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Review

Protective mechanisms of the gastrointestinal mucosa: a review

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ABSTRACT

The use of animal models to study the cause and cure for gastrointestinal mucosal injury has been pursued since the time of Pavlov through different methods. Fundamentally, there are natural causes like the incompetence of the pyloric sphincter allowing the duodenal contents to reflux into the stomach, delayed gastric emptying due to antral hypomotility, and the position and movement of muscle bands in the stomach, all contributing towards the production of gastritis and predispose to ulceration. Psychological stress and anxiety are associated with gastrointestinal dysfunction leading to gastric hyper-acid secretion and haemorrhagic lesions. Generation of reactive oxygen species (ROS) in tissues can lead to damage of cellular metabolic systems and subsequently, cause inflammation by initiation of lipid peroxidation reactions. Recent studies have brought new insight into the activities of *Helicobacter pylori* colonization in the stomach, leading to the pathogenesis of not only autoimmune and inflammatory diseases, but also apoptosis. This review discusses the pathogenesis of gastroduodenal erosions and how they are eradicated through suppression of the causes and the mechanisms involved. The mechanisms behind the protective effect of certain group of drugs like histamine H_2 receptor antagonists, proton pump inhibitors, growth factors and peptides, centrally active drugs and their molecular pathways of cellular activation are discussed in this review. The influence of prostaglandins, nitric oxide, bicarbonate, mucus, as well as cell restitution and proliferation on the epithelial cells, are delineated. In future, a combination of these biochemical and genetic approaches will bring a detailed understanding of the physiology and pathology of the gastrointestinal remedial system.

KEYWORDS: gastric ulcer, gastric protection, mucosal integrity, prostaglandins, H_2RAs, PPIs, CCBs, growth factors, peptides

INTRODUCTION

Gastric mucosal injury is a multifaceted complicated disease involving epithelial and sub-mucosal damage. Though many theories have been put forward towards the cause of mucosal injury, the etiological basis has not been fully understood. For the past 50 years, the search for a therapeutic target for ulcer disease is still going on. During this process the old dictum “no acid – no ulcer” lost its genuinity and a new slogan “no *Helicobacter pylori* – no ulcer” has evolved [1]. One principal cause of mucosal injury is the disturbance in either the interaction or the balance

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between aggressive factors and defensive factors in the body.

The gastrointestinal epithelium is a continuously changing cell renewal system. The production of new cells, their migration to the epithelial surface and the loss of damaged cells to the lumen, are a continuous and regular process in the stomach. Whenever there is a disturbance caused by any exogenous or endogenous factors, it leads to development of gastrointestinal ulcers.

There is a wide array of pathways leading to gastroduodenal mucosal injury in addition to protective defense mechanisms that counteract them to maintain homeostasis. Gastric damage is a consequence of many interacting factors such as decreased gastric motility, decreased gastric mucus production, decreased gastric mucosal blood flow, decreased prostaglandin production [2-5], increased free radical generation, increased acid back-diffusion, increased gastric vascular permeability, increased calcium (Ca^{2+}) influx, increased release of serotonin and histamine and increased production of leukotriene [6, 7].

The gastric mucosa maintains structural integrity and function despite continuous exposure to noxious factors, including hydrochloric acid (HCl) and pepsin that are capable of digesting tissues. Under normal conditions, mucosal integrity is maintained by defense mechanisms, which include pre-epithelial factors (mucus-bicarbonate-phospholipid barrier) [8, 9], an epithelial barrier (surface epithelial cells connected by tight junctions and generating bicarbonate, mucus, phospholipids, trefoil peptides, prostaglandins (PGs), cathelicidins and heat shock proteins) [8-14], continuous cell renewal accomplished by proliferation of progenitor cells (regulated by growth factors, PGE_2 and survivin, the anti-apoptosis protein) [15-20], an endothelial "barrier" (continuous blood flow through mucosal microvessels), sensory innervations, and generation of prostacyclin (PGI_2) and nitric oxide [21, 22]. Mucosal injury may occur when noxious factors "overwhelm" an intact mucosal defense or when the mucosal defense is impaired [23]. Nonsteroidal anti-inflammatory drug (NSAID)-associated injury is primarily related to inhibition of cyclooxygenase (COX)-mediated PG synthesis, and stress-related mucosal disease, which occurs with local ischemia [8, 23, 24].

The aggressive factors that are responsible for mucosal damage include substances like hydrochloric acid, pepsin, gastrin, protease, free radicals, leukotriene; psychological factors like stress; drugs like ethanol, nicotine and nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin, indomethacin; and the bacteria *Helicobacter pylori* (*H. pylori*) [15, 1, 25-28].

1. Natural causes of gastrointestinal ulcers

There is much evidence that gastric ulcer is not one disease, but is a heterogeneous group of disorders each with one or several causes. There are three causes whereby abnormal motor function might predispose to gastric ulceration namely the incompetence of the pyloric sphincter, delayed gastric emptying and the position and movements of muscle bands in the stomach wall. All of these have been postulated as factors contributing to the vulnerability of overlying mucosa to ulceration.

Stress induces the clinically termed 'stress ulcers' which encompasses both upper gastrointestinal hemorrhage and lesions as a consequence of trauma including burns, intracranial injures and septic shock [24, 27, 29]. Psychological stress and anxiety are associated with gastrointestinal dysfunction including abdominal pain and diarrhea, and also alterations in fluid and electrolyte absorption, which ultimately leads to ulcer disease by stimulating acid and pepsin secretion and/or by decreasing mucosal defense [30, 27]. The majority of the effects of stress are due to rebound stimulation of the parasympathetic nervous system following sympathetic activation [31, 32]. Activity of the dorsal vagal complex and vagal efferents has been shown to be the final pathway that induces stress ulcer [33]. Cold-restraint stress (CRS) and water-immersion stress (WIR) are complex phenomena that involve multiple physical and psychological factors. The endoplasmic reticulum stress [34] response is an important molecular mechanism of stress gastric ulcer pathogenesis. Activation of the hypothalamo-pituitary-adrenal axis accompanied by increases in plasma concentrations of adrenocorticotropic hormone (ACTH) and glucocorticoids [35, 36], as well as the release of thyrotropic-releasing hormone (TRH), mediated by both muscarinic and histaminergic H_2 systems [37] are typical characteristics.

Though the exact role of gastric acid in the pathogenesis of gastric ulcer is unclear, hypergastrinemia is known to cause increases in both basal gastric acid secretion and parietal cell mass, resulting in acidification of the stomach and duodenum, thus leading to ulceration [38, 39].

1.1. Free radical generation

Free radicals are continuously produced from the dissolved molecular oxygen under normal oxygen tension. Molecular oxygen is unable to oxidize other chemical compounds, but instead, it must be converted into an active form of oxygen called the oxygen free radical, namely superoxide free radical O_2^- and a peroxide radical hydrogen peroxide. The tissues also contain multiple enzymes that rapidly remove these free radicals including peroxidases, catalases and superoxide dismutases. In the haemoglobin-buffering system, when the protective mechanisms for the removal of free radicals fail, the enzyme systems swarm the free radicals for removing them and this in turn has serious destructive and lethal effects on cells. One of the serious effects during this process is the oxidation of the polyunsaturated fatty acids that are essential components of many of the membranous structures of the cells. Moreover, free radical generation oxidizes some of the cellular enzymes and damages the cellular metabolic systems. Inflammation causes activation of several metabolic pathways with liberation of free radicals, which cause tissue damage by initiation of lipid peroxidation reactions [40-42]. This is an important cause of damage to the cell membrane. In addition, membrane peroxidation can induce changes in membrane fluidity and permeability as well as protein degradation resulting in cell lysis. Lipid peroxidation is important in the pathogenesis of experimental gastric mucosal injury induced by stress, I/R and indomethacin [43].

1.2. Helicobacter pylori colonization

Helicobacter pylori (*H. pylori*) is a gram negative bacterium frequently detected in biopsy samples from patients suffering from chronic gastric and duodenal inflammatory diseases. It is characterized by a high increase in acidic activity, which metabolizes urea to NH_4 and NH_4 production is the basis of cytotoxicity [44]. NH_4 directly damages mucosal epithelial cells, increases the permeability of gastric mucosa and damages the gastric mucosal endothelial cells especially in sub-epithelial capillaries and venules, leading to vascular injury [45] through oxidant release and vasocongestion. Moreover, *H. pylori*-contaminated mucosa expressed neutrophil accumulation, which produced a factor that promotes chemotaxis [46, 47]. *H. pylori* can induce eosinophil migration through increased production of chemokines CCL2 and CCL5 as well as granulocyte-macrophage colony stimulating factor. These events are mediated by the *cag* pathogenicity island and by mitogen-activated protein kinases [48]. Neutrophil activation is proportional to the load of *H. pylori* and neutrophil adherence to the endothelium in gastric tissue could lead to protein leakage as a result of microvascular damage [49]. Moreover, *H. pylori* also produces haemolysin, generates platelet-activating factor (PAF), and a factor that alters parietal cell function [50]. In turn, this factor exacerbates mucosal damage and reduces blood flow [51, 52]. In addition, *H. pylori* is associated with a number of endogenous substances through many mechanisms including vacuolating cytotoxinA- and CagA-activities. The bacterium evades the host defense by remaining viable within epithelial cells and macrophages, causing a down-regulation of inflammation either through the activation of immunoregulatory T cells or by direct immunosuppression of T cells [53]. Inflammatory genes, including cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), are involved in *H. pylori*-induced gastritis. The colonization of gastric mucosa by *H. pylori* involves specific glycolipid receptors bearing acidic substituents, a process inhibited by gastric sulfomucins. A variety of extracellular enzymes such as proteases, lipases and phospholipases, elaborated by *H. pylori* cause the weakening of the integrity of the gastric mucus coat and render the underlying epithelium vulnerable to noxious luminal contents. The gastric epithelial integrity is compromised by the *H. pylori* cell-wall lipopolysaccharide resulting in the activation of the epithelial surface receptors thereby interfering with the laminin receptors, a family of trimeric glycoprotein present in the extracellular matrix and a major constituent of basement membranes. A cytotoxin-associated gene (*cag A*) has been isolated in approximately 65% of the bacteria that are associated with more severe gastritis, gastric ulcer, gastric cancer, and

lymphoma [48]. Systemic administration of endotoxin provokes the disruption of mucosal architecture with a significant increase in mucosal permeability, which results in the development of hemorrhagic lesions in the stomach and intestine. Figure 1 highlights the role of *H. pylori* on blood flow and vascular damage in the stomach.

