Review of Literature
In the present chapter, we have reviewed the existing literature related to the occurrence, symptoms, risk factors and pathogenesis of peptic ulcer disease, followed by the detailed mechanism of ulcer healing with separate briefings on each of the factors involved. In this context, we have also reviewed all substantial findings highlighting the role of COX-2 in ulcer healing with a special reference to the shift from the classical COX hypothesis and systematic comparison of COX-1 and COX-2 enzyme at genetic, protein and functional level. Finally, we have cumulated different existing therapeutic strategies against PUD. In order to have the better understanding of the process of ulcerogenesis, we have mentioned the normal physiology of stomach, in terms of its anatomy, secretions and regulation at first.

2.1 Normal Physiology of Stomach

2.1.1 Gastric anatomy

The stomach is an expanded section of the digestive tube between the esophagus and small intestine, located in the upper quadrant of the abdomen. Anatomically, the stomach is divided into four parts - cardiac (narrow conical portion distal to gastroesophageal junction), fundus (dome shaped proximal stomach), body or corpus (remainder of stomach), and a pyloric part (pyloric antrum and pyloric canal) (Guyton and Hall, 1999). The medial curvature of stomach is known as lesser curvature while the lateral curvature is called as greater curvature. The wall of the stomach is structurally similar to other parts of the digestive tube, with an extra oblique layer of smooth muscle inside the circular layer. It is composed of four layers-mucosa, submucosa, muscularis propria and serosa (Lippin, 1985; Forte, 1986).

The mucosa membrane lining the stomach is thick and vascular carrying a smooth, soft and velvety surface. The submucosa comprising of a dense connective tissue layer, supporting large plexus of blood and lymph vessels along with meissner’s nerve plexus, is relatively unspecialized. The muscularis propria is a thick layer that is composed of three muscular layers: outer longitudinal, middle circular and inner oblique. Serosal layer is the visceral peritoneum of the stomach, containing blood vessels, adipose tissue and nerve trunk. In the empty state, the stomach is contracted and its mucosa and sub-mucosa are thrown up into distinct folds called
rugae. The characteristic shape of stomach describing its different parts and the layers is shown in Figure 2.1 (a and b).

Figure 2.1: Different parts and layers of the normal stomach

(a) Gross view of stomach with its different parts; (b) Different layers of normal stomach

The gastric epithelial lining consists of rugae that contain microscopic gastric pits, each branching into four or five gastric glands made up of highly specialized epithelial cells. The makeup of the gastric glands varies with their anatomic locations. The gastric mucosa has three important types of tubular glands: cardiac, oxyntic or parietal and pyloric gland (Valle, 2005).

(A) **Cardiac glands:** They are heavily branched tubular glands, which mainly contain mucus-producing cells. A few of the secretory cells characteristic of the principal glands are also often present.

(B) **Oxyntic glands:** These glands are located inside the surface of the body and fundus of stomach, comprising 80% of the total stomach. Four major types of secretory epithelial cells, as shown in Figure 2.2, cover the surface of the stomach are found in oxyntic gland (Ito and Winchester, 1963).
Figure 2.2: Diagrammatic representation of the oxyntic gland

(i) Surface mucus cells that extend into rugae and secrete a neutral proteoglycan (mucin), which protects the epithelium against shear stress and acid. The continuous shedding and replacement is found in this layer.

(ii) The parietal / oxyntic cells are located on the upper end of the glands and secrete hydrochloric acid. Beside from activating pepsinogen, hydrochloric acid also effectively sterilizes the contents of the stomach. The resting or unstimulated parietal cells have prominent cytoplasmic tubulovesicles and intracellular canaliculi containing short microvilli along its apical surface (Figure 2.3). $\text{H}^+\text{K}^+\text{ATPase}$ enzyme is expressed in the tubulovesicle membrane, which on cell stimulation, along with apical membranes, transforms into dense network of apical canaliculi containing long microvilli.
(iii) The chief cells/zymogen cells are present in the deepest part. The chief cells secret pepsinogen, an inactive precursor of protein digesting enzyme pepsin.

(iv) A group of endocrine cells are also present in the epithelial lining of the glands, which are called enterochromaffin-like (ECL) cells or argentaffin cells. These cells are the sole source of gastric histamine involved in acid secretion.

(C) **Pyloric glands:** The pyloric glands mainly consist of mucous neck cells along with endocrine cells such as gastrin secreting-G cells and somatostatin secreting-D cells. G cells possess gastrin-containing granules while D cells present in the antral region of the stomach secrets somatostatin that regulate acid secretion. Glucagon producing cells are also found in the stomach.

### 2.1.2 Secretions of Gastric fluids

Gastric juice contains enzymatic (pepsinogen, gastric lipase, amylase and rennin), non-enzymatic (mucin, hydrochloric acid) secretions, intrinsic factor, lactic acid and inorganic ions such as Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\), HPO\(_4\)\(^{2-}\), SO\(_4\)\(^{2-}\) and HCO\(_3\)\(^{-}\). Out of these constituents, hydrochloric acid, pepsinogen, mucus, intrinsic factor and bicarbonate ions (HCO\(_3\)\(^{-}\)) play major roles in various physiological conditions. Gastric glands secrete about 2.5 liters of juice per day in humans, which contains 97-99% water.
In addition to mucus secreting cells that line the entire surface of the stomach, the major secretory portion of the stomach mucosa is comprised of two important tubular glands: the oxyntic and pyloric gland as the major secreting regions. The oxyntic gland secrete hydrochloric acid, pepsinogen, intrinsic factor and mucus, while the pyloric glands secret mainly mucus, little pepsinogen and very important gastrin. Gastric secretion occurs in three phases: cephalic, gastric and an intestinal phase.

(i) **Hydrochloric acid**: Hydrochloric acid is secreted by the stimulated parietal cells. Capacity of the stomach to secrete HCl is almost linearly related to parietal cell numbers. One of the hallmarks of the stomach is its ability to secrete large quantities of concentrated (0.16 mol/L) HCl, pH 1.8-2.5 (Wolfe and Sachs, 2000). The primary function of hydrochloric acid is to kill and/or minimize ingested bacteria with a notable exception of _H pylori_. Hypochlorhydria and achlorhydria predispose and exacerbate the severity of bacterial and certain parasitic infection (Wallace and Granger, 1996). Gastric acid plays a significant role in protein hydrolysis and other aspects of the digestive process.

Hydrochloric acid is one of the most important offensive factors in ulcerogenesis, which not only regulates normal functioning of stomach but also plays an etiologic role in producing various forms of discomfort and inciting gastroduodenal mucosal injury.

(ii) **Pepsinogen**: Pepsinogen, an inactive zymogen is a precursor of proteolytic enzyme pepsin and is secreted into gastric juice from both mucus cells and chief cells. Once secreted, pepsinogen is activated by stomach acid into active protease pepsin, which is largely responsible for the stomach ability to initiate digestion of the protein. Pepsinogen (42,500 Dalton) gets cleaved in presence of HCl to yield active pepsin molecule (32,500 Dalton) (Van Vunakanis and Harriott, 1956). Pepsin acts on proteins in highly acidic medium (pH 1.8-3.5). It also autocatalyses conversion of pepsinogen to active pepsin and gets inactivated above pH 5.0. Pepsin also acts as one of the aggressive factors involved in gastric mucosal injury (Allen et al., 1991). However, the gastric juice contains both HCl and pepsin and their level vary in parallel, therefore, it is difficult to determine the relative importance of each in ulcerogenesis.
(iii) **Mucus**: The most abundant epithelial cells are mucous cells, which cover the entire luminal surface and extend down into glands as mucous neck cells. These cells secret bicarbonate rich mucus that coats and lubricates the gastric surface and serves an important role in protecting the epithelium from acid, pepsin and other chemical insults (Allen, 1981; Allen et al., 1986; Neutra and Postner, 1987). Mucus is present mainly in two forms: insoluble and soluble. Insoluble mucus is viscous, slippery and covers most of the surface of gastric mucosa and is continuously secreted by the surface and gastric pit cells. The insoluble mucus forms a semi permanent layer and shows permeation of acid from the lumen and bicarbonate from the underlying epithelial cells establishing an unstirred buffer zone, which protects the underlying cell. Soluble mucus (mucin) is a degradative product of insoluble mucus formed by peptic action. This mixes with and lubricates food during gastric motility.

Mucus consist of about 1% by weight of salts and other dialysable components, 0.5-1 % of free proteins and a similar quantum of carbohydrate rich glycoprotein and 95% or more of the water. The glycoprotein component of mucus is responsible for its characteristic viscous gel forming property, believed to be important for the functional role of mucus (Goel and Bhattacharya, 1991). Mucus gel is capable of maintenance of pH gradient so that the pH of the cell membrane is slightly alkaline (approximately 7.3) on the epithelial side of the mucus layer and acidic (approximately 2.0) on the luminal side (Allen and Garner, 1980) There are 600 carbohydrate side chains per molecule of native glycoprotein composed of hexoses, hexosamines, fucose and sialic acid.

(iv) **Bicarbonate**: Bicarbonate ions, secreted by the surface epithelial cells of mucosal lining of the gastric mucosa, forms a pH gradient ranging from 1 to 2 at the gastric luminal surface and reaching 6 to 7 along the epithelial cell surface. Secretion of alkali by surface epithelial cells into the unstirred layer affords far more protection than mucus alone. This constitutes the first line of stomach defense against the damaging action of gastric fluid. Bicarbonate secretion is stimulated by calcium, prostaglandins, cholinergic input and luminal acidification.
(v) **Hormones:** The principle hormone secreted from the gastric epithelium is gastrin, a peptide that is important in control of acid secretion and gastric motility. Gastrin is a linear peptide hormone secreted into the blood by G cells that are located mainly in the pylorus of the stomach. Gastrin is found primarily in three forms: gastrin-34 (big gastrin), gastrin-17 (little gastrin), and gastrin-14 (minigastrin). The numbers refer to the amino acid count. Gastrin is produced in response to certain stimuli. Among these stimuli are stomach distension, amino acid stimulation, vagal stimulation (mediated by the neurocrine bombesin), the presence of partially digested proteins, and hypercalcemia. The presence of gastrin stimulates parietal cells of the stomach to secrete hydrochloric acid (HCl). It also causes chief cells to secrete pepsinogen.

It is important to regulate the release of gastrin, as too much gastrin secretion would, in turn, cause too much acid to be secreted. Therefore, via a negative feedback enzyme mechanism, the presence of acid (primarily the secreted HCl) in the stomach inhibits further release of gastrin by the G cells. Somatostatin also inhibits the release of gastrin.

(v) **Intrinsic factor:** The intrinsic factor, essential for the absorption of Vitamin B₁₂, in the ileum is also secreted from the parietal cells along with hydrochloric acid. When the acid producing cells of stomach are destroyed, which frequently occurs in chronic gastritis, the person develops not only achlorhydria but also often pernicious anemia.

