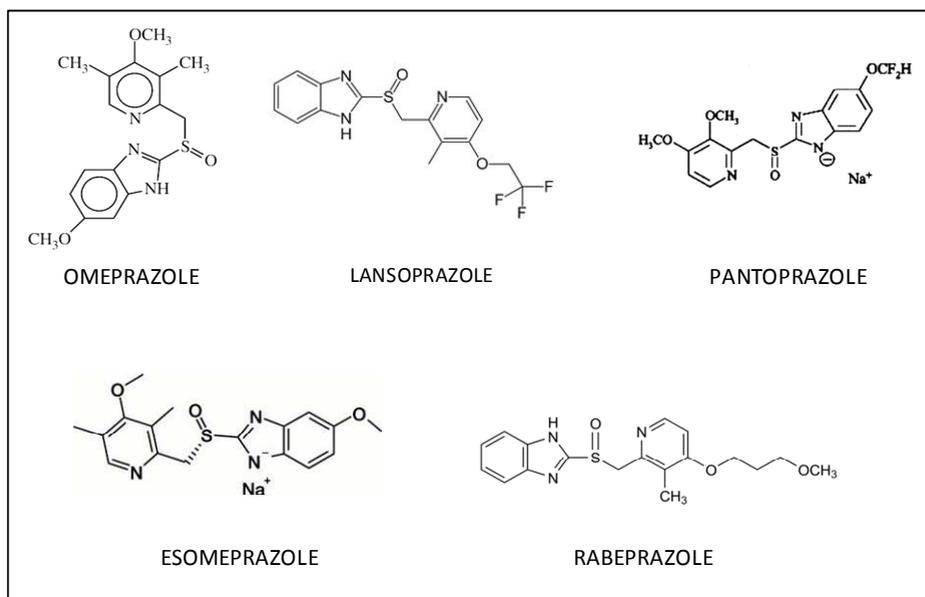


Fig. 3. Chemical structure of proton pump inhibitors (PPIs)

These drugs act primarily through the blockade of the enzyme H^+/K^+ -adenosine triphosphatase (H^+/K^+ -ATPase, the so-called "proton pump"), which is activated during the final step of acid secretion by the parietal cells of the stomach. PPIs are weak basic compounds, with acid dissociation constant (pKa) values ranging from 3.9 to 5.0. For this reason, they accumulate massively in the highly-acidic secretory canalicula of parietal cells, where they are rapidly converted into their active cyclic sulfenamide form. This highly reactive sulfenamide derivative binds sulfidryl groups of H^+/K^+ -ATPase, leading to permanent enzyme inhibition and subsequent potent reduction of acid secretion (Boparai et al., 2008). Some studies have suggested that the beneficial effects of PPIs on ulcer healing could be ascribed to a marked inhibition of acid secretion, which can lead to a consistent increase in plasma gastrin levels, a peptide actively involved in the regulation of mucosal cell proliferation (Koh and Chen, 2000). However, the evidence supporting the involvement of gastrin in the healing action of PPIs is conflicting and there is no general consensus on the significance of this mechanism. Ito et al. (1994) initially showed that omeprazole was effective in increasing the healing rate of acetic acid-induced gastric ulcers in rats, and that this effect was related to a marked increase in serum gastrin levels. In a subsequent study, Schmassmann and Reubi (2000) observed that both omeprazole, inducing hypergastrinaemia, and exogenous gastrin-17 enhanced cell proliferation in the ulcer margin, with an acceleration of the healing process. Since these ameliorative effects were reversed by treatment with a gastrin receptor antagonist, the authors suggested that

omeprazole promoted ulcer healing through an increase in cell proliferation secondary to hypergastrinaemia. However, Okabe and Amagase (2005) provided evidence that a somatostatin analogue significantly decreased the omeprazole-induced hypergastrinaemia in rats with gastric ulcers, while not affecting the ability of this PPI to stimulate ulcer healing. In addition, the healing effect of omeprazole was not modified by gastrin receptor antagonists, thus suggesting that gastrin, released in response to omeprazole, played a marginal role in the mechanisms underlying the ulcer healing action of this PPI.