1.3. Gastrointestinal epithelial response to injury and mucosal integrity

The actual events that initiate the mucosal response to injury remain a mystery. Restitution is the re-epithelialization of the denuded basal lamina by migrating epithelial cells. These migrating cells extend lamellipodia, flatten and reform cell-cell junctions to reseal the epithelial barrier. As a result, the superficial damage of the gastrointestinal mucosa is repaired [17].

2. Mitogen activated protein kinase (MAPK) signaling cascade

2.1. MAP kinases

MAP kinases are ubiquitous intermediates in transmitting signals from the cell surface. These pathways mediate many of the cellular responses to growth factors and cytokines and regulate numerous cellular events by phosphorylation of transcription factors, growth factor receptors, cytoskeletal proteins, phospholipases and protein kinases [16, 20]. So far, three parallel mammalian MAP kinase pathways have been established. The final kinases in each module, namely extracellular signal-regulated kinase (ERK), *c-Jun* amino terminal protein kinases (JNK) and p38/HOG1, are homologous and all require dual phosphorylation of both tyrosine and threonine for activity [54, 55].

2.2. Integrins

Integrins are a diverse family of heterodimeric transmembrane receptors that mediate cell extracellular matrix (ECM) interactions including adhesion and motility during epithelial restitution [56]. Many of the processes in which integrins participate have a requirement for strong adhesion coincident with times of mechanical stress. In addition to serving as transmembrane mechanical links, integrins in vertebrates synergize with a number of receptors including growth factor

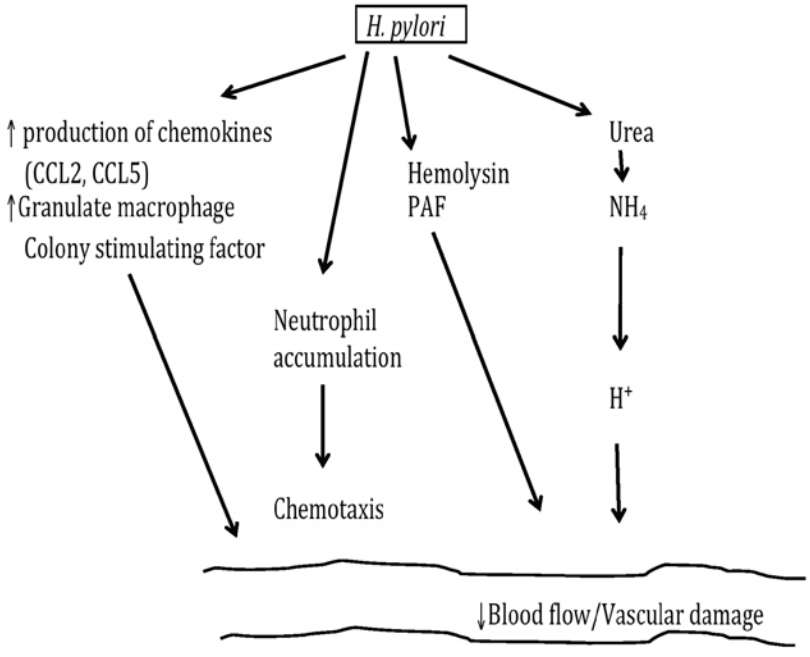

Figure 1. Flow diagram showing how *Helicobacter pylori* can produce multiple injurious materials near the gastric mucosal epithelium that damage epithelial cells, provoke release of inflammatory mediators, and cause neutrophil migration into the interstitium and the lumen of the stomach.

receptors to enhance responses. This leads to the activation of a large signaling network that affects cell proliferation and differentiation, as well as cell shape and migration [57]. Matrix metalloproteinases (MMPs) are suggested to play a critical role in extracellular matrix degradation and remodeling during inflammation and wound healing processes [58]. Integrin receptors can function as signal transducers by activation of the Ras-dependent MAP kinase pathway [59]. Expression of integrins is regulated at several levels of biological processes. These include transcription and other post-transcriptional regulatory events, translation in endoplasmic reticulum, dimerization, and transportation from endoplasmic reticulum to the cell surface. All these processes have the ability to integrate extracellular stimuli into intracellular signals and affect cell behavior [57].

2.3. Cytokine growth factors and phorbol esters

Cytokine growth factors and phorbol esters, which are known to stimulate epithelial cell monolayer repair, have the ability to rapidly activate MAP kinase. Generally, cytokines that augment epithelial wound repair also activate the MAP kinase pathways involving Ras and ERK [60]. Ras plays a central role in activation of MAP kinase and is necessary for migration in models of wound repair. Ras acts as a regulator of transcription factor AP-1, that is composed of *cFos* and *c-Jun*, and mediates inflammation and carcinogenesis [61]. Ras expression is a critical step in signal transduction pathways involving growth factor receptor tyrosine kinases such as fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) receptors. Wounding of the epithelial cell monolayer initiates a Ras-dependent pathway that results in *c-Fos* expression [62]. Ras is linked to the MAP kinase pathway of ERK and JNK by activation of Raf-1. In turn, this leads to the activation of ERK and MEK kinase, resulting in JNK activation. Ras activation of Raf-1 is potentially regulated by protein kinase A (PKA) [63]. Cyclic AMP blocks activation of MAP kinase cascade by preventing the Ras-dependent activation of Raf-1 [64]. It also inhibits the activation of the MAP kinase pathway using ERK and blocks restitution in mucosal restitutive models and motility in other cell systems [65].

MAPK pathways mediate expression of immediate response genes, including *c-Fos* and *c-Jun*, which are early events in several injury and wounding models suggesting an up-regulation of *c-Fos* expression and important roles for *c-Jun* and *c-Fos*. Epidermal wounds in rats can result in prompt nuclear expression of *c-Fos* [66]. Moreover, multiple cytokines are capable of enhancing monolayer wound repair in IL-6 cells. Transforming growth factor-alpha (TGFα), epidermal growth factor (EGF), interleukin-1β (IL-1β), and interferon-R act to increase bioactive TGF-β production suggesting a common TGF-β-dependent pathway mediating un-stimulated or cytokine-stimulated monolayer repair [67]. The IL-1β--PG--HGF pathway also plays a role in the repair process of gastric mucosa.

Rapid local release of endothelins (ET-1) was demonstrated prior to the development of gastric hemorrhagic erosions due to intragastric administration of either ethanol or HCl in rats [68], as well as in indomethacin-, stress- and ischemia-reperfusion-induced ulceration [69]. It was demonstrated that ET-1 induced a rapid expression of early growth response factor-1 (Egr-1) which seemed to be one of the chemical mediators of cell injury and Egr-1 expression resulting in elevated growth factors production [69].

2.4. Prostaglandins

Several prostaglandins (PGs) have been shown to exert major gastrointestinal actions in the body. These include inhibition of gastric acid secretion, antiulcer activity, cytoprotection for the stomach and stimulation of smooth muscle contraction and intestinal secretion through increase of cyclic AMP formation [70, 71]. Prostaglandin (PGE) derivatives are widely used for treating gastric mucosal injury and their receptors are classified into four subtypes, EP(1), EP(2), EP(3) and EP(4) [7]. Ethanol-induced mucosal injury was inhibited by EP(2) and EP(4) agonists [18, 7]. Hattori *et al.* [7] demonstrated that leukotriene antagonists reduced ethanol-induced mucosal injury and reductions in leukotriene C_4 (LTC_4) generation in response to EP2 and EP4 receptor signaling which may be relevant to the protective action of PGE_2. Moreover, PGE_2 has been shown to have a number of biological actions including vasodilatation, anti- and pro-inflammatory actions, decreased

gastric acid output and increased mucus and bicarbonate secretions [18, 7, 72]. During the process of ulceration, stimulation and inhibition of PGs in the gastrointestinal system normally take place. The increase in the bound PGE_2 levels in the gastric glandular tissues releases phospholipids especially since the gastric mucous cell is the source of surfactant phospholipids as well as mucin. Nevertheless, their synthesis and release play a critical role in the primary defense of gastric epithelium which may account for the antiulcer activity [73]. PGE_2 enhanced the migration of oral cancer cells through an increase in intracellular adhesion molecule 1 (ICAM-1) production [74].

The biosynthesis of PG is catalyzed by the enzyme cyclooxygenase (COX) leading to formation of the intermediate PG $(PG)G_2$ which is reduced to PGH_2 in a peroxidase reaction by the same enzyme. The metabolism of PGH_2 by various isomerases or reductases yields the different PGs and thromboxane. Two isoforms of COX have been characterized, a constitutively expressed COX-1 involved in gastric mucosal defense reactions, and an induced COX-2 involved in the repair of damaged gastric mucosa [70]. PGs accelerate gastro-duodenal ulcer healing by enhancing COX-2 expression and PGE_2 generation in the ulcer area. Up-regulated COX-2 at the ulcer margins plays a crucial role in ulcer healing by endogenous PGs, PPIs, growth factors, gut hormones and melatonin. In contrast, COX-1 and COX-2 inhibitors delay ulcer healing by suppressing PG generation and increasing COX-2 expression in the ulcer area [75]. PGs derived from both COX-1 and COX-2 pathways play a beneficial role in gastroprotection involving phospholipase C, protein kinase C and potassium sensitive ATP channels [71].