(vi) **Other enzymes:** Small quantities of other enzymes are also present in the stomach juice, including gastric lipase, gastric amylase and gelatinase. These enzymes play a role in the digestion process of different components of food.

### 2.1.3 Regulation of gastric secretions

Gastric acid secretion is a complex, continuous process controlled by multiple central (neural) and peripheral (endocrine and paracrine) factors (Schubert and Shamburek, 1990). Each factor attributes to a common final physiological event- the secretion of H⁺ by parietal cells. All three- Neuronal (acetylcholine, ACh), paracrine (histamine) and endocrine (gastrin) factors play important roles in the regulation of acid secretion as shown in [Figure 2.4](#). Their respective receptors (M₁ and M₃; H₂ and CCK₂) are localized on the basolateral membrane of the parietal cell.
The two major signaling pathways are present within the parietal cell: the cyclic AMP-dependent and the Ca$^{2+}$ dependent pathway. Histamine uses the first pathway, while gastrin and Ach exert their effect via later one. The cyclic-AMP pathway results in phosphorylation of parietal cell effector protein and the Ca$^{2+}$ dependent pathway leads to an increase in cytosolic Ca$^{2+}$. Both the pathways activate the H$^+$/K$^+$-ATPase (proton pump). This pump generates an intracellular pH of about 7.3 and an intracanalicular pH of about 0.8. (Hoogerwerf and Pasricha, 2001). All the three pathways under physiological conditions can be activated directly by stimuli originating in the brain or reflexively by stimuli originating in the stomach like mechanical stimulation (distention) or chemical stimulation (presence of proteins) after ingestion of a meal.
(a) Neuronal control

Neuronal regulation involves central and peripheral mechanisms. The most important structures in the CNS involved in central stimulation of acid secretion are dorsal motor nucleus of vagal nerve (DMNV), the hypothalamus and the nucleus tractus solitarius. Two nerve plexus are found on the stomach wall. Myenteric plexus is located between longitudinal and circular muscle layer and Meissner’s plexus is located on the submucosal layer. These are traversed by parasympathetic and non-cholinergic (NANC) neurons. These neurons collectively constitute the intrinsic nervous system, which helps in the regulation of gastric secretion. The neurons secrete noradrenaline (NA), Ach and vasoactive intestinal peptide (VIP) for controlling gastric acid secretion. Ach stimulates acid secretion while NA and VIP act as inhibitors of acid secretion (Pearse et al., 1977).

Efferent fibres originating in the DMNV descend to the stomach via vagus nerve and synapse with the ganglion cells of the enteric nervous system (ENS). Ach release from the postganglionic vagal fiber stimulates gastric acid secretion directly through the muscarinic cholinergic receptor (M₃), located on the basolateral membrane of the parietal cells. The CNS probably regulates the activity of ENS with Ach as its main regulatory neurotransmitter. CNS is also responsible for the initiation of gastric acid secretion in response to the sight, smell, taste and anticipation of food (cephalic phase).

(b) Hormonal regulation

The hormones of gastrointestinal tract include both endocrine and paracrine secretion. The basic neurotransmitters or hormones that stimulate secretion by the gastric glands are Ach, gastrin and histamine. Gastrin is an important endocrine secretion (Tache, 1988) whereas histamine is the most important paracrine secretion. Other hormones regulating gastric secretion are secretin and cholecystokinin (Guyton and Hall, 1999). Gastrin and histamine secreted by G cells and ECL cells/histaminocytes respectively, act as regulators of acid secretion through receptors on the parietal cells.
(i) **Acetylcholine:** Acetylcholine (Ach), a parasympathetic neurotransmitter is secreted by cholinergic neurons following vagus nerve stimulation. Ach activates parietal/acid secreting and chief/pepsinogen secreting cells by increasing the intracellular Ca\(^{2+}\) levels (Soll, 1981; Chew, 1985 and 1986). Ach exerts its effect by increasing IP\(_3\) (1, 4, 5- inositol trisphosphate) levels through a G-protein coupled pathway, which mobilizes Ca\(^{2+}\) from intracellular stores. Rise in Ca\(^{2+}\) or IP\(_3\) level correlates with the degree of stimulation of acid secretion from the parietal cells (Chiba et al., 1988).

Ach may also act on parietal cells directly through cholinergic M\(_3\) receptors present on the parietal cell membrane or it may act on endocrine cells of mucosa through M\(_1\) receptors. Activation of these receptors causes secretion of gastrin or histamine, which leads to the activation of parietal cells, and acid secretion.

(ii) **Gastrin:** Gastrin stimulates acid secretion from parietal cells either directly or indirectly by enhancing histamine release from ECL cells through gastrin/cholecystokinin (CCK\(_2\)) receptors (Waldum et al., 1991). Enhancement of inositol phospholipid turnover and the resulting increase in Ca\(^{2+}\) concentration play an important role in histamine releases from parietal cells induced by gastrin (Inomoto et al., 1993; Hersey and Sachs, 1995) indicating that similar type of signal transduction pathway is operative in both the cell types.

(iii) **Histamine:** Histamine is released from ECL cells through multifactorial pathways and is a critical regulator of acid production through the H\(_2\) subtype of receptor. A small amount of histamine is continuously formed in the gastric mucosa in response to acid in stomach. Histamine activates the parietal cell in a paracrine fashion. The ECL cells are the sole source of gastric histamine involved in acid secretion. Histamine binding to H\(_2\) receptor leads to an activation of adenylate cyclase, increases intracellular cyclic adenosine monophosphate (cAMP) content thereby causing activation of cAMP dependent protein kinase (Chew et al., 1989), which further activates proton pump. Involvement of histamine in gastric acid secretion has been convincingly demonstrated by the inhibition of acid secretion with the use of H\(_2\) receptor antagonists.
(c) **Paracrine regulation**

Somatostatin and PGE$_2$ secreted by the mucus cells of gastric mucosa act as inhibitors of acid secretion (Robert, 1976; Crutzfeldt and Arnold, 1978). Besides gastric inhibitory peptide (GIP), pancreozymin, enkephalins, VIP, 5 HT/serotonin and neurotensin secreted by gut also play a regulatory role in the gastric secretion.

### 2.2 Peptic Ulcer disease

Peptic ulcer disease (PUD) is one of the most prevalent gastrointestinal disorders that encompasses both gastric and duodenal ulcer. An ulcer is defined as the disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. The pathophysiology of PUD is due to an imbalance between offensive (acid, pepsin and *H pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) (Valle, 2005). PUD is caused in either of two ways:

(i) Excess secretion of acid and pepsin by the gastric mucosa or

(ii) Diminished ability of the gastroduodenal mucosal barrier to protect against the digestive properties of the acid-pepsin complex.

Gastric and Duodenal ulcers share many common features in terms of pathogenesis, diagnosis and treatment, but several factors and pathophysiology distinguish them from one another (Table 2.1). As in majority of duodenal ulcer, *H pylori* and NSAIDs are the causing agent. Basal and nocturnal acid output seems to be increased in duodenal ulcer whereas in gastric ulcer patients’ gastric acid output is normal or decreased. In both the cases impairment of defensive system is observed.
Table 2.1: Difference between gastric and duodenal ulcer

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Gastric ulcer</th>
<th>Duodenal Ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathophysiology</td>
<td>Decrease in defensive factors</td>
<td>Increase in acid production</td>
</tr>
<tr>
<td>Location</td>
<td>Lesser curvature of stomach</td>
<td>First portion of duodenum</td>
</tr>
<tr>
<td>Age</td>
<td>45-55 years</td>
<td>35-45 years</td>
</tr>
<tr>
<td>Gastric emptying</td>
<td>Delayed</td>
<td>Increased</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Epigastric pain increases with food intake, weight loss, hemmorrhagic lesion as a complication</td>
<td>Epigastric pain decreased with food intake, weight gain, bloating, excessive gas and nausea</td>
</tr>
</tbody>
</table>

2.2.1 Epidemiology of PUD

PUD represents a worldwide health problem because of its high morbidity, mortality and economic loss. This is probably most common chronic infection of humans. In the United States, approximately 4 million adults suffer annually from PUD. The available data states that approximately 15,000 deaths per occur as a consequence of complicated PUD. The financial impact of PUD has substantial, with an estimated burden on direct and indirect health care costs of ~10$ billion per year in the United States (Valle, 2005). In India, PUD is very common with about 10% of the total population gets affected with this gastro-intestinal disorder. High occurrence of PUD among Indians is also evident from the fact that antacids and anti-ulcer drugs share 6.2 billion rupees and occupy 4.3% market share of Indian Pharma pie. Occurrence of peptic ulcer in different countries corresponding to different geographical zones is shown in Table 2.2. For example in India ~14 million positive cases were reported out of ~1 billion tested (US Census Bureau, International Database, 2004).
<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Extrapolated Incidence</th>
<th>Population Estimated Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>North America</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>3,994,577</td>
<td>293,655,405</td>
</tr>
<tr>
<td>Canada</td>
<td>442,202</td>
<td>32,507,874</td>
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<tr>
<td><strong>Europe</strong></td>
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</tr>
<tr>
<td>Austria</td>
<td>111,200</td>
<td>8,174,762</td>
</tr>
<tr>
<td>France</td>
<td>821,947</td>
<td>60,424,213</td>
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<tr>
<td>Germany</td>
<td>1,121,217</td>
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<tr>
<td>Italy</td>
<td>789,752</td>
<td>58,057,477</td>
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<td>Switzerland</td>
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<td>7,450,867</td>
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<tr>
<td>United Kingdom</td>
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<td>60,270,708</td>
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<tr>
<td><strong>Asia</strong></td>
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<tr>
<td>Bangladesh</td>
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<td>China</td>
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<td>1,298,847,624</td>
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<tr>
<td><strong>India</strong></td>
<td>14,488,092</td>
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<tr>
<td>Japan</td>
<td>1,732,103</td>
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<td>Pakistan</td>
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<td>159,196,336</td>
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<tr>
<td>Sri Lanka</td>
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<tr>
<td><strong>Eastern Europe</strong></td>
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<td>Bulgaria</td>
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<tr>
<td>Russia</td>
<td>1,958,470</td>
<td>143,974,059</td>
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<tr>
<td><strong>Australasia and Southern Pacific</strong></td>
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</tr>
<tr>
<td>Australia</td>
<td>270,877</td>
<td>19,913,144</td>
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<tr>
<td>New Zealand</td>
<td>54,327</td>
<td>3,993,817</td>
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<tr>
<td><strong>Middle East</strong></td>
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<tr>
<td>Afghanistan</td>
<td>387,869</td>
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<td>Iran</td>
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<td>Iraq</td>
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<td>Israel</td>
<td>84,324</td>
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<td>Saudi Arabia</td>
<td>350,900</td>
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<tr>
<td><strong>South America</strong></td>
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<td>Brazil</td>
<td>2,504,316</td>
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<tr>
<td><strong>Africa</strong></td>
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<td>Kenya</td>
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<td>Nigeria</td>
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<td>12,575,356</td>
</tr>
<tr>
<td>South Africa</td>
<td>604,629</td>
<td>44,448,470</td>
</tr>
</tbody>
</table>
2.2.2 Symptoms of PUD

PUD is characterized by symptomatic heterogeneity where each individual may experience symptoms differently. Burning abdominal pain that exacerbated by fasting and improved with meals is a classical and most frequent symptom of PUD. Variation in the intensity or distribution of the abdominal pain as well as onset of associated symptoms such as nausea or vomiting may be indicative of an ulcer complication (Sanders, 1996). Other common symptoms include bloating and weight loss. Apart from specific PUD symptoms, PUD exhibits a variety of nonspecific symptoms that are collectively referred to as dyspepsia (symptom that are present even in the absence of ulcer), which is present in 30-50% of patient. Numerous studies have shown that the presence of dyspepsia is not indicative of an actual ulcer as determined by endoscopy. Symptoms that require immediate medical attention include sudden sharp pain, black or bloody tarry stools, or bloody vomit (Valle, 2005).