Besides the marked inhibition of gastric acid secretion, increasing evidence indicates that the beneficial effects of PPIs against NSAID-induced gastric injury could depend on acid-independent mechanisms. For instance, it has been shown that these drugs are able to counteract tissue oxidative damage in a direct or indirect manner (Lapenna et al., 1996; Natale et al., 2004). In particular, several *in vitro* experiments demonstrated a direct antioxidant activity of PPIs, showing that pantoprazole (Fornai et al., 2005) and lansoprazole (Blandizzi et al., 2005) concentration-dependently reduced copper-induced oxidation of human native low density lipoproteins (LDLs), while omeprazole behaved as a scavenger of hypochlorous acid (an oxidant compound generated by phagocytes) (Lapenna et al., 1996). Other studies have shown that pantoprazole is able to scavenge hydroxyl radicals, produced during a Fenton reaction, through the interaction with the hydroxyl radical generating system (Simon et al., 2006). Interestingly, *in vitro* experiments demonstrated that omeprazole and lansoprazole protected DNA from oxidative damage generated by hydroxyl radicals (Biswas et al., 2003). When considering the indirect antioxidant mechanisms, it has been observed that PPIs can significantly counteract the oxidative stress arising from polymorphonuclear cell activation. In this regard, omeprazole was shown to reduce neutrophil functions (Wandall, 1992), including adhesion processes to endothelial cells (Suzuki et al., 1999), phagocytosis and acidification of phagolysosomes (Agastya et al., 2000), and the production of ROS (Zedtwitz-Liebenstein et al., 2002). In addition, lansoprazole inhibited the release of free oxygen radicals from neutrophils activated by *Helicobacter pylori* (Suzuki et al., 1995). Recently, Martins de Oliveira et al. (2007) showed that omeprazole and pantoprazole inhibited H⁺K⁺-ATPase in neutrophils, resulting in cationic flow disturbances and subsequent suppression of migration and intracellular events, such as calcium influx and p38 MAPK activation. On the same line, Pastoris et al. (2008) demonstrated that, in addition to inhibiting acid secretion, the effects exerted by esomeprazole against indomethacin-induced gastric damage can be partly ascribed to a reduction in gastric oxidative injury.

It is also worthy to mention a novel mechanism contributing to the acid-independent beneficial effects of PPIs, which are able to induce and subsequently increase the catalytic activity of heme oxygenase-1 (HO-1) (Becker et al., 2006). The antioxidant, anti-inflammatory, anti-apoptotic, and vasodilatory properties of HO-1 pathway products, such as bilirubin and carbon monoxide, can counteract the main mechanisms of gastric damage. In particular, HO-1 plays a key role in the physiological tissue defense as well as in the modulation of ulcer healing process (Becker et al., 2006).

Mucosal depletion of sulphhydryl radicals has been found to take part to the pathogenesis of gastric lesions evoked by different NSAIDs (Villegas et al., 2002), and reduced glutathione (GSH) concentrations have been detected in mucosal biopsies from patients with NSAID-induced gastric bleeding (Savoie et al., 2001). Consistently with these findings, gastric injury evoked by indomethacin in rats was shown to be associated with a significant decrease in mucosal GSH concentration, and treatment with esomeprazole protected the

gastric mucosa against indometacin-induced damage by restoring mucosal GSH levels (Pastoris et al., 2008).

The involvement of cyclooxygenase/prostaglandin pathways in the ulcer healing mechanisms activated by PPIs has been investigated with conflicting evidence. Some reports suggested that gastric mucosal levels of PGE₂ were unaffected by treatment with PPIs (Natale et al., 2004; Fornai et al., 2011). By contrast, Tsuji et al. (2002) reported that lansoprazole increased gastric COX-2 expression and PGE₂ production after repeated administrations in rats, and suggested that such increments resulted from a lansoprazole-induced increase in gastrin secretion.