It was also postulated that peroxisome proliferator-activated receptor gamma (PPARγ) modifies PG formation and exerts gastroprotective action [76]. Similarly, proteinase-activated receptor-1 (PAR1) reveals endogenous prostanoid-dependent gastroprotection. Ciglitazone, a thiazolidinedione PPARγ agonist, dramatically facilitated the PAR1-triggered PGE_2 production and up-regulation of COX-2. Inhibitors of MEK, p38 MAP kinase (p38MAPK) and PI3-kinase (PI3K), but not JNK, blocked this effect [76]. PI3K is essential for several cellular signal transduction pathways including cystic fibrosis transmembrane conductance regulator (CFTR) activity in intestinal epithelial cells. Tuo *et al.* [77] have demonstrated that PI3K pathways play an important role in the regulation of cAMP- and cGMP-induced duodenal epithelial CFTR channel activity and intracellular trafficking.

2.5. Nitric oxide

Endothelial cells secrete an unstable factor that induces relaxation of adjacent smooth muscle cells when stimulated by vasodilators such as acetylcholine (ACh). The mediator originally named as endothelium-derived relaxing factor (EDRF), is now known to be nitric oxide (NO). It has multiple actions in the gastrointestinal tract and is now the subject of extensive studies with respect to its role in the physiology and pathogenesis of gastrointestinal mucosal injury and defense. It influences mucus secretion, mucosal blood flow and enteric nerve function, all of which can have an impact on resistance to injury. Suppression of NO synthesis renders the gastric mucosa more susceptible to injury [78], while administration of NO donors protects the stomach from injury [79]. NO is associated with mucosal damage and modulates hypoxia inducible factor 1 (HIF-1) activity [80]. Evidence suggests that the inducible nitric oxide synthase (iNOS)-derived NO associated with NSAID-induced gastric injury is implicated in mucosal restitution via the HIF-1-mediated induction of TFF. NO released from vascular epithelium, epithelial cells of gastrointestinal tract and sensory nerves can influence many of the same components of mucosal defense, as do prostaglandins [81].

2.6. Bicarbonate and mucin

The secretion of bicarbonate (HCO_3^-) in both the stomach and duodenum was increased in response to PGE_2 as well as mucosal acidification with the latter occurring with concomitant enhancement of mucosal PG generation [9]. Thus, the HCO_3^- stimulatory action of PGE_2 in the duodenum is mediated by both EP3 and EP4 receptors being coupled intracellularly with both Ca^{2+} and cAMP while that in the stomach is mediated by EP1 receptors, coupled with Ca^{2+} [82]. The results clearly demonstrate the involvement of EP3

receptors, in addition to EP4 receptors, in the regulation of HCO_3^- secretion and maintenance of mucosal integrity of the duodenum against acid injury [9, 83]. The specific protective mechanisms involve luminal bicarbonate secretion, intracellular pH buffering and interstitial buffering [84]. Epithelial cell intracellular pH regulation, rather than secreted extracellular bicarbonate, is the principal means by which duodenal epithelial cells are protected from acidification and injury. RT-PCR studies of mouse gastrointestinal tract mRNAs demonstrated that a transporter known as anion exchanger isoform 4 (AE_4), an apical Cl^-/HCO_3^- exchanger in gastric mucous cells and duodenal villus cells, is expressed in both the stomach and the duodenum. On the basis of its function and location, it is proposed that AE_4 plays an important role in mucosal protection [85].

Mucins, growth factors, and trefoil factors are involved in accelerating gastric injury healing through reduction of gastric acid secretion and epithelial reconstruction through PG and NO pathways [8, 11, 86, 87]. Muc1, a cell surface mucin containing a large glycoprotein, restricts access of *H. pylori* to the epithelial surface, thus reducing exposure of the host to pro-inflammatory bacterial products [88]. *H. pylori* inhibits total mucin synthesis *in vitro* and decreases the expression of MUC5AC and MUC1 [89, 90].

3. Experimentally induced gastric injury

3.1. Acid as the stimulant of gastric injury

Gastric mucosal damage can be increased naturally due to stress, or induced by water-immersion-, or cold restraint-stress or by administering ethanol, acetic acid, hydrochloric acid or any irritant into the stomach. In water-immersion stress-induced gastric mucosal injury lafutidine, an H_2 receptor antagonist, decreased susceptibility to acid-induced gastric mucosal injury in rats by inhibiting neutrophil activation [91]. Ethanol administration into the stomach increases vascular permeability and vascular damage in capillaries near the luminal surface and not in the deeper muscularis mucosa that might indicate a role for impaired blood flow in the production of gastric lesions. It increased 4-hydroxy-2-nonenal levels, a byproduct of oxidative stress in the luminal part of the gastric mucosa [92] and significantly increased gastric ulcer index concomitantly with increased cellular apoptosis, accompanied by increase of calcitonin gene-related peptide (CGRP) expression. The capsaicin pathway is an acid-sensing pathway that promotes hyperemia and mucus secretion in response to luminal acid [93]. Previous studies have demonstrated that capsaicin-sensitive sensory nerves are involved in the protection of gastric mucosa against damage by various stimuli and CGRP is a potential mediator in this process [94]. The MEK-ERK1/2 signaling pathway is believed to be involved and it included an early growth response gene called *Egr-1*, which is activated upon acid exposure [95]. In turn, this up-regulated vascular endothelial growth factor (VEGF) expression, which was inhibited by *Egr*-1 antisense oligonucleotide. Luminal acid stimulation significantly increased 5-hydroxytryptamine (5-HT) release from the duodenal mucosa and it plays a physiological role in acid-stimulated bicarbonate secretion via a Ca^{2+} signaling pathway, in which the plasma membrane Na^+ transporter, as well as intermediate calcium-activated potassium ($IK(Ca^{2+})$) and cystic fibrosis transmembrane conductance regulator (CFTR) channels, are involved [96]. SB204070, a selective 5-HT_4-receptor antagonist, dose-dependently reduced luminal acid-stimulated HCO_3^- secretion of mice *in vivo*.

Luminal acid sensing consists of ecto- and cytosolic carbonic anhydrases, epithelial ion transporters and acid sensors expressed on the afferent nerves of the gastric mucosa. These luminal chemosensors help to activate mucosal defense mechanisms in order to maintain mucosal integrity [97], prevent mucosal injury and modulate sensory nerve activity [98]. Acid challenge of the gastric mucosa is signaled to the brainstem. The gastritis-evoked increase in the gastric acid-evoked *c-Fos* expression in the nucleus tractus solitarius (NTS) is related to disruption of the gastric mucosal barrier, mucosal inflammation, mucosal acid influx and enhanced activation of the afferent stomach-NTS axis [99].

3.2. Ischaemia/reperfusion injury (I/R injury)

I/R injury caused significant accumulation of gastric luminal fluid that was alkaline and rich in protein, glucose and bicarbonate content when compared with sham controls. It caused gastric surface epithelial cell injury and significant

increase in serum and antral gastrin levels. In addition, I/R inhibited basal acid secretion in the gut and blunted the acid secretory response to pentagastrin [100]. I/R damages gastric mucosa via reactive oxygen species activity and increased gastric microvascular permeability and hydrogen peroxide (H_2O_2) production in rats. Pepsin plays a pivotal role in the pathogenesis of I/R-induced gastric lesions. This process is associated with back-diffusion of acid mediated through a vagal-cholinergic pathway [101]. In turn, this induces significant inflammation and immune-mediated mucosal damage. Moses *et al.* [102] have revealed that toll-like receptor-4 (TLR4) is a critical receptor in the induction of inflammatory responses and in turn this plays an important role in intestinal homeostasis. TLR4 stimulation of COX-2 activation of PGE_2 production is necessary, but not sufficient for intestinal I/R-induced damage and inflammation. Yoshida *et al.* [103] have demonstrated that protease activated receptors (PARs) are widely recognized for their modulatory properties during inflammation and they also demonstrated that PAR-2 plays an important role in the pathogenesis of I/R-induced intestinal injury. PAR-1 is protective against *H. pylori*-induced gastritis, mediated by the suppression of pro-inflammatory pathways. Angiotensin (AT1) receptor blockers also suppress I/R-induced gastric injury in rats [104]. NADPH oxidase is an enzyme that converts molecular oxygen into reactive oxygen species, causing severe damage in the gastric mucosa induced by I/R. The activity of this enzyme is related to the upregulation of COX-2 [105] and the mucosal protective drug, polaprezinc, which is a chelate compound that exhibits ROS-quenching-like activities [106, 107].

3.3. Nonsteroidal anti-inflammatory drugs (NSAIDs)

It is now well established that nonsteroidal anti-inflammatory (NSAID) agents can cause mucosal damage [23] and the basis for gastric injury facilitated by animal models correlated well with disease in humans [108]. NSAIDs including indomethacin and aspirin represent a class of drugs that produced gastric epithelial cell damage by two major mechanisms. One of them is by blocking the COX pathway thereby inhibiting the endogenous PG production [109]. The second mechanism is called ‘ion trapping’ and results from the acid dissociation of NSAID (pKa = 3.5-4.0) in the comparatively neutral intracellular environment (pH = 7) of mucosal cells. NSAIDs can cause damage to the gastroduodenal mucosa due to several changes, including changing the high pH environment of the mucoid cap leading to topical irritant effect on the epithelium, impairment of the barrier properties of the mucosa, suppression of gastric PG synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury. All these, in turn, contribute to the pathogenesis of NSAID-induced ulcers and bleeding, by impairing the restitution process, interfering with homeostasis and inactivating several growth factors that are important in mucosal defense and repair [110].