Complications of PUD

Complications of peptic ulcers include upper gastric bleeding especially in NSAIDs user, perforation and gastric outlet obstruction with the most common complication being upper gastric bleeding (Hawkins and Hanks, 2000). As a complication there is a hole in the wall of the stomach or duodenum, and bacteria and partially digested food can spill through the opening into the sterile abdominal cavity (peritoneum) and cause peritonitis, an inflammation of the abdominal cavity and wall. Other than this sometimes, ulcers located at the end of the stomach (where the duodenum is attached) can cause swelling and scarring, which can narrow or close the intestinal opening. This obstruction can prevent food from leaving the stomach and entering the small intestine, resulting in vomiting the contents of the stomach.

Gastrointestinal bleeding occurs in approximately 15% of patients. Continued bleeding of an untreated ulcer can lead to anemia and weakness and may require surgical or endoscopic intervention. Second most common ulcer related complication is perforation, being reported in 6-7% of PUD patients. Gastric outlet obstruction is the least common ulcer related complication, occurring in 1-2 % of patients (Valle, 2005).
The symptom-based diagnosis of PUD is not reliable so confirmed diagnosis is done either with radiographic (barium study) or an endoscopic procedure. Endoscopy is the most sensitive and specific approach for examining the gastric ulcer, as it not only provides direct visualization of the mucosa but also facilitates photographic documentation of a mucosal defect. Tissue biopsy for the detection of *H. pylori* is also done during the procedure of endoscopy.

### 2.2.3 Pathophysiology of PUD

Discontinuity in the gastric mucosa marks the major physiological event that is observed in gastric ulcer. Despite the constant attack on the gastroduodenal mucosa by noxious or aggressive agents (acid, pepsin, pancreatic enzyme, alcohol, smoking, ischemia, drugs as NSAIDs, and bacteria as *H. pylori*), integrity is maintained by an intricate system that is defensive system (mucus, bicarbonate, prostaglandins, blood flow and growth factors), which provides mucosal defense and repair (Abdel-Salam et al., 2001). Overall, the imbalance between the aggressive and defensive factors as shown in Figure 1.1 contributes in the causation of PUD.

**Aggressive factors:** These factors are those to which the gastric mucosa is constantly exposed and if removed, they would either prevent or greatly lessen the likelihood damage to mucosa. Details of various aggressive factors have been explained in detail in the section of “gastric secretions” and “risk factors of PUD”

**Defensive factors:** These are the factors or properties normally present endogenously that allow the stomach to resist the potentially noxious insults to which it is continually exposed. If one or more of these factors is missing or is present to a less than normal degree, the aggressive mucosal factors, which do not usually damage the mucosal lining, are likely to do so (Miller, 1987). Details of various defensive factors and their role in protection of gastric mucosa have been elaborated in the section of “gastric secretions” and “ulcer healing”.

### 2.2.4 Risk factors for PUD

Peptic ulcer disease is a chronic disease with a high rate of relapsing. As it is a multifactorial disease, lots of factors contribute in its progress. Development of peptic ulcer is attributed to the cumulative effect of several factors that acts synergistically to
cause gastric injury. The two predominant causes are *H. pylori* and NSAID ingestion. However, the major factors responsible for pathogenesis of ulcer vary from environmental, physiological, and genetic to bacterial infection. All these factors summarized in Figure 2.5 are discussed below in detail.

**Figure 2.5: Risk factors for Peptic Ulcer Disease**

- **Environmental factor**
  - Dietary habits
  - Smoking
  - Drugs
  - NSAIDs
  - Cortisosteroids
  - Coffee and alcoholic beverages
  - Stress
- **Psychological factor**
  - Altered gastric motility
- **Physiological factor**
  - Duodenal reflux
  - Acid hypersecretion and pepsin
- **Genetic factor**
  - **Bacterial factor**
    - *Helicobacter pylori*
      - Platelet activating factor
      - Lipid mediators
        - Leukotrienes
        - Thromboxane
      - Neuropeptides
        - Substance P
        - Endothelins
      - Biogenic amines
        - Histamine
        - Serotonin
- **Reactive oxygen species**
- **Endogenous mediators**
(A) Environmental factors

The environmental factor that leads to the pathogenesis of PUD includes various aspects of normal individual lifestyle that involves dietary habits, cigarette smoking and consumption of caffeine, alcohol and various drugs like NSAIDs and corticosteroids.

(i) Dietary habits: Diet has always been attributed to contribute in causation of PUD, where certain foods are reported to cause dyspepsia, but no convincing studies indicate an association between ulcer formation and specific diet. Large amounts of food should be avoided in ulcer patients because stretching or swelling of the stomach can result in painful symptoms. A diet rich in fiber may cut the risk of developing ulcers in half and speed healing of existing ones; however studies conducted with spices and peppers have shown conflicting results.

(ii) Smoking: Cigarette smoking is strongly associated with pathogenesis of inflammatory bowel diseases (Guo et al., 2001). Gastric and duodenal ulcers occur more frequently in smokers than in non-smokers. Ma et al. (2000) has shown that cigarette smoking is associated with occurrence and recurrence of PUD and delay of ulcer healing, yet the exact mechanism has not been fully elucidated. The adverse action shown by cigarette smoking may be because of stimulation of acid secretion, decreased bicarbonate secretion, alteration of blood flow or gastric motility, reduction of prostaglandins content and generation of free radicals. The major component of cigarette, nicotine, has been reported to tilt the balance of aggressive and defensive factors, favoring more towards aggressive factors (Wu and Chow, 2004). It has been also reported that H pylori infection is positively associated with cigarette smoking, which is further confirmed by the observation that success rate of H pylori eradication is significantly lower in smokers during the medication (Matsuo et al., 2003).

Smoking has also been shown to decrease the ulcer healing, impair response to the therapy and increase ulcer related complications such as perforation. Exposure to cigarette’s smoke delay healing by decreasing angiogenesis, which is associated with a reduction of constitutive NO synthase (eNOS) activity (Ma et al., 1999). Along with these deleterious effects, cigarette smoking has been found to impair EGF expression, which alters VEGF expression during ulcer healing process (Tsai et al., 1995).
(iii) **Drugs**

(a) **Non-steroidal anti-inflammatory drugs**

Several drugs are known to induce gastric ulcer. Most important among them are NSAIDs, which represents one of the most commonly used medications worldwide because of their broad range of applications. In 1989, Fries and his coworkers demonstrated that patients consuming NSAIDs are more prone to develop gastrointestinal complications than patients not using this agent. The spectrum of NSAID-induced morbidity ranges from nausea and dyspepsia to serious gastrointestinal complication such as frank peptic ulceration (∼15%) complicated by bleeding and perforation. About 20,000 people die each year from serious gastrointestinal complication from NSAIDs (Valle, 2005).

Chronic administration of NSAIDs produces gastroduodenal mucosal erosions in 35-60% of patients, ulcerations in 10-25%, and severe bleeding, hemorrhages or perforation in <1% (Hawkey, 1990). Moreover, NSAIDs exhibits considerable variability in their intrinsic potential to damage the gastroduodenal mucosa. Among the various NSAIDs, low incidence of gastroduodenal toxicity was observed with ibuprofen and high risk of side effects was observed with azapropazone. The NSAIDs spectrum with regard to their ability of causing gastroduodenal toxicity is shown in Figure 2.6 (Garcia and Jick, 1994; Henry et al., 1996).

**Figure 2.6:** Spectrum of NSAIDs according to their ability to cause gastroduodenal toxicity.

![Diagram showing the spectrum of NSAIDs](image)

Ibuprofen ... Diclofenac... Naproxen... Indomethacin .... Piroxicam ..... Azapropazone

(2-3)*  (4)*  (3-9)*  (6-11)*  (13-18)*  (23-31)*

("Numbers in the parentheses relate to the relative risk of peptic ulcers")

The adverse side effect of NSAIDs on gastric mucosa is through inhibition of cyclooxygenase (COX) pathway of PG synthesis. This is the basis of anti-inflammatory action but is also responsible for the development of side effects in other gastrointestinal actions. As inhibition of PG synthesis is central to both the beneficial
and toxic effects of NSAIDs, hence PGs are often regarded as “Double edged sword” (Cipolla et al., 2002).

Other mechanism through which NSAIDs cause gastromucosal injury is independent of COX inhibition, which mainly includes: neutrophils, gastric hypermotility, microcirculatory disturbances, oxygen derived free radicals and luminal acid (Singh, 1998; Wolfe et al., 1999). Different mechanism through which NSAIDs contributes to the genesis of mucosal damage are shown in Figure 2.7.

Figure 2.7: Mechanism of NSAID induce gastric injury

- **Epithelial injury**
  - Due to Prostaglandin depletion
    1. Mucin ↓
    2. HCL secretion ↑
    3. HCO₃↓
    4. Surface active phospholipid secretion ↓
    5. Mucosal proliferation ↓

- **Direct Toxicity**
  - Ion Trapping

- **Microvascular Injury**
  - Increased adhesion molecule expression ↓
  - Neutrophil adherence ↓
  - Stasis ↓
  - Microvascular Ischemia ↓
  - Free radical formation ↓

**Acid (HCl)**

**Erosion**

**Gastric Ulcer**
NSAIDs cause gastric injury by various different means such as topical application increases permeability of the mucosa, allowing aggressive factors access to the mucosa. Most of the NSAIDs are weak organic acid so in the acidic milieu they are converted to more lipid soluble unionized acids that penetrate into the gastric epithelial cells. There at neutral pH, they are reionized and trapped within the cell causing local injury (Hogben et al., 1957). Other action of NSAIDs on different cell types (Scheiman, 1996; Halter et al., 2001) is exhibited in Figure 2.8.