Some studies have investigated the modulating effects of PPIs on several molecular markers of cell proliferation and apoptosis, in order to better characterize the mechanisms contributing to their ulcer healing actions. In this respect, Colucci et al. (2009) observed that the ability of esomeprazole to counteract the detrimental action of indomethacin on ulcer repair was ascribable to an enhancement of NF- κ B activation and to a decrease in caspase-3-dependent apoptosis. Interestingly, these effects were found to likely depend on acid-independent mechanisms, since they were not reproduced by the histamine H₂ receptor antagonist famotidine, administered at an equivalent acid-inhibiting dose. More recently, in another experimental model of gastric ulceration, elicited by chronic indomethacin administration, it was confirmed that esomeprazole can exert antiapoptotic actions on gastric mucosal cells in the setting of ulcer repair (Fornai et al., 2011). In the same study, treatment with esomeprazole was also associated with a significant increase in mucosal expression of PCNA and Ki-67, both regarded as markers of cell proliferation. The beneficial influence of esomeprazole on ulcer repair has been related to mechanisms which are likely to be independent from the inhibition of acid secretion and ascribable to antioxidant properties (Fornai et al., 2011). This view is in line with previous studies reporting that both the antioxidant compound ascorbic acid and omeprazole enhanced the expression of growth factors, including TGF- α , in the gastric mucosa of rats treated with aspirin (Jainu and Mohan, 2008). In addition, these preclinical findings are consistent with the clinical evidence provided by Tsuji et al. (1995), who showed that lansoprazole, but not famotidine, induced the expression of bFGF in the gastric ulcer margin, and that PPI was more effective than famotidine in promoting ulcer healing. Other reports have suggested that several growth factors are involved in the ulcer healing effects of PPIs. In this regard, Kinoshita et al. (1998) observed that the gastric levels of HGF were enhanced by omeprazole in rats with indomethacin-induced gastric damage. Moreover, the expression of EGF was found to be increased in the gastric mucosa of mice with indomethacin-induced injury, and further enhanced by omeprazole (Banerjee et al., 2008).

6.1 Effects of PPIs on gastric ulcer healing: clinical evidence

Several clinical studies have been performed to investigate the efficacy of PPIs in promoting the healing of mucosal lesions in patients who unavoidably need to continue NSAID therapy. In a multicentre study, a subgroup of 68 gastric ulcer patients, who continued using NSAIDs, showed rapid ulcer healing when receiving omeprazole 20 and 40 mg/day, with a therapeutic advantage of 31% and 43%, respectively, after 8 weeks as compared with ranitidine 300 mg (Walan et al., 1989). Subsequently, in the ASTRONAUT trial, two doses of omeprazole (20 and 40 mg/day) were compared with ranitidine (150 mg twice daily) in patients with both gastric and duodenal ulcers. In this study, treatment with omeprazole was more effective than the H₂-receptor antagonist in terms of ulcer healing (Yeomans et al.,