Indomethacin interferes with three lines of mucosal defense. PGs regulate mucus cell secretion of mucin and surface active phospholipids, and COX inhibition leads to decreases in the mucus barrier function [10, 23]. Indomethacin inhibits basal bicarbonate secretion from gastric and duodenal mucosa, a defect which can be reversed by exogenous PGE_2 and known to inhibit the mucosal proliferation, critical to ulcer healing [111]. The fall in gastric mucosal blood flow induced by indomethacin in doses sufficient to inhibit PG formation in the mucosa could suggest a role for endogenous PGs in the local regulation of gastric microcirculation; PGE and A series have been shown to increase resting mucosal blood flow. Small changes could reflect intense focal ischemia and such areas would be the sites of subsequent erosion especially in the presence of acid. It also has a topical action of a physico-chemical nature. This is unrelated to PG inhibition, but it is mainly due to the change in potential difference in the cell [112]. Of the two COX isoforms, COX-1 is constitutively expressed and is important in the maintenance of normal gastric function and COX-2 is an inducible form that is up-regulated in areas of inflammation. NSAIDs reduce both of these isoforms leading to beneficial (antipyretic, anti-inflammatory) and toxic (gastrointestinal injury) effects.

Activated neutrophils and oxidative stress seem to play a significant role in NSAID-induced gastric mucosal damage [113, 114]. This effect occurs

through activation of neutrophil-derived free radicals, proteases or various lipid mediators and up-regulation of adhesion molecule-1 (ICAM-1) [115]. Naito and Yashikawa [116] have demonstrated that reactive oxygen species (ROS) produced by activated neutrophils after indomethacin treatment can cause gastric mucosal injury resulting in epithelial cell apoptosis. This damage to the gastric mucosa involves ROS-mediated oxidation of important biomolecules such as lipid, protein and DNA [40, 117]. Figure 2 illustrates the mechanism(s) whereby the NSAIDs can induce injury in the stomach.

4. Protection of gastric ulcers

Gastric mucosal defense consists of a complex network of components that function in concert with one another. These extra-mucosal components include acid, mucus, surface-active phospholipids, bicarbonate, the epithelial barrier, the microcirculation, the sensory afferent neurons beneath the epithelium, the mucosal immune system and more importantly how fast the mucosa can repair itself [118]. This review places much emphasis on the factors that contribute to gastrointestinal mucosal defense and the cellular and molecular mechanisms through which mucosal defense is modulated.

4.1. Histamine H_2 receptor antagonists

The histamine H_2 receptor antagonists (H_2RA) block the H_2 receptors located on the parietal cells so as to reduce the gastric acid secretion evoked by histamine. They include cimetidine, ranitidine, famotidine, nizatidine, lafutidine, etc., that differ in structure and potency, but they all have been frequently used in clinical practice for treatment of gastric ulcers. H_2RA have pleiotropic effects. They not only block the secretion of gastric acid, but also inhibit cell-cell adhesion, resulting in inhibition of metastasis. Cimetidine, ranitidine, and famotidine improved restitution of the gastric mucosa contributing to the healing process of the gastric damage. They exert their antagonistic effects by reverting the ethanol-induced properties like diminution of some phospholipids in order to increase cholesterol and to decrease the activity of

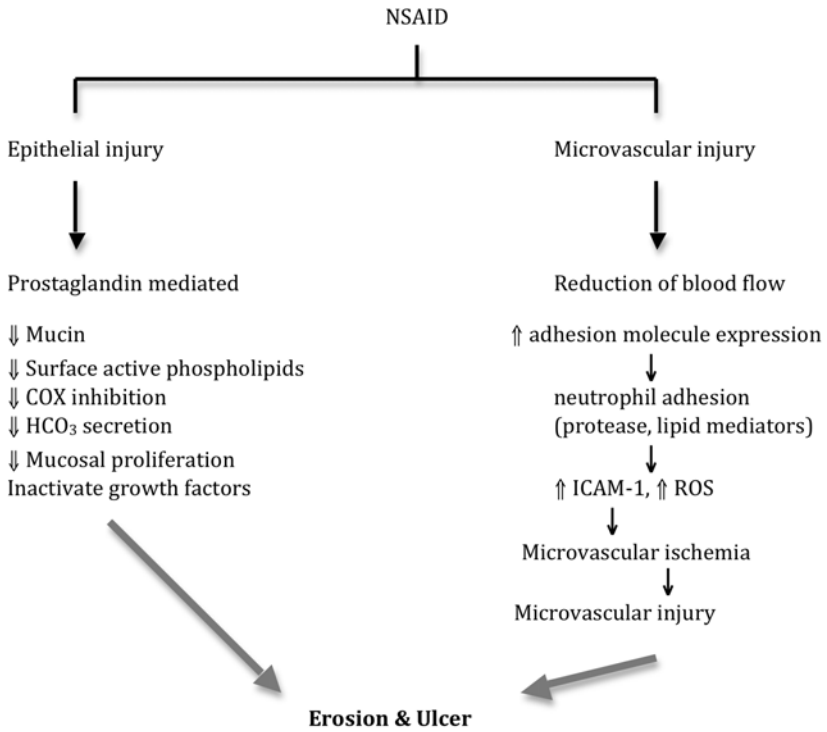

Figure 2. Diagram illustrating the mechanism of NSAID-induced epithelial and microvascular injury, and potential sites of action.

5'-nucleotidase of H_2RAs on gastric damage, in addition to their cytoprotective effect [119]. Moreover, they can also prevent indomethacin-induced gastric injury by decreasing the lysosomal enzymes N-acetylglucosamine, acid phosphatase and beta-glucuronidase thereby contributing to lysosomal membrane protection [120].

4.1.1. Ranitidine

Ranitidine acts as a potent antiulcer agent acting against stress-induced ulcers in rats through the release of PG and activation of the NO pathways [24] by inhibiting neutrophil activation *in vitro* and *in vivo*. It prevents indomethacin-induced ulcer formation with attenuation and inhibition of mucus, catalase and superoxide dismutase [121]. Ranitidine can improve the macro-circulation of gastric mucous membrane by decreasing plasma endothelin levels and increasing the cGRP levels in stressed patients [122].

4.1.2. Cimetidine

Cimetidine has been shown to act directly on endothelial cells to inhibit high glucose-induced expression of adhesion molecules and neutrophil adhesion mediated by increasing endothelial NO production, but not by inhibiting protein kinase C (PKC) [123]. Cimetidine exerts a protective effect against acute gastric mucosal injury induced by I/R. Its antagonistic effect is not only due to suppression of gastric acid secretion, but also to its antioxidant action when it is present at a high concentration in the intragastric environment [124]. Cimetidine, a partial agonist for H_2-receptor, has a pharmacological profile different from ranitidine and famotidine, possibly contributing to its antitumor activity on gastrointestinal cancers [125].

4.1.3. Famotidine

Famotidine is effective in the prevention of gastric and duodenal ulcers, upper gastrointestinal bleeding and erosive esophagitis induced by aspirin and indomethacin. Famotidine pretreatment reduced water restraint stress-induced [126], and aspirin-induced mucosal injury in the stomach by suppressing gastric mucus cell function and reducing mucin [127]. Singh *et al.* [128] found a novel matrix metalloproteinase-9 (MMP-9)-mediated pathway for the inhibition via pro-inflammatory cytokines by famotidine in ethanol-induced gastric ulceration. Matrix metalloproteinases have the ability to cleave and remodel the extracellular matrix. Nizatidine contributed towards antiulcer activity by increasing bicarbonate secretion in the rat duodenum mediated by vagal-cholinergic mechanism, the action being associated with the anti-acetylcholineesterase (AChE) activity of this agent [129].

4.1.4. Roxatidine

Roxatidine has a different chemical structure than cimetidine, ranitidine and famotidine, but it can suppress indomethacin-induced small intestinal injury in rats through a mechanism of increased intestinal mucus mediated by NO, but not by PG [130]. As roxatidine does not block gastric first-pass metabolism of ethanol, it is considered as a safe H_2RA in individuals who continue consuming alcohol even when they are under treatment for gastroduodenal ulcer disease [131].

4.1.5. Lafutidine

Lafutidine, a newly developed H_2RA having potent antisecretory and gastroprotective properties against noxious agents-induced gastric mucosal damage, acts through capsaicin-sensitive afferent nerves [132, 133]. It reduced WIR stress-induced gastric mucosal injury not only by inhibiting acid secretion, but also by inhibiting neutrophil activation through enhancement of sensory neuron activation [91]. Moreover, it can augment cGRP release from the rat stomach when administered before the induction of WIR stress [126].

4.1.6. Ebrotidine

Ebrotidine exerts a unique cytoprotection against injury by various ulcerogens including ethanol, ammonia, lipopolysaccharides (LPS), stress and aspirin or acidified taurocholate. Ebrotidine exerts its protective effect by stimulating mucus secretion and increasing the quality of adherent mucus gel and gastric mucosal blood flow (GBF). These effects of ebrotidine are possibly due to its ability to enhance mucosal formation of PGE_2 and NO [134]. This hypothesis was supported and demonstrated by Palop *et al.* [135] who showed that during the process of gastroprotection by ebrotidine in ethanol- as well as indomethacin-induced gastric damage, endogenous NO and sulfhydryl compounds, but not PG, played a crucial role. Ebrotidine possesses the ability to

counteract the *H. pylori* interference with a somatostatin-regulatory effect on gastric acid secretion [136]. It also exerts modulatory effect on mucosal inflammatory responses by interfering with the events propagated by NOS-2 and caspase-3 [137]. The ulcer healing was accompanied by a marked elevation in mucosal expression of gastric mucosal proliferating cell nuclear antigen (PCNA) and cyclin-dependent kinase (Cdk2). These findings implicate cell cycle regulatory proteins in the processes leading to mucosal repair suggesting that erbotidine exerts a similar effect on the expression of proteins that control cell cycle progression [138]. The protection of ebrotidine against indomethacin-induced mucosal damage occurs through two mechanisms. Firstly, it inhibits epithelial cell apoptosis triggered by the enhancement in the mucosal TNFα expression [139]. Second, it enhances gastric mucosal proliferative activities associated with ulcer healing through the stimulation of EGF and PDGF (platelet derived growth factor) receptor expression. In turn, this has the ability to protect the cellular integrity from calcium imbalance by modulating the EGF-stimulated gastric mucosal calcium channel phosphorylation [140]. Among the most potent agents capable of countering the proteolytic activity of *H. pylori* are nitecapone, ebrotidine and sulglycotide, while ebrotidine and sulglycotide were found to be the most effective inhibitors of *H. pylori* lipolytic activities. The interference of the lipopolysaccharides with the laminin receptor was found to be most efficiently countered by ebrotidine, sulglycotide and sucralfate, whereas sulglycotide is the most potent in the reversal of the inhibitory effect of the lipopolysaccharides on mucin receptor binding [141]. Two antiulcer agents bearing sulfated sugar groups, namely sucralfate and sulglycotide, have been demonstrated to possess the ability to interfere with the *H. pylori* colonization process. Moreover, they are also potent inhibitors of *H. pylori* glycosulfatase activity directed against indigenous mucosal defenses.