**Figure 2.8: Diagrammatic presentation of mechanism of action of non-steroidal anti inflammatory drugs**

- **Topical irritant properties**
  - Increase in gastrointestinal permeability
  - Drug trapment in gastric cells
  - Uncoupling in oxidative phosphorylation in mitochondria
  - Loss of cytoskeletal control over tight junctions
  - Decrease in gel hydrophobicity
  **Overall effect:** Gastrointestinal erosions/ulcers

- **Inhibition of repair mechanism**
  - Inhibition of cell proliferation
  - Increase in cell apoptosis
  - Inhibition of angiogenesis
  **Overall effect:** Impairment of repair

- **Damage to Blood vessels**
  - Increase in adhesion molecule
  - Accumulation of leukocytes
  - Injury to endothelial cells
  **Overall effect:** gastro-intestinal erosion

- **Inhibition of PG synthesis**
  - Decrease in mucus and bicarbonate secretion
  - Vasoconstriction
  **Overall effect:** Impairment of mucosal defense and repair

- **Various other effects**
  - Inhibition of other enzymes as phospholipase
  - Reactive oxygen species
  - Direct effect on gene
  - Interaction with iNOS and NO

- **Inhibition of thromboxane production in platelets**
  - Inhibition of platelet aggregation.
  - Increase in bleeding time
  **Overall effect:** Gastrointestinal bleeding
(b) **Corticosteroids**

The treatment with large doses of corticosteroids (Ogilhara et al., 1991) and glucocorticoids (Tsukada et al., 1994) is associated with an increased incidence of ulceration and has reported to have different adverse effects on gastric mucosa (Bandyopadhyay et al., 1999). Long-term treatment with dexamethasone has been shown to increase gastric secretion and acidity (Gray et al., 1951), which may be because of degranulation of the mast cells that releases histamine, responsible for increased acid secretion (Rasmussen, 1962). Prednisolone has been shown to cause more than 3 folds increase in pepsin secretion in dogs (Tsukada et al., 1994).

Glucocorticoids are also reported to delay ulcer healing (Carpani de Kaski et al., 1995) as they deplete prostaglandins and VEGF, which inhibits angiogenesis (Luo et al., 2004).

(iii) **Coffee and alcoholic beverages**

Nicotine (Qui et al., 1991), alcohol (Macmath, 1990) and caffeine containing beverages such as tea, coffee etc (Parmar et al., 1985) also leads to mucosal injury and gastric ulceration.

Ethanol (50-100%) rapidly penetrates the gastric mucosa and apparently causes plasma membrane damage, that result in increased membrane permeability leading to intracellular accumulation of sodium and water. The leaky membrane is a well-known stage in cell injury. When the increased membrane permeability fails to maintain the normal electrolyte distribution between extra-cellular and intracellular compartments, the massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. In the gastric mucosa these changes results in cell death and exfoliation in the superficial epithelium, i.e., erosion (Laine and Weinstein, 1988). Further, gastric lesions caused by ethanol have been attributed to free radical damage, which results in lipid peroxidation products (Pihan et al., 1987; Szelenyi and Brune, 1988). Role of several factors like products of arachidonate metabolism, e.g. leukotrienes (Peskar et al., 1986) and mast cell secretory products (Oates and Hakkinen, 1988) have been demonstrated in ethanol-induced gastric ulcers. Neutrophils migration also contributes significantly in the gastric damage induced by ethanol.
(B) **Physiological Factor**

(i) *Altered gastric motility*

Gastric motility regulates the gastric emptying of its content into duodenum. It is controlled centrally by vagus stimulation and locally by neuro-hormonal control of muscles. Factors that influence gastric motility may be myogenic, neural and chemical. Peristaltic movements are increased with vagal activity, gastrin and motilin whereas these movements get decreased with gastric secretion. Changes in these movements create a high-pressure gradient at gastroduodenal junction with pyloric sphincter playing a central role in the co-ordinate activity of emptying its content into duodenum. Incapability of pyloric sphincter may further lead to gastric and duodenal ulcer.

(ii) **Duodenal reflex**

Under normal conditions, duodenal contents are prevented from back-diffusion into stomach by the pyloric sphincter but defective motor activity may allow passage of duodenal contents in stomach. Duodenal contents include bile acids, salts, lysolcithin and pancreatic enzymes which have been implicated in the pathogenesis of gastritis and gastric ulceration. Duodenal content also stimulate secretion of gastrin in the stomach, which leads to hyperacid secretion thereby causing gastric injury (Hunt, 1983) and also duodenal contents damage the gastric mucosal barrier covering epithelial lining, which results in increased diffusion of hydrogen ions thereby further reducing the protective layer and predispose the gastric lining for ulceration.

(iii) **Acid hypersecretion and Pepsin**

Acid hypersecretion and peptic activity are two of the most important factors in ulcerogenesis. The importance of hydrochloric acid in the pathogenesis of gastric ulcer is very well established. It aggravates the mucosal lesion mainly by back diffusion and by activating pepsinogen to pepsin, which increase the size of the lesion by its proteolytic action (Leonard and Allen, 1986). Acid back diffusion releases histamine, which causes inflammation and further increase in the acid output to cause more gastric damage. However, mucosal injury by luminal H⁺ may not normally occur unless the integrity of the mucosa is disrupted by other aggressive factors along with the loss of mucosal cytoprotection. The ultimate result is the damage of mucosa with
rupture of capillaries, interstitial hemorrhage and formation of frank mucosal lesion with bleeding into the gastric lumen (Morris and Wallace, 1991). Drugs cannot heal mucosal lesion caused by any of these damaging factors or due to the loss of cytoprotection, unless the acid secretion is blocked.

(C) **Psychological stress**: Stress ulceration is associated with clinical condition like trauma, head injury (Cushing’s ulcer), burns (Curling’s ulcer), shock, sepsis and neurological disorder (Vaile, 2005); and is now regarded as multifactorial phenomenon. It is reported to result from interactions between mucosal, vascular and neuro-humoral factors and the autonomic nervous system (Desai et al., 1997). Elevation of acid production and breakdown of protective barrier plays an important role in the pathogenesis of stress-induced ulcers.

Stress causes both sympathetic and parasympathetic stimulation of stomach, which induces increased motility and muscular contraction leading to vascular compression and mucosal ischemia. Stimulation of gastric mucosa, due to stress is transmitted by cerebral marginal system and hypothalamus to the medulla oblongata and spinal cord. Medulla oblongata stimulates the vagus, which increases the gastric secretions and augments gastric motility (Heelihy, 1994). The spinal cord causes the stimulation of splanchnic nerve to produce a disturbance in circulation due to functional constriction of the gastric vessels; which leads to diminution of gastric blood flow. The function of anterior pituitary also gets disturbed due to stress releasing adrenocorticotropic hormone (ACTH), which ultimately leads to increased gastric secretions and reduced gastric mucosal resistance. Circulatory disturbances and the nutritional deficiency are thus induced in the local tissue, which is then followed by a rapid appearance of a deep ulcer (Desai et al., 1997).

Electrical stimulation of different regions of the limbic area modulates gastric acid secretion, motility and mucosal blood flow- all of which are important factors for stress ulcer development. The central nervous system and more importantly, the brain-gut axis are important mediators of stress ulcerogenesis. Many more neurotransmitters are involved in stress induced ulcers namely dopamine, norepinephrine, acetylcholine, gamma-aminobutyric acid and several other neuropeptides.
Das and Banerjee (1993) has shown role of oxygen free radicals in stress induced ulcerogenesis, which is associated with increase lipid peroxidation, inactivation of gastric peroxidase and activation of superoxide dismutase. Each of these conditions is suitable for generation of H₂O₂.

(4) Genetic factors

The role of genetic predisposition has not been completely understood, but it is also considered to play a role in ulcer development. First degree relatives of the duodenal ulcer patient are three times more likely to develop an ulcer; however, the potential role of H pylori infection remains a major consideration. Increased frequency of blood group O and non-secretor status have also been implicated as genetic risk factors for peptic diathesis (Valle, 2005). Some studies suggest that hereditary factors are important in the pathogenesis of peptic ulcer disease, as a higher prevalence of PUD is seen in certain genetic syndromes. However, the exact possibility of peptic ulcer as a genetic trait is not yet clearly understood.

(5) Bacterial infection

Gastric infection with the bacterium H pylori accounts for the majority of PUD. This organism also plays a role in the development of gastric mucosal associated lymphoid tissue (MALT) lymphoma and gastric carcinoma (Blaser, 1992). Although the entire genome of H pylori (1.65 billion base pairs, encoding ~1500 proteins) has been sequenced but still the exact pathogenic mechanism of this organism is not clear.

The prevalence of H pylori varies throughout the world and depends to a great extent on the overall standard of living in a particular region. In the developing part of the world, 80% of the population may be infected by the age of 20 years, whereas the prevalence is 20-50% in industrialized countries. Approximately 90-95% and 70-75% of gastric ulcers and duodenal ulcers are attributable to infection with H pylori (Ernst, 2000). Two factors, which predispose to higher colonization rate of H pylori include poor socioeconomic status and less education.

In 1983, Warren has first time demonstrated the presence of H pylori in gastritis. H pylori is a small spiral shaped, flagellated, gram -ve organism that resides under microaerobic conditions in a neutral microenvironment between the mucus and
the superficial epithelium of the stomach (Ernst, 2000). Motility, urease enzyme and adhesions pedestals are important factors for the colonization of bacterium inside the host (Hazel et al., 1986; Doig et al., 1991; Turbett et al., 1992). *H pylori* neutralize the acid secreted in the gastric lumen through the ammonia produced by the enzyme urease. The organism remains in the stomach for long periods, possibly for life with its ability to be inaccessible to the host immune defenses (Lee et al., 1993), immune invasion (Blaser, 1992) and by the secretion of substances that stimulates inflammatory mediators of the host body (Hatz et al., 1992).

*H pylori* induced mucosal damage is characterized by active inflammation accompanied with epithelial damage (Godwin et al., 1986), reduced blood flow (Cho, 1994), decrease bicarbonate production, neutrophils infiltration of the gastric epithelium (Nielsen and Andersen, 1992) and microvascular leakage (Kurose et al., 1994). Neutrophil activation induced by *H pylori* provokes release of free radicals and reactive oxygen species such as superoxide (Mooney et al., 1991), and NO (Mc Call et al., 1989), which interact together to form free radicals and thereby causing cell injury. *H pylori* is also known to intensify the activities of protease, phospholipase and lipase, as a result of which mucosal cell permeability is increased and that predispose the mucosal cell to the hydrochloric acid.

The inflammatory response to the *H pylori* includes recruitment of neutrophils, lymphocytes (T and B), macrophages, and plasma cells. The pathogen leads to local injury by binding to class II MHC molecule expressed on epithelial cell surface leading to apoptosis. *H pylori* infection can also lead to both mucosal and systemic humoral responses, which does not lead to eradication of the bacteria but further compounds the epithelial cell injury. *H pylori* infection might induce increased acid secretion through both direct and indirect actions of H pylori and proinflammatory cytokines (IL-8, TNF and IL-1) on gastrin (G), delta (D) and parietal cell.