1998). The clinical effectiveness of omeprazole has also been documented in comparative studies with other protective drugs. For example, a therapeutic gain of 18% in gastric ulcer patients and 22% in duodenal ulcer patients taking NSAIDs has been estimated when comparing omeprazole with sucralfate (Bianchi Porro et al., 1998). By contrast, the OMNIUM study did not display significant differences between omeprazole (20 and 40 mg/day) and misoprostol (200 µg four times daily) in terms of ulcer healing (Hawkey et al., 1998). Similar results were observed for lansoprazole (15 or 30 mg/day) in comparison with ranitidine (150 mg twice daily). Both doses of lansoprazole were significantly more effective than ranitidine for promoting the healing of gastric ulcers, in patients taking NSAIDs, after 4 and 8 weeks of treatment. In particular, after 8 weeks, the healing rate was 74% in patients treated with lansoprazole 30 mg, and 50% in patients treated with ranitidine 150 mg twice daily (Agrawal et al., 2000; Campbell et al., 2002). In a double-blind, placebo-controlled, randomized trial, patients, treated with low-dose aspirin and affected by upper digestive symptoms, were assigned to treatment with rabeprazole (20 mg once daily) or placebo for 4 weeks. At the end of this period, 47% of patients treated with rabeprazole and 43% of patients given placebo reported a complete relief of upper gastrointestinal symptoms (Laheij et al., 2003). Subsequently, two studies were performed to compare esomeprazole (20 or 40 mg once daily) with ranitidine (Goldstein et al., 2005; 2007). In the first study, gastric ulcer healing occurred in significantly higher proportions of patients treated with either 20 or 40 mg of esomeprazole, as compared with ranitidine at both 4 and 8 weeks. In particular, at the end of the 8-week treatment, the healing rate was 74% in the ranitidine group, 88% with esomeprazole 20 mg and 92% with esomeprazole 40 mg (Goldstein et al., 2005). The second study, performed by the same Authors, highlighted a significant difference in favor of both esomeprazole doses only after 4 weeks. By contrast, after 8 weeks, the healing rates were similar for esomeprazole (20 and 40 mg/day) in comparison with ranitidine (Goldstein et al., 2007).

7. Novel therapeutic options for prevention and treatment of gastric ulcer

Although the control of gastric acid secretion represents a cornerstone for the promotion of ulcer healing, an increasing interest is growing up about the characterization of the mechanisms supporting the process of ulcer repair, and the possibility that both the speed and quality of ulcer healing can be pharmacologically modulated.

At present, novel pharmacological strategies are being investigated to counteract the detrimental actions of traditional NSAIDs on the gastrointestinal tract. The main options currently under active evaluation are: (i) dual inhibitors of cyclooxygenase and 5-lipoxygenase (5-LOX), in order to prevent the mucosal injury resulting from the enhanced biosynthesis of leukotrienes, arising from the shift of arachidonic acid metabolism towards the leukotriene pathway as a consequence of cyclooxygenase inhibition; (ii) traditional NSAIDs associated with phosphatidylcholine, to minimize the destabilizing action of these drugs on the extracellular mucosal lining of zwitterionic phospholipids; (iii) NO donating NSAIDs, designated as cyclooxygenase inhibitors/NO donors (CINODs) and aimed at preventing the injurious actions of NSAIDs through the gastroprotective activity of exogenous NO; (iv) NSAIDs releasing H₂S, a gaseous mediator actively involved in the maintenance of digestive mucosal integrity and blood flow (Blandizzi et al., 2009).

Some of the above mentioned drugs are under clinical development. In particular, licofelone, a dual cyclooxygenase/5-LOX inhibitor, has been shown to spare the human gastric mucosa (endoscopic endpoint) when administered for 4–12 weeks to healthy

volunteers or patients with osteoarthritis in phase II or phase III trials controlled with placebo or naproxen (Bias et al., 2004; Becker et al., 2004). In a 4-day study, performed on healthy volunteers, the gastric injuring action of aspirin, assessed through endoscopic examination, was significantly reduced in subjects administered with soy phosphatidylcholine, although in both treatment groups prostaglandin levels in gastric biopsies were significantly reduced (Anand et al., 1999). Recently, Lanza et al. (2008) evaluated the digestive safety of ibuprofen chemically combined with phosphatidylcholine in osteoarthritic patients, observing a better tolerability of this association in comparison with ibuprofen alone.