4.1.7. Pibutidine hydrochloride (IT-066)

Pibutidine induced gastric mucosal protection against acidified ethanol-induced lesions and this involved endogenous NO and PGs. The endogenous PGs in turn are believed to contribute to the protective effect of NO [142]. Similarly, Z-300 and IGN-2098 [143], two newly developed H_2RAs, have been shown to exert marked protective effect on gastric mucosa from WIR-stress-, indomethacin-, aspirin-, acidified ethanol-, and pylorus ligation-induced lesions via their potent antisecretory and mucosal protective activities which are 8 times more potent than roxatidine.

4.2. Proton pump inhibitors (PPIs)

Proton pump inhibitors (PPIs) have been widely used as acid inhibitory agents for the treatment of disorders related to gastric acid secretion for over 15 years [144]. They are substituted benzimidazoles and inhibit acid secretion by acting on the hydrogen-potassium exchanger (H^+K^+ATPase) of the apical plasma membranes of the gastric mucosa. PPIs are acid-activated prodrugs that convert to sulfenic acids or sulfenamides that react covalently with one or more cysteines accessible from the luminal surface of the ATPase. All PPIs give excellent healing of peptic ulcers and when combined with antibiotics eradicate *H. pylori*. Subtle differences have emerged between the old and the new proton pump inhibitors in their pharmacokinetics, pharmacodynamics and efficacy profiles. The increased efficacy and use of PPIs against gastric ulcer provide support that the enzyme inhibition is superior to receptor antagonism as a therapeutic approach. Five such agents include omeprazole, lansoprazole, pantoprazole, rabeprazole and esomeprazole, which are now available for therapeutic use. Figure 3 illustrates the binding of PPIs to the alpha subunit of the gastric proton pump.

4.2.1. Omeprazole

Omeprazole is known to function not only as a proton pump inhibitor, but also as an anti-inflammatory, antioxidant, antiapoptotic molecule and stimulator of gastric mucus secretion [145]. Omeprazole promotes gastric epithelial cell migration [146, 147]. Biswas *et al.* [148] showed that it blocks stress-induced increased generation of $*OH^-$ and associated lipid peroxidation and protein oxidation, and also prevents DNA fragmentation indicating its antioxidant role in oxidative damage and antiapoptotic role in blocking cell death during ulceration. Omeprazole reduced the mucosal inflammatory changes elicited

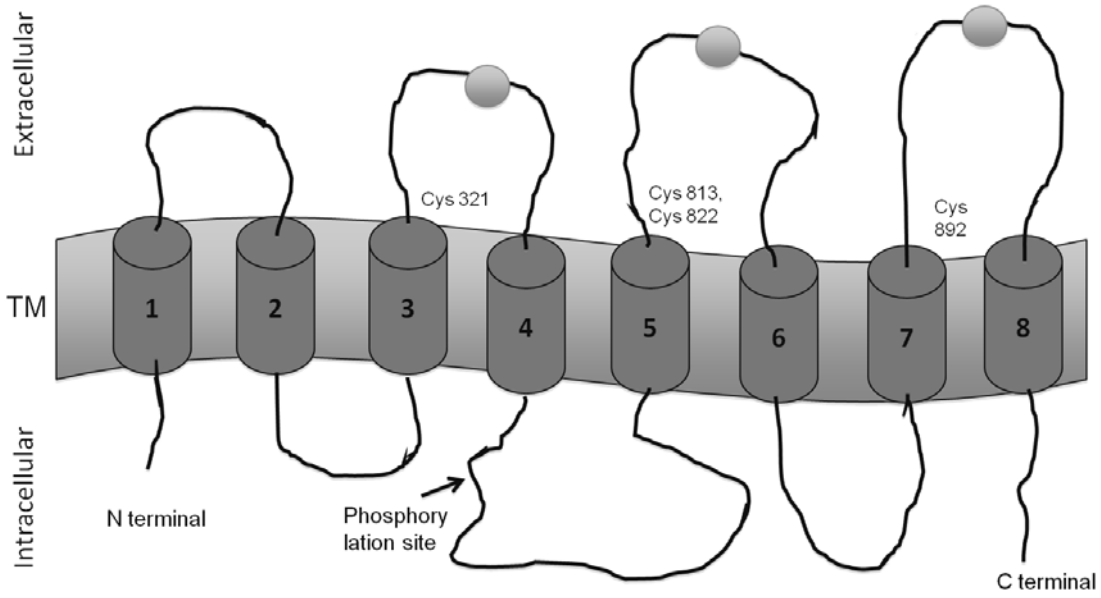

Figure 3. Representation of the common site of binding to the ATPase by the PPIs (●) illustrating reaction of the drugs with the cysteine at positions 813, 822, 892 and 321 in the amino acid chain of the α-subunit of the H^+K^+-ATPase and showing the cys 822 is deeper within the membrane domain. Both C- and N-terminals are intracellular and the bulk of the sequence is located in the cytoplasm of the parietal cell. TM = transmembrane.

by *H. pylori* lipopolysaccharides, decreased epithelial cell apoptosis, decreased caspase-3 activity leading to the inhibition of nitric oxide synthase-2 (NOS-2) [149]. Omeprazole-dosed rats have shown up-regulation of genes known to protect the cell against oxidative stress. These include endothelial PAS domain protein-1 (*Epas1*), carbonyl reductase-1 (*Cbr1*) and spleen tyrosine kinase (*syk*). Theseproteins are involved in the molecular mechanisms underlying acid-independent gastroprotective effects of PPIs [150].

4.2.2. Lansoprazole

Lansoprazole has a protective action on gastric mucosa. This is mediated by a decrease in oxidative stress and a concomitant increase in antioxidants resulting in an increased bioavailability of mucosal sulfhydryl compounds [151]. It is well known that mucosal cell apoptosis plays a crucial role in the pathogenesis of gastric ulceration as recent studies have shown that endoplasmic reticulum (ER) stress is an important pathway leading to cellular apoptosis [34]. Lansoprazole significantly promoted the cell restitution rate after wounding. The addition of MEK inhibitor significantly attenuated the cell restitution rate in the lansoprazole group suggesting that mechanism of cell proliferation and migration promoted by lansoprazole might involve the activation of p44/p42 MAPK [152]. It is proposed that lansoprazole does not exert modulatory effects on the gastric expression of COX isoforms as well as on the activity of NO pathways [153, 154]. Lansoprazole increased gastric mucosal PGE_2 and reduced gastric damage caused by ethanol [153]. A specific COX-2 inhibitor blocked the lansoprazole-induced increase in mucosal PGE_2 and mucosal protection. Activation of gastrin receptors by endogenous gastrin has a pivotal role in the effects of lansoprazole on COX-2 up-regulation and mucosal protection in the rat stomach [155].

4.2.3. Esomeprazole

Esomeprazole is the first PPI to be developed as an optical isomer for the treatment of patients with acid related disorders and it has been shown to reduce or prevent NSAID-induced gastrointestinal injury [156, 157]. The beneficial effects of esomeprazole can be ascribed largely to its ability to maintain sustained inhibition of gastric acid secretion at a steady-state than other PPIs [158], although there is evidence to suggest that

pharmacodynamic properties unrelated to acid inhibition also contribute to the gastroprotective effects of this agent [159]. Esomeprazole treatment was found to increase the level of PGE_2 in the glandular portion of the stomach [157]. In turn, this resulted in a release of phospholipids, as the gastric mucous cell is the source of surfactant phospholipids as well as mucin all of which play a critical role in the primary defense of gastric epithelium [160]. The healing by esomeprazole on indomethacin-induced gastric ulceration is ascribed to acid-dependent reduction of pro-apoptotic signaling and acid-independent restoration of proliferating/repairing pathways [161]. The acid-independent actions are related to decrease in tissue oxidation and apoptosis and to enhancement of nuclear factor-kB activation [162]. The indomethacin-induced activation of caspase-3 was prevented by esomeprazole [162].

4.2.4. Pantoprazole

Pantoprazole showed inhibitory activity on gastric ulcers induced by stress and alcohol, but was ineffective on pylorus ligation-induced ulcers. This observation indicates that PPIs, including pantoprazole, might reveal highly different effects according to the type of ulcer inducers [163]. The protection afforded by pantoprazole against NSAID-induced gastric damage depends on a reduction in mucosal oxidative injury, which may also account for an increment of sulfhydryl radical mucosal bioavailability. It is also suggested that pantoprazole does not influence the down-regulation of gastric prostaglandin production associated with NSAID treatment [164]. Like omeprazole and lansoprazole, pantoprazole not only binds at cysteine 813, but additionally at cysteine 822. Both of these sites are located in the proton transport pathway, though cysteine 822 is found deeper in the membrane domain than cysteine 813 [165].