(F) **Endogenous mediators**

Several substances that are formed endogenously under normal physiological conditions and by various inflammatory processes have been implicated in gastric ulceration. These can be lipid metabolites, amines, reactive oxygen species, proinflammatory cytokines or chemoattractants.
(a) Lipid mediators

(i) Leukotrienes:

Leukotrienes are synthesized from arachidonic acid (Figure 2.9). Leukotrienes exert a number of proinflammatory effects on the gastric mucosa and are involved in mediating NSAIDs and ethanol induced gastric damage (Peskar et al., 1986; Wallace et al., 1989).

Figure-2.9: Synthesis of various lipid mediators by lipid membrane

Leukotrienes are potent vasoconstrictors of gastric mucosal and submucosal circulation and stimulator of granulocytes margination into gastric microcirculation (Ivey, 1988; Wallace, 1992). Leukotrienes also induce pepsinogen secretion from chief cells (Fiorucci et al., 1995). Leukotrienes increase reactive oxygen species contributing to mucosal injury (Vaananen et al., 1992).
(ii) Platelet activating factor (PAF):

PAF is released by most of cell types and can exert effects on a wide range of target cells and organs (Synder, 1990). Among its most potent actions, is ability to modulate smooth muscle tone and to activate neutrophils. PAF is also reported to be extremely potent ulcerogenic agent (Rosam et al., 1986). PAF stimulates leukocyte adherence to the vascular endothelium and activates granulocytes to release reactive oxygen metabolites (Wallace et al., 1990; Sun et al., 1996). PAF can themselves act as adhesion molecule.

(iii) Thromboxane A₂:

Thromboxane A₂ is generated by the sequential degradation of arachidonic acid by the enzyme cyclooxygenase and thromboxane synthetase (Figure-2.9). Thromboxane A₂ is a very potent vasoconstrictor and renders gastric mucosa susceptible to the injury by gastric irritants. It also produces ischemia, which in tum causes injury due to decreased supply of nutrients and energy. Takahashi et al. (1999) has also demonstrated that thromboxane A₂ synthesis in the stomach was significantly elevated in ulcerated tissue, and a thromboxane synthase inhibitor markedly accelerated ulcer healing by promoting regeneration of the mucosa

(b) Neuropeptides

Neuropeptide includes a variety of peptides that are found in neural tissue; e.g. substance P and endothelins. Substance P reduces mucosal blood flow and influences leukocyte activities (Payan, 1989). It has also been demonstrated to cause mast cell degranulation resulting in the release of histamine (Goetzi et al., 1985), thromboxane A₂ and oxygen free radicals (Hartung et al., 1986). These changes lead to mucosal injury. Endothelins are secreted from vascular endothelial cells. They exert their vasoactive and growth regulatory action through G-protein coupled receptors (Slomiany et al., 2000). Role of endothelins has been demonstrated in ethanol and aspirin induced gastric ulcers, which act by potentiating acid secretion and vasoconstriction (Wallace et al., 1989) and in ischemia reperfusion induced injury (Hassan et al., 1997).
(c) Biogenic amines

Biogenic amines are a group of naturally occurring biologically active amines, such as norepinephrine and serotonin that act primarily as neurotransmitters and are capable of affecting various physiological processes. Nonepinephrine/NA, a central nervous neurotransmitter, plays a major role in stress induced ulcers. Evidence based on the various studies suggests that it has a protective role in stress induced gastric pathology (Glavin et al., 1991). Serotonin/5-hydroxytryptamine (5-HT) is secreted from endocrine/ECL cells present in gastric mucosa and is a potent vasoconstrictor, which results in reduced mucosal blood flow thereby rendering gastric mucosa susceptible to injury (Ohta et al., 1999). It is known to play a role in ethanol induced ulcers by reduction in mucus secretion.

(G) Reactive Oxygen Species

Oxygen taken in by the aerobic organism is fully reduced to water during the process of mitochondrial respiration. However, a small percentage (5%) of the O$_2$ consumed is converted to semireduced species i.e., the superoxide anion radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and the hydroxyl radical (OH$^-$). These species are collectively referred as reactive oxygen species (ROS) or free radicals. They are highly reactive chemical species with unpaired electron on their outer orbit (Cheeseman and Slater, 1993).

Involvement of ROS in pathogenesis of gastric ulceration is well established (Perry et al., 1986). A growing body of experimental and clinical evidence suggests that gastric mucosal damage by ethanol (Szelenyl and Brune, 1988), NSAIDs (Yoshikawa et al., 1993), H pylori (Davies et al., 1994) and stress-induced ulcers are all ROS mediated. Lipid peroxidation in the membrane has been shown to be initiated by hydroxyl radicals (Halliwell and Gutterdge, 1990).

In biological system, oxygen free radicals are either derived from the xanthine oxidase hypoxanthine reaction (Granger, 1988) or reduced by NADPH oxidase system located on the neutrophils membrane (Robinson et al., 1984). Activation of neutrophils by microorganism or receptor ligand binding leads to augmentation in production of free radicals by neutrophils (Konturek et al., 1993). Role of neutrophils generated free radicals has been implicated in the ischemia and reoxygenation induced
gastrointestinal mucosal damage (Yoshikawa et al., 1989; Grisham et al., 1990). Neutrophil may also cause microvascular damage by action of released oxidants or protease (Elasbach and Weiss, 1988).

2.3 Defense system of gastric mucosa

The gastric epithelium is under a constant assault by a series of endogenous noxious factors including HCl, pepsin and bile salts etc. In addition, a steady flow of exogenous substances such as medications, alcohol and bacteria also encounter the gastric mucosa. The ability of gastric mucosa to resist injury by endogenous secretions and by ingested irritants is attributed to a number of factors, which are collectively termed as mucosal defence (Wallace, 2001). These factors collectively constitute five lines of defense that acts either against ulcerogenesis or mediates ulcer healing. Each of these factors is best characterized in stomach. Structurally, the gastric mucosal defence system is divided into three barriers: pre-epithelial, epithelial and subepithelial as shown in Figure 2.10. In pre-epithelial barrier, first level of defence consisting of acid, bicarbonate and mucus secretion plays its role. The second level of defence occurs in epithelium, damage to epithelium can be repaired very quickly through a process known as restitution, which involves migration of healthy epithelial cells from the gastric pits over the denuded region. Several growth factors including EGF, bFGF and transforming growth factor (TGF) modulate the process of restitution. PGs play a central role in gastric epithelial defense system. The elaborated microcirculation in subepithelial barrier system, representing the third level of mucosal defense, is the key component at this level. A rich microvascular blood supplies HCO₃⁻, which neutralizes the acid generated. Moreover, this microcirculation provides adequate supply of micronutrients and oxygen while removing toxic metabolic by products. The fourth level of defence is mucosal immune system that consists of various immunocytes residing in the lamina propria. Finally, fifth and the last level of defence come into play when an ulcer is formed, where it constitute and promote ulcer healing or repair mechanism. The ulcers are repaired through growth and redevelopment of gastric glands, growth of new blood vessels (angiogenesis), and reinnervation of the mucosa by extrinsic and intrinsic nerves (Wallace and Ma, 2001).
2.4 Healing of gastric ulcer

Prevention of gastric mucosal lesions and the mechanism of gastric ulcer healing are multifactorial processes (Sandor et al., 1995). The gastric mucosa develops efficient tissue injury repair mechanism whenever gets exposed to aggressive insults (Susuna et al., 2004). The gastrointestinal tract, containing a complex interplay of epithelial, vascular cells and connective tissue, is one of the most complicated structure of the organism. Pathogenesis of gastric ulcer involves sequential or parallel damage to several components. Repair process includes contributions from epithelial, endothelial and connective tissue cells as these are the main responders to mitogenic and migratory stimuli (Szabo et al., 1998).

The cellular mechanism of mucosal repair/ulcer healing depends on the depth of the lesion i.e., erosion versus ulcer. Erosion is superficial damage to the mucosal surface, which does not reach to muscularis mucosa. Gastric or duodenal ulcers are deep necrotic lesions penetrating through the entire mucosal thickness and muscularis
mucosa (Tarnawski et al., 2001). The process of mucosal repair is therefore divided into two important phenomenon– restitution and regeneration (Table 2.3) where former is mainly confirmed to repair of erosion while later one is for ulcer repair.

Table-2.3: Lesions and their repair in gastrointestinal tract

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Defect</th>
<th>Repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erosion</td>
<td>Epithelial/Mucosal</td>
<td>Mainly restitution</td>
</tr>
<tr>
<td>Ulcer</td>
<td>Mucosal and smooth muscle</td>
<td>Involves regeneration:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♦ Granulation tissue formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♦ Angiogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♦ Connective tissue formation</td>
</tr>
</tbody>
</table>

Epithelial restitution refers to rapid cell migration (from surviving gastric mucus neck cells) to cover the superficial mucosal defect caused by intraluminal damaging agents (Lacy and Ito, 1984) and is a part of natural defense mechanisms. It can not be directly stimulated by gastroprotective agents such as PGs etc, but if these compounds maintain microvascular integrity and mucosal blood flow, the energy dependent epithelial restitution will passively but rapidly ensues (Szabo et al., 1985; Pihan et al., 1986).

Regeneration refers to cell division or multiplication and may involve epithelial, endothelial and mesenchymal cells. If the mucosal injury is superficial (e.g., erosion), the lost tissue is replaced by the original (e.g., epithelial) cells and if the damage is extensive, in the form of deep ulcer, the lost tissue is replaced by granulation tissue which consists of rapidly proliferating fibroblasts, capillaries, deposited collagen and chronic inflammatory cells (lymphocytes, plasma cells etc). Eventually as the healing progress, the loose granulation tissue becomes a dense fibrous framework, which provides the basis of re-epithelialization by restitution and regeneration (Szabo et al., 1995).

2.5 Ulcer healing dynamics

Ulcer healing, an active and complicated process of filling the mucosal defect by proliferating and migrating cells (Perini et al., 2003), involves inflammation, tissue formation (granulation tissue formation, angiogenesis and re-epithelialization) and
tissue remodeling. The ulcer healing curve (healing dynamics) follows a gradual ulcer size reduction characterized by an organic decay curve (Halter et al., 1995). This curve has mainly three phases: early lag phase, a rapid healing phase and late lag phase as shown in Figure 2.11.

Figure 2.11: Dynamics of ulcer healing

Vascular, microvascular changes, endothelial injury, thrombi formation and vascular constriction are the earliest events occur during the development of ulcer.

(i) The early lag phase is a phase when the healing mechanism dominates the ulcerogenic factors. It is initiated by replacement of necrotic tissue. Once the mucosa and submucosa become necrotic, polymorphonuclear leukocytes (PMNL), mainly neutrophils and macrophages are attracted to the injured area by a variety of signals such as growth factor released from platelets and fibrin degradation. The early lag phase ends when granulation tissue forms below the ulcer crater. Granulation tissue develops at the ulcer base within 48-72 hrs of ulceration (Tarnawski et al., 1990) and is essential for ulcer healing because it supplies microvessel for the restoration of microvasculature within the ulcer scar
and connective tissue to restore the lamina propria (Tarnawski et al., 1990, 1993).