CINODs have been developed exploiting the concept that NO, released locally in the gastric mucosa, would enhance the mucosal blood flow and reduce leukocyte adherence in the gastric microcirculation. Based on this assumption, aspirin and other traditional NSAIDs have been coupled to a nitroxybutyl or nitrosothiol group to yield novel anti-inflammatory entities which release discrete amounts of NO (Fiorucci et al., 2007). At present, the pharmacokinetic profile of these novel pharmacological entities remains unclear and deserve further investigations. However, encouraging results about the gastric safety profiles of these novel drugs arise from studies performed on healthy volunteers. In this regard, an endoscopic study demonstrated that healthy subjects treated for 7 days with NCX-4016, an NO-donating aspirin, did not display gastrointestinal toxicity (Fiorucci et al., 2003). On the same line, a trial performed on 31 healthy volunteers showed that upper gastrointestinal endoscopic events following oral administration of AZD 3582, a novel NO donating naproxen, for 12 days were significantly reduced in comparison with traditional naproxen (Hawkey et al., 2003). Moreover, Wilder-Smith et al. (2006) investigated the effects of equimolar doses of AZD3582 and traditional naproxen in healthy volunteers treated for 12 days, observing that treatment with the CINOD was endowed with a better gastroduodenal safety profile in comparison with naproxen. Clearly, further clinical studies are needed to establish whether CINODs confer actual advantages over traditional NSAIDs in terms of upper digestive safety.

Recently, an increasing attention has been paid to the beneficial effects of H₂S on the gastric mucosa. This gaseous compound, previously regarded as a toxic agent, is emerging as an endogenous modulator which seems to share almost all the beneficial actions of NO on several physiological processes. In particular, it has been demonstrated that H₂S is produced by the gastric mucosa, and that it contributes to the ability of this tissue to resist against damage induced by luminal agents (Fiorucci et al., 2007). Interestingly, several lines of evidence have shown that H₂S donors can prevent the decrease in gastric blood flow induced by NSAIDs, and reduce NSAID-induced leukocyte accumulation and adhesion in gastric microvessels, thus providing a rationale for the synthesis of H₂S-releasing NSAID derivatives as novel anti-inflammatory drugs (Fiorucci et al., 2007). As previously observed with CINODs, an H₂S-releasing derivative of diclofenac was shown to be better tolerated, in terms of gastric damage, than traditional NSAIDs. Moreover, the addition of the H₂S-releasing moiety has been found to increase the anti-inflammatory activity of diclofenac (Wallace, 2007; Li et al., 2007). Additional strategies for the prevention of NSAID-induced upper digestive damage include the ongoing clinical development of pharmaceutical products containing fixed combinations of a NSAID with a gastroprotective drug, such as naproxen/omeprazole, naproxen/lansoprazole, naproxen/esomeprazole and ibuprofen/famotidine (Blandizzi et al., 2009).

Several studies have focused their attention toward novel approaches to promote the healing of gastric ulcer. It has been widely recognized that the healing process requires

angiogenesis in the granulation tissue at the ulcer base, followed by a sustained proliferation of epithelial cells in ulcer margins and a subsequent re-arrangement of tissue architecture (Wallace, 2005). As discussed in this chapter, this complex process is finely regulated. In particular, it has been demonstrated PARs play important roles in the modulation of ulcer repair. In particular, preclinical studies have suggested PAR1 as a potential therapeutic target for promoting ulcer healing (Ma et al., 2005).

8. References

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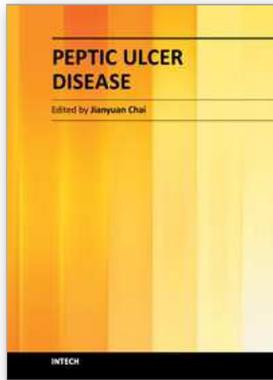
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Peptic ulcer disease is one of the most common chronic infections in human population. Despite centuries of study, it still troubles a lot of people, especially in the third world countries, and it can lead to other more serious complications such as cancers or even to death sometimes. This book is a snapshot of the current view of peptic ulcer disease. It includes 5 sections and 25 chapters contributed by researchers from 15 countries spread out in Africa, Asia, Europe, North America and South America. It covers the causes of the disease, epidemiology, pathophysiology, molecular-cellular mechanisms, clinical care, and alternative medicine. Each chapter provides a unique view. The book is not only for professionals, but also suitable for regular readers at all levels.

How to reference

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