4.2.5. Rabeprazole

Rabeprazole significantly inhibited the secretion of acid and pepsin from the stomach and increased gastric mucin secretion [147]. The observations made against dexamethasone plus pylorus ligation-induced ulcer model, showed that rabeprazole is the most effective gastric ulcer healing agent as compared to omeprazole and lansoprazole [147]. Moreover, it has an added benefit of having a consistent efficacy profile and low drug interaction potential due to its predominantly non-enzymatic metabolism [166]. Okazaki *et al.* [167] have shown that the COX-2 mRNA expression increased in the rabeprazole group more than that in the H_2RA group during the ulcer-healing stage. Rabeprazole has a cytoprotective effect against ethanol-induced gastric mucosal damage mediated via NO, but not via prostaglandins [168].

Among various substituted benzimidazoles including E3810, methoxy derivative of E3810, RO 18-5364, picoprazole and timoprazole, only a few have a good correlation between them [169]. E3810 has an antibacterial effect against *H. pylori* and is mediated through direct binding to *H. pylori* [170]. CS-526 has a curative effect on gastro-esophageal reflux disease via its potent antisecretory and antiulcer actions [171]. RO 18-5364 is a potent inhibitor of the gastric H^+K^+-ATPase [172].

PPIs not only block acid secretion, but also enhance mucosal protective factors including reduction of gastric oxidative injury and bioavailability of mucosal sulfhydryl compounds [173, 154].

4.3. Growth factors and peptides

4.3.1. Trefoil factors

Trefoil factor family (TFF) is a group of peptides synthesized and secreted by mucosal epithelia and composed of 3 members, namely TFF1, TFF2 and TTT3. In the gastrointestinal tract TFF peptides are involved in mechanisms of defense and repair by interacting with mucins to form the mucus barrier and to promote the process of restitution and healing [11, 174]. TFF increased the barrier properties of the pre-epithelial mucus gel [175, 176]. Mucin-1, a cell surface mucin is an important barrier to gastrointestinal infection. TFFs are pivotal for gastric restitution after surface epithelial damage. They activate epithelial repair via Na^+/H^+-exchangers (NHE2), which are implicated in cellular migration [177]. TFFs promote epithelial restitution via a novel mechanism that does not require cyclooxygenase activation [178]. In stress-induced ulceration, TFFs may not only participate in the early phase of epithelial repair known as restitution (marked by increased cell migration), but they also play an important role in the subsequent, protracted phase of glandular renewal made by cell proliferation [16, 179]. Growth

factors, such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), trefoil peptides, platelet-derived growth factor (PDGF) and other cytokines produced locally by regenerating cells, control re-epithelialization and the reconstruction of glandular structures [20]. These growth factors, most notably EGF, trigger epithelial cell proliferation via signal transduction pathways involving EGFR-MAP (Erk1/Erk2) kinases [180]. Vascular endothelial growth factor (VEGF), bFGF, and angiopoietins promote angiogenesis, thereby allowing reconstruction of microvasculature in the mucosal scar, which is essential for delivery of oxygen and nutrients to the healing site [181]. The primary trigger to activate expression of angiogenic growth factors and their receptors appears to be hypoxia. Angiogenesis is followed by proliferation of connective tissue fibroblasts that deposit collagen over which adjacent surviving and dividing epithelial cells migrate to complete the healing and also reduction of inflammatory cytokines [182]. Szabo *et al.* [183] have postulated that stimulation of angiogenesis alone might be sufficient to accelerate ulcer healing in the GI tract. The molar potency of angiogenic growth factors was 2-7 million times better than the antiulcerogenic effect of antisecretory H_2RAs.

4.3.2. EGF and TGFα

The presence of EGF receptors localized at the basolateral membranes of parietal cells [184-186] and the absence of apical EGF receptors in the gastric epithelium suggest that these peptides may act through the paracrine/autocrine pathways [128]. Scheiman *et al.* [187] suggested that the absence of apical EGF receptors in the gastric epithelium and TGFα that enters the lumen do not have any physiological function in the intact gastric mucosa. In turn, this explains the ineffectiveness of these peptides when given intraduodenally. Interaction of TGFα with the EGF receptor leads to tyrosine kinase activation and induces mitogenesis, suggesting TGFα and EGF stimulate peptide phosphorylation through the same EGF receptor system [188]. Moreover, the inhibitory activity of EGF requires all three disulfide loops to bring about significant binding and activation of the EGF receptors [189]. Similarly, polyamines namely spermine, spermidine and putrescine, have been shown to stimulate cellular growth and differentiation and they act as primary mediators of EGF-induced gastroprotection [190]. A correlation exists between regulation of cell proliferation and polyamine metabolism and their influence in the distribution of microtubules during damage *in vivo*, indicating a partial mechanism for the dependency of mucosal healing [191]. Polyamine protection depends on its anti-peroxidative properties [192] and mediation in epithelial cell proliferation [193].

TGFα, EGF and urogastrone provide cytoprotection against ethanol-induced lesions and moreover, they have a mitogenic effect. Bastaki and co-workers [189] found that the antisecretory and antiulcer effects of TGFα and EGF do not exert their effects in parallel and the antiulcer activity was unrelated to their ability to inhibit acid secretion. The dose-dependent inhibition of ulcer formation by acid-supplemented ethanol indicates that the protective effect of these peptides is unrelated to their ability to inhibit acid secretion. It was also speculated that the mechanisms may involve stimulation of PG secretion and/or NO release, subtle effects on mucosal blood flow, and changes in mucosal cell microenvironment. Another possible explanation is that the luminal membranes of non-secreting parietal cells may be more resistant to ethanol damage than those of actively secreting cells. The use of indomethacin pretreatment before application of an ulcerogen in animals can reduce the cytoprotection to zero, indicating that prostanoids could be involved in their cytoprotective action. In the same manner, N^G-nitro L-arginine methyl ester (L-NAME)-pretreated animals show reduction in their cytoprotective effects indicating the involvement of endogenous NO. TGFα, EGF and urogastrone are equally effective against ethanol-induced gastric ulcer and bring about their cytoprotective action through reduction of gastric acid secretion and through prostaglandin and NO pathways. The inhibitory effect of TGFα on dimaprit- and pentagastrin-induced gastric acid secretion suggests that the growth factor may interfere with protein kinase C, Ca^{2+} and cyclic AMP stimulus-secretion coupling pathways [189].

Growth factors are the most important enterotrophic molecules for both normal cell renewal and

healing after cell damage. They promote cell proliferation, stimulate cell migration and inhibit gastric acid secretion, thereby playing a pivotal role in ulcer healing. EGF family consists of four members namely TGFα, amphiregulin, heparin-binding EGF, poxvirus growth factors, cripto and heregulin and they all bind to the same EGFR. During the ulcer healing process of the antiulcer agent, rebamipide, significant up-regulation of pro-angiogenic genes encoding VEGF, heparin binding epidermal growth-like factor (HB-EGF), and fibroblast growth factor receptor-2 (FGFR2) were observed [194]. Heregulin is a new member of the growth factor family involved in the COX-2 dependent ulcer repair process [195]. Moreover, Guo *et al.* [196] have shown that an evolutionarily conserved enzyme called mammalian insulin-degrading enzyme (IDE) can degrade TGFα. This interesting observation has provided an explanation for the reversal of antisecretory activity to normal level within a short time.

4.3.4. BPC 157

BPC 157 is a peptidergic agent proven in clinical trials to be both safe in inflammatory bowel disease and wound healing and stable in human gastric juice, with no toxicity being reported. It has prominent effect on alcohol-, and NSAID-induced lesions [197] and it can improve healing of both the skin and stomach mucosa and closure of fistulas [198]. It protects against both acute and chronic alcohol-induced lesions in the stomach and liver [199]. The interactions of this pentadecapeptide with many important systems namely, dopamine-, NO-, prostaglandin-, and somatosensory neurone-systems, could provide a basis for the observed protective effects [200]. Continuous application of BPC 157 in chronic acetate-induced gastric ulcer accelerates rebuilding of glandular epithelium and formation of granulation tissue [201].

4.3.5. Ghrelin

Ghrelin is a gut-brain peptide, an endogenous ligand for growth hormone secretagogue receptor that regulates growth hormone secretion, increases appetite and contributes to energy homeostasis. Ghrelin reversed the ethanol-induced PGE_2 surge and reduced the increase in COX-2 expression, which concluded that COX-1 derived PGs are involved in ghrelin gastroprotection [202]. Ghrelin exerts a potent protective action on the gastric mucosa and accelerates the healing of I/R-induced lesions, and these effects depend upon activation of sensory nerves, hyperemia mediated by NO, increased angiogenesis due to expression of VEGF and the anti-inflammatory properties of this peptide [203]. Deactivation of sensory nerves with capsaicin or inhibition of constitutively expressed endothelial nitric oxide synthase (cNOS) by N^G-nitro-L-arginine (L-NNA) significantly attenuated the protective activity of ghrelin and accompanying increase in the GBF.

4.3.6. Leptin

Leptin is an ob gene product of adipocytes, and its receptors have been revealed in the gastric mucosa and pancreas. Moreover, both the gastric mucosa and the pancreas can release leptin upon stimulation with either CCK or gastrin. A number of studies have shown the involvement of leptin in the cytoprotection of gastric mucosa [204]. Konturek *et al.* [205] demonstrated that leptin accelerates ulcer healing by mechanisms involving the up-regulation of TGFα and increased production of NO due to up-regulation of cNOS and iNOS in the ulcer area. It protects against I/R-induced gastric injury through increasing tissue histamine content, which in turn maintains the gastric mucosal blood flow [206]. It was shown that either peripheral or central leptin administration reduced gastric lesions induced by ethanol, indomethacin or acetic acid and the ulcer prevention ability involves cyclooxygenase and NO pathways against ethanol-induced ulcers. Leptin is believed to act only via the cyclooxygenase pathway in indomethacin-induced ulcers [204] and through interference with neutrophil infiltration, NO production and oxidative stress [207].