(ii) The transition from the initial lag phase to the phase of rapid healing is characterized by migration of regenerated epithelial cells to re-epithelialize the ulcer crater and by intensive epithelial cell proliferation in the ulcer margin. During the rapid healing phase, granulation tissue undergoes continuous remodeling and changes in the cellular composition. This phase mainly involves cell migration, proliferation and angiogenesis. Initially macrophages and inflammatory cells are found in abundant, while at the later stage fibroblasts dominate (Tarnawski et al., 1991). PMNL phagocytize necrotic tissue and releases pro-inflammatory cytokines such as tumor necrosis factor (TNF-α) and interleukin (IL-1α and IL-1β), which in turn activate local fibroblasts, endothelial and epithelial cells (Martin, 1997). Macrophages remove necrotic debris by phagocytosis and when activated, release a variety of growth factors and cytokines (Benett and Schultz, 1993; Martin, 1997). Macrophages are essential for ulcer healing as evident by the fact that inhibition of macrophage infiltration impairs and delays ulcer healing. Mature granulation tissue consisting of proliferating connective tissue cells, i.e., fibroblasts, macrophages and endothelial cells forms new blood vessels through angiogenesis. Granulation tissue gradually allows reepithelialization of the ulcer bed. Migration of actively proliferating cells in the ulcer margin is also observed in this phase. The transition from the rapid healing phase to late lag phase is characterized by shrinkage of the granulation tissue in the ulcer bed, which gets converted into fibrous tissue.

(iii) In the late lag phase also called as early remodeling phase, the ulcer is completely reepithelialized by poorly differentiated epithelial cells. The granulation tissue becomes mature scar tissue and glandular architecture gets slowly reconstructed. Division and differentiation of mature specialized cells like parietal, mucus or chief cells, occur in late remodeling phase. The sequential events that occur during the process of ulcer healing are mentioned in Table 2.4.
Table 2.4: Time sequence of gastric ulcer healing

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Phase</th>
<th>Morphological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>Ulcer development</td>
<td>Generalized tissue necrosis, prominent inflammatory infiltration of serosal layer.</td>
</tr>
<tr>
<td>4-5</td>
<td>Early lag phase</td>
<td>development of granulation tissue, formation of ulcer margin</td>
</tr>
<tr>
<td>5-12</td>
<td>Rapid ulcer healing</td>
<td>Rapid migration of epithelial cells, intensive proliferation of epithelial cells, intensive angiogenesis in granulation tissue, contraction of granulation tissue in ulcer bed</td>
</tr>
<tr>
<td>12-20</td>
<td>Early remodeling phase, late lag phase</td>
<td>Complete re-epithelialization of the ulcer crater, reestablishment of glands, connective tissue and microvessels in the scar</td>
</tr>
<tr>
<td>20-100</td>
<td>Late remodeling phase</td>
<td>Maturation and differentiation of specialized (parietal, mucus, chief) cells</td>
</tr>
</tbody>
</table>

2.6 Factors and mechanisms involved in ulcer healing

The complex sequence of events that occur during the ulcer healing requires a high degree of co-ordination and is regulated by several factors and mechanism. Important factors include luminal factors (H⁺ secretion, pepsin, mucus and bicarbonate secretion) and ulcer healing promoting factors such as prostaglandins, growth factors, cell proliferation and blood flow etc, whereas restitution and angiogenesis are important mechanism involved in the process of ulcer healing. Tissue repair is initiated with aggregation of platelets, formation of fibrin clot and the release of growth factors from the activated coagulation pathways, injured cells, platelets and extracellular matrix followed by migration of inflammatory cells to the ulcerated site. Thereafter epithelial cells migrate over the damage tissue angiogenesis is initiated where fibroblasts deposit and remodel the granulation tissue.

2.6.1 Growth factors

Ulcer healing involves cell migration, proliferation and angiogenesis. All these processes are triggered and regulated by a sequential expression of growth factors.
(Tarnawski et al., 2001) that stimulates reconstruction of damaged mucosal architecture. Growth factors are synthesized and secreted by many types of cells involved in tissue repair including platelets, inflammatory cells, fibroblasts, epithelial cells and vascular endothelial cells. These growth factors may act on the producer cells (autocrine stimulation), adjacent cell (paracrine stimulation) or distant cells (endocrine stimulation).

All the growth factors initiate their effects by binding to and activating specific, high affinity receptor protein located in the plasma membrane of target cells. Activation of the receptors eventually results in stimulating a number of processes including those involved in the ulcer healing (Bennett and Schultz, 1993). Specificity and potency of different growth factors vary (Szabo and Vincze, 2000). There are five major growth families that contribute significantly to the ulcer healing process namely epidermal growth factor (EGF), transforming growth factors-β (TGF-β), Insulin growth factors (IGF), platelet derived growth factors (PDGF) and fibroblast growth factor (FGF).

1. **EGF family**

The EGF family comprised of epidermal growth factor (EGF), transforming growth factor-α (TGF-α), ampiregulin and heparin binding epidermal growth factors. These peptides are similar in structure, bind to same cell membrane receptor (EGF receptor) and have similar, but not identical biological effects.

1.1 **EGF:**

EGF, a 6 KDa pleiotropic polypeptide, is structurally related to human β-urogastrone, which is a potent inhibitor of stimulated gastric acid secretion (Cohen and Carpenter, 1975; Gregory, 1975). Plasma level of EGF is undetectable, but the platelets contain substantial level of EGF (approximate 500pmol/10^{12} platelets) (Pesonen et al., 1989). After clotting, the concentration approaches to 130pmol/Lt, which is sufficient to induce mitosis and migration of cells. This suggests that EGF released by platelets acts locally during the early phase of ulcer healing. EGF provides a variety of gastrointestinal protective effects along with acceleration of ulcer healing. Further, EGF stimulates migration and division of epithelial cells and increases the
synthesis of proteins such as fibronectin that aid in cell attachment and migration (Nishida et al., 1984; Ma et al., 2000).

Gastric mucus enhances the binding of EGF and other growth factors to their receptors (Szabo and Hollander, 1989). EGF has been demonstrated to stimulate gastric mucus synthesis (Yoshida et al., 1987; Kelly and Hunter, 1990).

1.2 TGF-α:

TGF-α is synthesized by a large variety of normal cells including activated macrophages, hepatocytes, gastrointestinal cells and brain cells. TGF-α shares 30% amino acid identity with EGF. TGF-α function by autocrine/paracrine mechanisms during the later stages of healing. Like EGF, TGF-α is a acid stable protein that stimulates epithelial cell migration (Barrandon and Green, 1987), promote cell proliferation (Takagi et al., 1992) and suppress acid production (Konturek et al., 1984; Rhodes et al., 1986; Lewis et al., 1990). All together these observations suggest that EGF and TGF-α play a pivotal role in the maintenance of mucosal integrity and participate in the reparative events following acute and chronic gastric mucosal injury.

1.3 Heparin binding EGF:

It has approximately 40% homology to other members of EGF family (Higashiyama et al., 1991). Although not much is known about the role of heparin binding EGF in ulcer healing, but its production by macrophage and its ability to bind a component of extracellular matrix suggest that it may be involved in healing process.

2. TGF-β family

Members of TGF-β family are synthesized by a wide variety of cell including platelets, macrophage, lymphocytes, fibroblasts and bone cells. Nearly all cells have TGF-β receptors. Thus, TGF-βs are probably the most broadly acting of all the families of growth factors. Activation of TGF-β during ulcer healing is thought to occur by additional proteolytic processing by plasmin or by exposure to the low pH environment of the ulcerated tissue (Sato and Rifkin, 1989). Two of the important actions of TGF-βs in the context of tissue repair are their ability to stimulate synthesis
of extracellular matrix and to increase mucus secretion. These properties suggest that TGF-βs are important regulator of in-vivo ulcer healing (Bennett and Schultz, 1993).

3. IGF family

Both IGF-I and IGF-II are synthesized by a wide range of tissues. IGF-I is found in substantial levels in platelets and is released during clotting along with the other growth factors present in platelets and is a potent chemotactic agent for vascular endothelial cells (Grant et al., 1987). IGF-I released may promote migration of vascular endothelial cells into ulcerated area, resulting in increased neovascularization.

4. PDGF family

The PDGF family comprises two major proteins: platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) (Raines et al., 1990; Westermark., 1990). These two growth factors are important component of ulcer healing process. PDGF and VEGF have similar structures, but they bind to different receptors and stimulate different actions.

4.1 PDGF:

PDGF molecule (24 KDa) shows various pharmacologic effects both in-vitro and in-vivo. It acts as mitogen for connective cells, glial cells, chondrocytes, vascular smooth cells, endothelial cells and epithelial cells (Hart et al., 1990). Platelets are the major source of endogenous PDGF that migrate at the site of gastric lesion and releases PDGF, which then becomes available to stimulate repair process (Guglietta et al., 1992) and enhances healing by stimulating polyamine synthesis (Thyberg and Fredholm, 1987), activating phospholipases releasing free arachidonic acid, formation of new eicosanoids mainly PGs (Raines et al., 1990), modulation of ion reflux (Paris and Pouyssegur, 1984) and thereby stimulating angiogenesis.

4.2 VEGF:

VEGF/ vascular permeability factor (VPF) is a 45 KDa dimeric protein; synthesized by vascular endothelial cells and exerts both acute (increased vascular permeability) and chronic actions (angiogenesis) (Ferrara and Davis-Symth, 1997; Pimentel, 1994). It is a major regulator of endothelial cell proliferation, angiogenesis and capillary permeability in both physiological and pathological process. VEGF plays a dual role in both acute gastroprotection as well as in chronic ulcer healing. The
slightly increased vascular permeability accompanies by acute gastroprotection, which is due to the perivascular dilution barrier, while the subsequent endothelial cell proliferation and migration (i.e., angiogenesis) are followed by enhanced granulation tissue production and ulcer healing.

5. FGF family

FGF family consists of at least seven structurally related polypeptides. Three proteins of FGF family are thought to be important regulators of ulcer healing: acidic FGF (aFGF), basic FGF (bFGF) and keratinocyte growth factor (KGF) (Baird and Bohlen, 1990; Gospodarowicz, 1990). Both aFGF and bFGF are potent mitogen and they share many similar biochemical and biological properties. They are stored within the basal membrane or extracellular matrix and are released in active form to stimulate tissue repair and healing (Nakamura et al., 1991). bFGF stimulate proliferation of epithelial cells, induces cell migration, neovascularization and formation of granulation tissue in animal models (Konturek et al., 1993).

Overall, the four important growth factors: bFGF, EGF, VEGF and PDGF influence different parameters, which alter the rate and extent of gastric ulcer healing (Szabo et al., 1995). A comparison of the effect of these growth factors on gastric ulcer healing mechanism is summarized in Table 2.5.

Table 2.5: Comparison of bFGF, PDGF, EGF and VEGF in ulcer pathogenesis, prevention and healing

<table>
<thead>
<tr>
<th>Actions</th>
<th>bFGF</th>
<th>PDGF</th>
<th>EGF</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric acid secretion</td>
<td>No change</td>
<td>No change</td>
<td>Inhibition</td>
<td>No</td>
</tr>
<tr>
<td>Duodenal alkaline secretion</td>
<td>No</td>
<td>No</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Angiogenesis stimulation</td>
<td>Potent</td>
<td>Mild</td>
<td>No/Mild</td>
<td>Potent</td>
</tr>
<tr>
<td>Smooth muscle regeneration</td>
<td>Yes</td>
<td>?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Neural regeneration</td>
<td>Yes</td>
<td>?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Epithelial restitution</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Epithelial proliferation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Secreted normally</td>
<td>No</td>
<td>±</td>
<td>Yes</td>
<td>±</td>
</tr>
<tr>
<td>Released after injury</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Acute gastroprotection</td>
<td>No</td>
<td>Mild</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Chronic gastric ulcer healing</td>
<td>Potent</td>
<td>Potent</td>
<td>Mild</td>
<td>Potent</td>
</tr>
</tbody>
</table>
2.6.2 Restitution

The rapid proliferation of mucosa plays a pivotal role in mucosal protection both under normal conditions as well as after mucosal damage. The term restitution refers to the epithelial repair process, which involves rapid migration of healthy cells over the denuded basement membrane (Silen and Ito, 1985). The process of ulcer healing is initiated by restitution whose response can be observed in-vivo as well as invitro (Wallace and Mcknight, 1990). In-vitro restitution depends on supply of HCO$_3^-$ to the basolateral membrane, whereas in-vivo restitution is mainly dependent on uninterrupted mucosal blood flow (Wallace and Granger, 1996).

Epithelial restitution is a fundamental protective mechanism that allows the gastrointestinal mucosa to reestablish its functional and structural integrity following the superficial injury. When the epithelium is damaged and luminal pH is low, basement membrane that acts as a template along with restituting epithelial cells, migrate to the site of damage. This is accomplished by the formation of mucoid cap over the site of damage that consists of cellular debris, mucus and plasma (including proteins such as fibrin and albumin). The mucoid cap provides a microenvironment conducive to epithelial restitution. The pH within the mucoid cap can be maintained at a greater than 5, despite the luminal pH being 1. The maintenance of the relatively high pH microenvironment is dependent on continuous supply of blood to this region. Certain growth factors such as bFGF (Paimela et al., 1993) and TGF-β (Dignass et al., 1994) have been shown to influence process of restitution.

2.6.3 Mucosal blood flow

Microcirculation / mucosal blood flow is one of the important component of mucosal defence system. Regulation of blood flow plays a very significant role in the maintenance of the integrity of gastric mucosa and protection against further mucosal injury. Underlying the surface epithelium of the stomach is a dense network of capillaries. In addition to supplying nutrients and oxygen to the epithelium, the microcirculation also removes, dilutes and neutralizes toxic substances that diffuse into mucosa from the lumen. The mucosal vascular architecture is ideally suited for the delivery of bicarbonate to the epithelium.
Mucosal blood flow is extensively modulated by nervous system and number of inflammatory mediators (Wallace and Ma, 2001). Among the most important of these are calcitonin gene-related peptide (CGRP), PGs and nitric oxide (NO). CGRP and NO play critical roles in mediating an essential component of mucosal defense. A constant delivery of plasma from the sub-epithelial blood vessels is crucial to the maintenance of a repair – conducive microenvironment. Even a very brief interruption of blood flow results in a rapid decrease in the pH at the site of injury, leading to disruption of the repair/healing process and progression of damage to deeper layers of the mucosa (Wallace and Mcknight, 1990). In addition to NO, PGs are also important factors in the maintenance of blood flow during the restitution process.

2.6.4 Inflammatory mediators

Inflammation of the mucosal layer of the gastrointestinal tract is not only associated with ulceration, but also plays an important role in healing of ulcers (Wallace and Ma, 2001). When all the superficial level of mucosal defence fails, the next level of defense, which comes to play its role, is acute inflammatory response. Most important inflammatory mediators include NO, eicosanoids (PG, LT, TX), neuropeptides, cytokines and proteinases. Many of these mediators are considered potential therapeutic targets for the treatment of ulcerative diseases. Mast cells and macrophages residing within the lamina propria act as “alarm cell”, sense the presence of foreign substance and liberates an array of inflammatory mediators and cytokines that can alter blood flow and enhance the recruitment of granulocyte in the affected region (Wallace and Granger, 1996). In Table 2.6, important inflammatory mediators and their effect on the gastric mucosa have been summarized.

Neutrophils are recruited from the circulation to the site of injury to facilitate repair and to reduce the entry of microbe into the systemic circulation (Granger and Kubes, 1994). Release of chemotactic factors such as leukotriene B4 and PAF from the mucosal immunocytes such as mast cells and macrophages is the key signal that leads to the extravasation of neutrophils and their migration towards the site of injury. In addition to the remaining damaged cells, foreign matters and microbes, neutrophils also participate in the formation of granulation tissue, which is critical for repair process.
Table 2.6: Mechanism of action of inflammatory mediators on the gastric mucosa

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Primary Source</th>
<th>Effect on blood flow</th>
<th>Effect on leukocyte</th>
<th>Other actions</th>
<th>Resistance to mucosal injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE\textsubscript{2}</td>
<td>Most cells</td>
<td>↑</td>
<td>Inhibits adherence and activation</td>
<td>Inhibits acid secretion, promotes mucus and bicarbonate secretion.</td>
<td>↑</td>
</tr>
<tr>
<td>PGI\textsubscript{2}</td>
<td>Endothelial cells</td>
<td>↑</td>
<td>Inhibits adherence and activation</td>
<td>Inhibits platelet aggregation.</td>
<td>↑</td>
</tr>
<tr>
<td>NO</td>
<td>Endothelial, mast and epithelial cells</td>
<td>↑</td>
<td>Inhibits adherence; scavenges free radicals, Inhibits acid secretion</td>
<td>Promotes mucus secretion, Promotes tissue repair</td>
<td>↑</td>
</tr>
<tr>
<td>TXA\textsubscript{2}</td>
<td>Platelets</td>
<td>↓</td>
<td>No effect</td>
<td>Increases platelet aggregation.</td>
<td>↓</td>
</tr>
<tr>
<td>LTB\textsubscript{4}</td>
<td>Neutrophils</td>
<td>⇔</td>
<td>Chemotactic for neutrophil, Releases free radicals</td>
<td>Promotes TNF, IFN, IL-2 release</td>
<td>⇔</td>
</tr>
<tr>
<td>LTC\textsubscript{4}/D\textsubscript{4}</td>
<td>Mast cells</td>
<td>↓</td>
<td>Increases vascular permeability</td>
<td>Contracts smooth muscle</td>
<td>↓</td>
</tr>
<tr>
<td>PAF</td>
<td>Mast and endothelial cells</td>
<td>↓</td>
<td>Increases vascular permeability and release of free radicals</td>
<td>Chemotactic for eosinophils</td>
<td>↓</td>
</tr>
<tr>
<td>Histamine</td>
<td>Mast and ECL cells</td>
<td>↑</td>
<td>Increases vascular permeability</td>
<td>Stimulates acid secretion</td>
<td>↑</td>
</tr>
<tr>
<td>Endothelins</td>
<td>Endothelial cells</td>
<td>↓</td>
<td>Increases vascular permeability</td>
<td>Contracts smooth muscle</td>
<td>↓</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>Mast cells, macrophage</td>
<td>⇔</td>
<td>Promotes adherence</td>
<td>Cytotoxic</td>
<td>↓</td>
</tr>
</tbody>
</table>
2.6.5 Nitric Oxide

In the gastrointestinal tract, there is considerable controversy regarding the predominant role of NO: protective or damaging. NO is synthesized inside the cells by semi essential amino acid, L - Arginine in presence of enzyme nitric oxide synthase (NOS) and performs various different functions in the body. The constitutive forms of NOS, neuronal NOS (nNOS) and endothelial NOS (eNOS) are very important in the normal functioning of the gastrointestinal tract. On the other hand, the inducible NOS (iNOS), which produces large amounts of NO under certain pathological conditions, contributes to mucosal injury and dysfunction (Fischer et al., 1999).

Suppression of NO synthesis renders the gastric mucosa more susceptible to injury (Whittle et al., 1990) as it performs various different functions in the gastrointestinal tract. NO plays a protective role through its vasodilating property that helps in regulating the mucosal blood flow and gastrointestinal motility (Fischer et al., 1999). NO also serves as a neurotransmitter in NANC neurons (Stark and Szurszewski, 1992) in animals as well as in humans, protects the stomach from microvascular injury (Fischer et al., 1999), inhibits the gastric acid secretion mediated through efferent vagocholinergic pathway (Yokotani et al., 1997; Kitamura et al., 1999) and stimulates mucous secretion via cGMP- mediated pathway (Brown et al., 1993). NO is also been shown to stimulate PG synthesis in cultured rabbit cells (Uno et al., 1997).

Involvement of NO has also been implicated in cytoprotective mechanism as well as drug mediated ulcer healing. Cytoprotection provided by sucralfate (Konturek et al., 1992), CGRP (Clementi et al., 1994), aluminum containing antacids (Konturek et al., 1992), teprenone (Nishida et al., 1998) and lansoprazole (Murakami, 1996) has suggested the involvement of NO in drug mediated cytoprotection. Konturek et al. (1993) and Akiba et al. (1998) have found delayed healing following inhibition of NOS, whereas Brzozowski et al. (1997) has shown that L-Arg, a NO precursor, potentiates the ulcer healing process.
2.6.6 Angiogenesis

Angiogenesis or neovascularization is formation of new blood vessels from the existing one and is very essential factor for tissue regeneration and ulcer healing. Angiogenesis occurs through the migration and division of endothelial cells, which gradually forms a tubular structure that eventually becomes the new blood vessel. It is a complex process involving extensive interplay between cells, soluble factors and extracellular matrix (ECM) components. The construction of a vascular network requires different sequential steps which include release of proteases from activated endothelial cells, degradation of the basement membrane surrounding the existing vessel, migration of the endothelial cells into the interstitial space, proliferation of endothelial cell, generation of new basement membrane, fusion of the newly formed vessels followed by blood flow.

Just as there are array of growth factors that drive epithelial proliferation, there are numerous factors that regulate the process of angiogenesis. Endothelial cells and vascular smooth muscle cells produce some of these factors in the region of injury. Other angiogenic factors are delivered at the site of injury via the platelets (Susuna et al., 2004).

Angiogenesis is fundamental process involved in ulcer healing. Stimulation of angiogenesis in granulation tissue has been shown to accelerate ulcer healing. Blood vessels are especially important during tissue injury. Following acute gastric mucosal necrosis such as erosion or ulcer, it is very important to reconstruct the microvascular network. When there is ulceration, blood delivers nutrients, growth factors, and immunocytes to the site of injury, whereas waste products are removed from there. The growth of new microvessel through angiogenesis is promoted by angiogenic growth factors such as bFGF, VEGF, PDGF and angiopoietin (Tarnawski, 2002).