4.3.7. HSP70

In recent years, heat shock proteins (HSPs) have been implicated to be an additional factor utilized for the gastric defense mechanisms at the intracellular level [208, 209]. HSPs are highly conserved ubiquitous proteins expressed by eukaryotic and prokaryotic cells, and hence, they are considered to have essential functions for the survival of cells and developmental process. HSPs are generally

considered to improve cellular recovery by either refolding partially damaged functional proteins or by increasing delivery of precursor proteins to important organelles. These include mitochondria and endoplasmic reticulum, through which they complete their efficient mucosal defense mechanisms and achieve ulcer healing, most probably protecting key enzymes related to cytoprotection [13]. HSPs, which function mainly as the molecular chaperones, have been shown to be involved in diverse biological activities such as rescuing from apoptosis, escape from carcinogenesis, protection from cytotoxic damages of NSAIDs and acceleration of ulcer healing. Heat shock protein 70 (HSP70) protects the gastric mucosa through inhibition of apoptosis, pro-inflammatory cytokines, and cell adhesion molecules (CAMs) [210].

Apoptosis in epithelial cells, as a result of mitochondrial injury, is an important pathogenesis, especially in indomethacin-induced gastric mucosal injury. It up-regulates the expression of gastric mucosal HSP70 and its over-expression potentiates resistance to apoptosis and oxidative stress in gastric epithelial cell injury [211]. Suemasu *et al.* [212] provided genetic evidence demonstrating that NSAID can induce gastric lesions which in turn can affect mucosal apoptosis that is mediated by the endoplasmic reticulum stress response. In turn, this leads to the activation of *Bax* and the expression of HSP70 resulting in an amelioration of gastric protection via an inhibitory effect on the activation of *Bax*. In addition, the over-expressed *Bax* can accelerate apoptotic cell death induced by cytokine deprivation. Moreover, the anti-apoptosis protein, survivin, promotes cell survival and mitosis [19]. It plays a mediatory role in the cytoprotection against ethanol-, NSAID- and *H. pylori*-induced gastric injury by a mechanism that is dependent on p34 (cdc2), the cell-cycle dependent kinase [213-215].

4.4. Calcium channel blockers (CCBs) in gastroprotection

Calcium channel blockers (CCBs) like nifedipine, verapamil (a phenylalkylamine), diltiazem (a benzodiazepine), nitrendipine (a dihydropyridine) and mibafradine (a T-type CCB) have been found to have anti-ulcerogenic activities against CRS-, I/R-, and ethanol-induced gastric lesions. Their protecting effects are associated with smooth muscle relaxation and increased mucosal blood flow (MBF).

4.4.1. Nifedipine

Nifedipine protected gastric mucosal injury induced by I/R and potentiated the protective effect of the antioxidant activity of alpha-tocopherol [216]. Nifedipine markedly prevented the acidified ethanol-induced gastric mucosal injury and increased the content of thiobarbituric acid-reactive substances in the injured mucosa showing that it possesses free radical-scavenging properties in rats [217]. Moreover, the unique effect of nifedipine in inhibiting mast cell degranulation has clearly demonstrated the potential value of this drug in the management of peptic ulcer disease [218].

4.4.2. Verapamil

Verepamil attenuated stress-induced gastric ulceration [219] and enhanced mucus secretion, reduced total acidity and lipid peroxidation and decreased non-protein sulfhydryl content [220]. Verapamil, its analogues devapamil and gallopamil, and nicardipine were studied against CRS-induced ulcers and they were found to promote healing by increasing gastric lipid peroxidation by decreasing glutathione levels [221].

4.4.3. Nitrendipine

Nitrendipine, a derivative of the dihydropyridine group of CCBs, was used in stress-induced ulcers and it was found to be an effective antiulcer agent [222]. Similarly, diltiazem inhibited the gastric secretion and lipid peroxidation induced by oxygen free radicals of gastric mucosa to enhance the antiulcer effect [223], but does not stimulate prostaglandin production by gastric cells nor does it increase the cellular level of protective sulfhydryls [224]. Moreover, diltiazem competed with luminal high affinity K^+ site of the H^+K^+-ATPase and K^+ stimulated p-nitrophenyl phosphatase reactions [225].

4.4.4. Flunarizine and cinnarizine

The gastroprotective effect of flunarizine involves inactivation potential of mitochondrial permeability transition pore opening. This process is normally

associated with anti-oxidative, calcium regulatory, as well as with anti-apoptotic effects [226]. In contrast, the effect of cinnarizine against ethanol-induced ulcers was investigated, but no clear mechanism for its protective action was observed [227]. It was also demonstrated that both flunarizine and cinnarizine had no significant effect on gastric histamine content [226, 227].

4.4.5. Mibefradil

Pretreatment of animals with mibefradil, a T-type CCB, significantly reduced ethanol-induced macroscopic, pathologic, and biochemical changes in the gastric mucosa [228]. Mibefradil plays a role in attenuating I/R injury of the small intestine by depressing free radical production and mucosal injury score. It also regulates post-ischemic intestinal perfusion while restoring intestinal microcirculatory blood flow and encountered histological injury [229].

4.5. CCK B/gastrin receptor antagonists

The gastrin/CCK receptors can mediate the physiological functions of gastrin in the stomach, including stimulation of acid secretion and cellular proliferation and migration. Gastrin exerts a pro-inflammatory effect in rats through CCK_2 receptor activation that contributes to the inflammation induced by *H. pylori*. This inflammation is believed to be associated with the transcription of CCK_2 receptors which can be increased by gastrin through PKC and MEK cascades. CCK_2 receptor expression increased progressively in the regenerating mucosa adjacent to the ulcer repair margin [230] and enhanced trophic effects during wound healing process [231]. PD-136450, an anxiolytic agent, is a cholecystokinin CCK_2/gastrin receptor antagonist which can evoke antiulcer activity against acidified ethanol-, indomethacin- and stress-induced gastric lesions acting through PG and NO pathways [153, 24, 232].

Several other CCK_2 receptor antagonists, like RP 73870, S-0509, CR 2945, YM022, L-365260, L-740093, and YF-476 were developed and tested for their antisecretory and antiulcer properties and most of them were found to be acting through antisecretory mechanisms. Relative to other CCK_2 antagonists, RP 73870 demonstrated greater affinity to gastrin binding sites, and possessed a unique spectrum of *in vivo* biological activities appropriate for an antiulcer indication [233]. Similarly, S-0509 was examined on acetic acid-induced ulcers and pylorus ligated rats and showed promising results in the treatment of peptic ulcers [234]. CR 2945 was as efficacious as ranitidine against indomethacin- and ethanol-induced gastric ulcers and cysteamine-induced duodenal ulcers [235]. YM022 prevented gastric and duodenal lesions induced by acidified ethanol, WIR, and CR-stress in rats and its action was mediated through PG pathway [236].

4.6. Adenosine receptor modulation (protective)

Adenosine is a purine nucleoside, which functions as a neuromodulator centrally, as well as peripherally. Gerber *et al.* [237] have shown that adenosine A_1 receptors are found in the gastric parietal cells and activation of these receptors results in an inhibition of gastric acid secretion. A_1 receptors inhibit cAMP-mediated gastrin release via a pertussis toxin-sensitive mechanism, whereas A_2 receptors potentiated the response to cAMP-independent stimulation of gastrin release. Enhancement of gastrin release by adenosine antagonists suggests functional restraint by endogenous adenosine [238]. Adenosine A_1 receptor agonist, 8-phenyl isopropyl-adenosine, reduced restraint stress-induced gastric lesions which was blocked by 8-phenyltheophylline, an antagonist of A_1 receptors. Moreover, Geiger and Glavin [239] have also shown that central nervous system (CNS) adenosine A_1 receptors are involved in blocking and methylxanthines in exacerbating stress-induced gastric pathology. Cho and Ogle [240] have demonstrated that the mechanism behind the gastroprotective effect of adenosine is to increase mucosal blood flow. The activation of adenosine receptors results in the release of somatostatin while at the same time it inhibits the release of gastrin [241]. Recently, adenosine A_{2a} and A_3 receptors have emerged as therapeutic targets in inflammatory bowel diseases and gastroprotection. Moreover, they can prevent purinergic receptor abnormalities. A specific adenosine A_{2a} agonist, ATL-146e, inhibits stress-induced gastric inflammation and damage. Adenosine A_{2a} agonist compounds may not only be useful for preventing ulcers, but may block gastric inflammation as well [242].

4.7. Dopamine receptor activation

Dopamine (DA) and its analogues have been shown to reduce ethanol- as well as stress-induced gastric lesions [243] through central and peripheral dopamine pathways. Dopamine agonists prevent, whereas antagonists augment stress and chemically induced gastrointestinal ulcers in preclinical models. Agonists of the peripheral DA_1 receptors present in rat stomach exerted potent anti-stress, antisecretory and mucus preserving effects. DA_1 antagonists worsened stress-induced ulcers and augmented acid secretion. Glavin and Szabo [244] explained the mechanism behind the protective effect of dopamine in the gut, demonstrating that it is mediated through the peripheral DA_1 receptors. The gastroprotective effect of amylin in reserpine-induced gastric lesions involves, at least in part, the dopaminergic transmission, interfering with both the DA_1 and DA_2 receptor subtypes [245]. Puri *et al.* [246] have shown the gastric cytoprotective role for DA and they further suggest that DA_1-DA_2 receptor interactions are crucial during dopaminergic regulation of gastric mucosal integrity during stress. Dopamine D_1 and D_2 receptors have opposing effects on gastric and duodenal ulcers. Stimulation of dopamine D_1 receptors by fenoldopam and SKF 38393 (D_1 receptor agonist) inhibits the formation of gastric and duodenal ulcers, whereas dopamine D_2 receptor antagonist sulpiride elicited significant reduction of ulcer index [247]. Glavin and Hall [248] showed that DA agonists, particularly DA_1/D_1 receptor, are powerful gastroprotective agents. Moreover, D4 receptor blockade by clozapine and activation of dopamine D_3 receptors by 7-hydroxy-N, N-di-n-propyl-2-aminotetralin (7-OHDPAT) are also associated with antisecretory and gastroprotective effects. Saxena *et al.* [249] have shown that risperidone and sulpiride, two D_2 receptor antagonists, exhibited marked gastroprotective effect against CRS-induced lesions, which is mediated by endogenous NO, sulfhydryl group, PG and ATP-sensitive K^+ channels. Hunyady *et al.* [250] have shown that out of five different subtypes, mRNA of D_5 (= D_{1b}) dopamine receptors are very abundant in the gastric epithelium and D_1 receptor-selective dopamine agonists have been shown to protect against experimental gastro-duodenal lesions induced by cysteamine. These workers concluded that D_5 receptor subtype is indeed likely to be involved in the protective effects of dopamine in the stomach. Roland and Grijalva [251] have also shown that apomorphine, a central and peripheral dopamine agonist, provided protection against lateral hyopothalamic (LH) lesion-induced gastric erosion formation. In contrast, domperidone, a peripheral dopamine antagonist had no effect, indicating that neurochemical mechanisms are involved in the development of erosions.

4.8. Neurotensin

Intracisternal administration of neurotensin (NT) prevents stress-induced gastric ulcers in rats, which is mediated by the central nervous system. Hernandez *et al.* [252] have shown that pre-treatment with intra-cerbroventricular haloperidol, a dopamine (DA) receptor antagonist, totally blocked the cytoprotective effect of NT. In addition, pretreatment with methylphenidate, a DA receptor agonist, produced cytoprotection similar to NT. On the basis of their experiments, they concluded that NT-induced cytoprotection is not mediated by either 5-HT, GABA, ACh (muscarinic) receptors, or endogenous opiate systems, but via interactions between brain DA systems and NT. However, this observation suggests an inter-relationship that may comprise the brain-gut-axis. The centrally-induced gastroprotective effect of neuropeptides may be partly due to a vagal-dependent increase of gastric mucosal resistance to injury. It is well known that activation of vagal cholinergic pathway can mediate the release of mucosal PG and NO. Furthermore, sensory neuropeptides (CGRP, tachykinins) released from capsaicin-sensitive afferent fibers are also involved in the centrally-induced gastroprotective effect of neuropeptides [253].

4.9. Opiates

Mu and *delta* opioid receptors exist in rat muscularis mucosa and submucosal plexus [254] and they have been identified through autoradiography. Morphine and encephalin analogues augmented CRS-induced gastric lesions and in some cases, morphine can protect the lesions [255]. Morphine has been shown to potentiate indomethacin-induced gastric lesions, which is mediated through the opiate receptors leading to an increase in leukotriene C4 and decrease in

PG-like activities in the gastric mucosa [256]. Morphine also has an anti-secretory activity, in addition to antiulcer activity, through its inhibition of gastrointestinal motility. Centrally administered morphine (opiates) can block the formation of stress ulcers. Naltrexone, an opiate antagonist, has a cytoprotective effect against stress-induced ulceration [257]. This effect appears to be due to blockade of peripheral, rather than central endogenous opiates and is not related to the central inhibitory effect of opiates on gastric acid secretion. Opiates have complex effects on gastric mucosal blood flow which may explain their role in stress ulceration.

Bilateral microinjections of the opiate antagonist naloxone into the central nucleus of the amygdala (CeA) produced a significant potentiation of CRS-induced gastric pathology in rats. The opiate agonist, beta-endorphin, on the other hand, can inhibit stress-ulcer formation in a dose-related manner. Attenuating effects can also be seen in intra-CeA injections of the enkephalin analogues [D-Ala2, D-Leu5] enkephalin and [D-Ala2] Met-enkephalinamide. Pretreatment of rats with naloxone completely antagonized and even reversed the gastric cytoprotective effects of beta-endorphin. This interesting observation indicates that the CeA is important in the gastric cytomodulatory effects of endogenous opiates during stressful experiences [258, 259].

Naloxone protected indomethacin- and HCl-induced ulcers, but not ethanol-induced ulcers. Morphine increased ulcers in HCl models, but not in the other two models. The ulcer aggravating effect of morphine in HCl model was blocked by naloxone, and both naloxone and morphine significantly decreased gastric acid secretion in pylorus- ligated rats [260] as well as CRS-induced rats by altering gastric motility [261]. Naloxone has a significant protective effect in stress-ulcer model and not in indomethacin- or cysteamine-induced duodenal ulcer models [262]. In pylorus-ligated rats, morphine affects gastric acidity through central and peripheral opiate receptors, whereas mucus synthesis appears to be regulated through peripheral opioid pathways [263].

The role of local sensory neurons in modulating the extent of gastric mucosal damage induced by close-arterial infusion of platelet-activating factor (Paf) has been investigated in the anaesthetized rat. Paf-induced gastric damage potentiated by morphine was inhibited by administration of the opioid antagonists, naloxone or the peripherally acting N-methyl nalorphine. Peripheral opiate-sensitive afferent sensory neurons seem to play a physiological defensive role in the mucosa, attenuating the extent of gastric damage induced by Paf [264].

CONCLUSION

The stomach is in a state of continuous exposure to potentially hazardous insults and agents. HCl and pepsin constitute major and serious threats and insults to the gastric mucosa. Reflux of alkaline duodenal contents containing bile and pancreatic enzymes, alcohol, cigarette smoking, aspirin and aspirin-like drugs, and steroids are among endogenous and exogenous mucosal irritants that can inflict mucosal injury. In addition, both food and ageing can also enhance gastric mucosa damage. The ability of the stomach to defend itself against these noxious agents/insults has been ascribed to a number of factors constituting the gastric mucosal defense. These include mucus and bicarbonate secreted by surface epithelial cells, prostaglandins, sulfhydryl compounds and gastric mucosal blood flow, the microcirculation in particular. These factors are considered by several researchers to be of paramount importance in maintaining gastric mucosal integrity. The exact mechanism(s) of action of the anti-ulcerogenic drugs is still under debate. There is much evidence that elevation or ‘shift’ in the cAMP:cGMP ratio is linked to the anti-ulcerogenic and cytoprotective processes in the stomach at cellular, subcellular and molecular levels. H_2RAs act through a mechanism that involves nitric oxide. Cimetidine and ranitidine (widely used H_2RAs) administered at doses that are too low to interfere with gastric acid secretion, cause an elevation in the cAMP:cGMP ratio, an effect that is also observed with other prostaglandin derivatives and anti-ulcerogenic drugs [265].

Multiple types of prostaglandin E synthases, including membrane-bound prostaglandin E synthase-1, mediate gastroduodenal bicarbonate secretion, a key process that aids in preventing acid-peptic injury. The bicarbonate stimulatory

effect of PGE_2 in the duodenum is mediated by both EP_3 and EP_4 receptors, coupled intracellularly with Ca^{2+} and cAMP, and EP_1 coupled with Ca^{2+} in the gastric mucosa [72].

H_2RAs (for example, ranitidine and cimetidine) induce mucosal defense through a number of regulatory processes involving the release of PG and NO which in turn inhibit neutrophil adhesion, reduce mucin and decrease gastric acid secretion, decrease lysosomal enzymes, increase bicarbonate secretion, increase gastric mucosal blood flow, improve restitution, augment CGRP release and act through capsaicin-sensitive afferent nerves. Likewise, PPIs bring about their mucosal defense by inhibiting acid secretion and promoting epithelial cell migration, cellular restitution and re-epithelialization. PPIs also have several important therapeutic roles including antioxidant, antiapoptotic, antibacterial effects against *H. pylori* and the up-regulation of genes against oxidative stress. Moreover, they can increase the endogenous levels of PGE2 and phospholipids in the mucosa. Its enzyme inhibition is superior to receptor antagonism. Growth factors, trefoil factors and certain peptides act through paracrine/autocrine pathways. They induce healing of gastric mucosal injury through restitution, cellular migration and cell proliferation via signal transduction pathways as well as COX2 dependent pathways. They induce angiogenesis to bring about the reconstruction of the epithelium. NO and CGRP, as well as some gut hormones including gastrin and CCK, and certain calcium channel blockers, all of which have been found to protect gastric mucosa against the damage induced by corrosive substances. This protective action of the gut hormones and peptides have been attributed to the release of PG, increased mucus secretion, inhibition of apoptotic proteins and the activation of sensory nerves, since all these effects could be abolished by the pretreatment with indomethacin or large neurotoxic dose of capsaicin. In turn, they can all be restored by the addition of exogenous PGE_2 or CGRP, respectively. The defensive mechanisms of actions for such peptides such as leptin, ghrelin, gastrin-releasing peptide and HSP70 involve cyclooxygenase and NO pathways, up-regulation of TGFα, cNOS and iNOS, and increase in gastric mucosal blood flow and angiogenesis. Subtypes of DA receptors (agonists and antagonists) also protect mucosal injury through activation of either central or peripheral dopaminergic pathways.

Dent *et al.* [266] surveyed thirty six such gastric mucosa defense mechanisms and the most support went to therapies aimed at enhancement of mucosal blood flow, epithelial restitution and mucosal alkaline secretion or inhibition of luminal pepsin activity. Gastric mucosal injury is a dynamic process, and further insights into these defense mechanisms will throw further light on the inadequate understanding of the processes of mucosal injury and repair, leading to safer therapeutic approaches in future.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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