Among all the growth factors, bFGF and VEGF are very important in regulating angiogenesis. bFGF is a direct mitogen for vascular endothelial cells, fibroblast and smooth muscle cells (Shing et al., 1984). bFGF expression is upregulated in the submucosa during early ulcer healing stage (Pohle et al., 1999) and its effect in ulcer healing via stimulating angiogenesis has been well established (Folkman et al., 1991; Szabo et al., 1994). VEGF expression is elevated during
granulation tissue formation (Frank et al., 1995). This growth factor acts specifically on vascular endothelial cell proliferation, migration and tube formation (Szabo and Vinceze, 2000). VEGF significantly accelerates gastric ulcer healing by enhancing angiogenesis at the ulcer site (Jones et al., 2001).

2.6.7 Anti-oxidants

Aerobic life is characterized by the continuous production of oxidants balanced by powerful antioxidant system (Rice-Evans and Diplock, 1993). Antioxidants are intimately involved in the prevention of cellular damage. Under normal conditions anti-oxidant level is quite low (Oshima et al., 1990; Salim, 1991). A shift of the balance on oxidant site may trigger a cascade of reaction leading to the formation of highly reactive cytotoxic compounds such as reactive oxygen species (ROS) or free radicals, which deregulate the cellular functions leading to various pathological conditions including ulcerations. Anti-oxidants provide defense against gastric mucosal cell injury induced by free radicals generated either by neutrophils or xanthine-xanthine oxidase system by preventing oxidation of macromolecule. Antioxidants can act either by inhibiting free radical generation or by free radical scavenging (Buettner, 1993).

(1) **Enzymatic anti-oxidants**

Major antioxidant enzymes involved in defense against O$_2^-$ and H$_2$O$_2$ mediated injury are superoxide dismutase (SOD) and catalase (CAT). The enzymes thereby form the protective mechanism, which maintain the lowest possible level of ROS inside the cell (Sies, 1997).

(a) **Superoxide dismutase**: SOD is an endogenously produced intracellular enzyme present in essentially every cell of the body. SOD first reduces (adds an electron to) the superoxide radical (O$_2^-$) to form hydrogen peroxide (H$_2$O$_2$) and oxygen (O$_2$).

$$2O_2^- + 2H \rightarrow \text{SOD} \rightarrow H_2O_2 + O_2$$

Exogenous administration of SOD has been found to reduce ulcer formation whereas SOD inhibitors pre-treatment has shown to produce antral ulcer formation (Oka et al., 1991). Thus preservation of mucosal SOD activity seems to be essential in
the prevention and healing of ulcer (Rastogi et al., 1998). Excessive superoxide inhibits glutathione peroxidase and catalase to modulate the equation from $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$. Further catalase and glutathione peroxidase are responsible for reducing $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$. The respective enzymes that interact with superoxide and $\text{H}_2\text{O}_2$ are tightly regulated through a feedback system.

(b) **Catalase (CAT):** Catalase, a heme containing protein, is found to act $10^4$ times faster than peroxidase. It is present in both peroxisomes (80%) and cytosol (20%). It catalyses decomposition of $\text{H}_2\text{O}_2$ to water and oxygen.

$$2\text{H}_2\text{O}_2 \rightarrow \text{CAT} \rightarrow \text{H}_2\text{O} + \text{O}_2$$

An increase in the production of SOD without a subsequent elevation of catalase or glutathione peroxidase leads to the accumulation of hydrogen peroxide, which gets converted into the hydroxyl radical.

(c) **Glutathione peroxidase (GPx):** GPx is present mainly in the cytosol and also found in small amount inside the mitochondria. It acts on $\text{H}_2\text{O}_2$ as well as on organic peroxidase (Hirashi et al., 1993; Michielis et al., 1994). One of the essential requirements for GPx is glutathione as a co-substrate. GPx reduces $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$ by oxidizing glutathione (GSH) (Equation A). Re-reduction of the oxidized form of glutathione (GSSG) is then catalysed by glutathione reductase (Equation B). These anti-oxidant enzymes also require trace metal co-factors for maximal efficiency (Halliwell, 1995).

$$\text{H}_2\text{O}_2 + 2 \text{GSH} \rightarrow \text{GSSG} + 2 \text{H}_2\text{O} \quad \text{(A)}$$

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{GSH} + \text{NADP}^+ \quad \text{(B)}$$

Inhibition of GPx leads to accumulation of lipid hydroperoxide resulting in lipid peroxidation and ultimately ulceration. Together, these entire enzymatic anti-oxidants repair oxidized DNA, degrade oxidized protein, and destroy oxidized lipids (fat-like substances that are a constituent of cell membranes).

(2) **Non-enzymatic antioxidants**

This category includes those anti-oxidants, which help in removal of free radicals. These can be sulphhydryl compounds or other antioxidant:

(a) **Glutathione:** Reduced glutathione (GSH) reacts with free radicals to prevent their deleterious effects (Karmeli et al., 1996). Protective role of GSH has been
demonstrated in gastric injury caused by ischemia, ethanol (Avila et al., 1996) and pylorus ligation induced ulcers (Rastogi et al., 1999).

(b) **Vitamin A:** Vitamin A is a fat soluble vitamine, which is essential for growth, maintenance of visual function, reproduction and differentiation of epithelial tissue. Vitamin A and its metabolite play a crucial role in regulating the differentiation and proliferation of epithelial cells; thereby in repair process.

(c) **Vitamin C:** Vitamine C (ascorbic acid) is an important water soluble antioxidant found in biological fluids and is an essential micronutrient required for metabolic functioning of the body. Vitamine C mainly helps in regeneration of α-tocopherol from α-tocopheroxyl radical. It is also reported to enhance host immunological responses.

(d) **Vitamin E:** Vitamin E occurs in plasma as variety of tocopherols, where tocopherols alpha and gamma isomers are usually the major one. Tocopherols are important chain breaking anti-oxidants. Vitamin E intercepts lipid peroxy radical (LOO·) formed during lipid peroxidation thereby terminating chain reaction leading to the production of toxic peroxides (Sies and Murphy, 1991; Yoshikawa et al., 1991). The resulting tocopheroxy radical is relatively stable and is incapable of inducing lipid peroxidation. Cytoprotective effect of vitamin E against ischemia reperfusion induced mucosal injury and alcohol induced ulcer model has been demonstrated to occur via inhibition of lipid peroxidation and interference with neutrophil infiltration (Naito et al., 1999).

Apart from these vitamine, CoQ10 (Coenzyme Q10) also known as ubiquinone, uric acid, albumin, several drugs such as xanthine oxidase inhibitors (allopurinol and folic acid), NADPH inhibitors (adenosine and calcium channel blockers) and inhibitors of iron redox cycling (deferoxamine, apotransferin and ceruloplasmin) (Halliwell, 1995) are also known to posses antioxidant properties.

Role of antioxidants as a defensive factor against mucosal injury has been shown in various agents induced ulcers such as acetic acid induced injury (Ito et al., 1998; Hiraishi et al., 1999).
2.6.8 Prostaglandins

PGs are one of the most important components of gastric mucosal defence and are key molecule in healing mechanism. PG was first recognised in 1930s as a substance in semen that caused contraction of smooth muscle of prostate gland hence named prostaglandins. PGs are members of a family of lipid mediators derived from 20 carbons containing polyunsaturated fatty acid- arachidonic acid (AA) and are also termed as Eicosanoids (Eicosa=20). Eicosanoids, the biologically active metabolite are derived from membrane phospholipids bilayer through the action of enzyme phospholipases (PL) (especially PLA2) and converted to PGH2 by PGH- synthase or cyclooxygenase (COX) enzyme, which catalyses a two-step reaction, first cycling AA to form PGG2 and than reducing the 15-hydroperoxy group of PGG2 to form PGH2. Cell specific PG synthase catalyses the conversion of PGH2 to different biologically active end products including PGE2, PGF2α, PGD2 PGI2 and thromboxanes (TXA2), which all are collectively known as prostanoids. In addition, AA can be metabolised by different lipooxygenase (LOX) and get converted to hydroxyeicosatetraenoic acid (HETE) and LT (Pareente and Perreti, 2003). The diagrammatic representation of prostanoid synthesis and their functions is shown in Figure 2.12.

PGs are well known for their dual role. They help in maintaining homeostatic balance of the normal gastric mucosa as well as are responsible for other pathological conditions like inflammation etc, hence they are often called as a double-edged sword. PGs modulate or in some instances, mediate the action of variety of biological responses. They are produced on demand rather than they are released from stores in the cell origin. They mainly act in autocrine manner and have very short half lives. The diversity of PG function is achieved by cell specific and tissue specific generation of different stable PGs, multiple PG receptor linked to different intracellular pathways and PG production involving enzymes that are induced to increase local PG production. The major subclasses of PG are TXA2, PGD2, PGE2, PGF2α, and PGI2.
The prostanoid exert their biological effect by interacting predominantly with cell surface receptors. The prostanoid receptors are G protein linked receptor. PGE\(_2\) and PGI\(_2\) are the most important prostanoid found in gastrointestinal tract and play a very important role in modulating gastrointestinal mucosal defense and repair (Wallace and Granger, 1996; Wallace and Ma, 2001). PGE\(_2\) acts through four different types of receptors i.e., EP\(_1\), EP\(_2\), EP\(_3\) and EP\(_4\). The EP\(_1\) receptor is coupled to Ca\(^{2+}\) channels and its activation results in increased intracellular Ca\(^{2+}\) concentration. The EP\(_2\) and EP\(_4\) receptors increases intracellular cAMP, whereas most abundant and important receptor EP\(_3\) decreases cAMP concentration which is mainly involved in the reducing the acid secretion as well as in enhancing the cytoprotective factors of gastric mucosa as shown in Figure 2.4 (Toh et al. 1995; Valle, 2005). Different eicosanoids, their receptors, distribution and their effects have been illustrated in Table 2.7.
### Table-2.7: Membrane based Eicosanoids

<table>
<thead>
<tr>
<th>Eicosanoids</th>
<th>Receptor</th>
<th>Tissue distribution</th>
<th>Transductional signal</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂</td>
<td>EP₁</td>
<td>SM*, Fibroblasts</td>
<td>Ca²⁺ mobilization</td>
<td>SM* contraction</td>
</tr>
<tr>
<td>PGE₂</td>
<td>EP₂</td>
<td>SM*, epithelial cell, mast cells, neurons, Fibroblasts</td>
<td>cAMP increase</td>
<td>SM* relaxation</td>
</tr>
<tr>
<td>PGE₂</td>
<td>EP₃</td>
<td>SM*, Adipocytes, Neurons, Epithelial cell, Kidney</td>
<td>cAMP decrease</td>
<td>SM* contraction, neurotransmitter release</td>
</tr>
<tr>
<td>PGE₂</td>
<td>EP₄</td>
<td>Fibroblasts, SM*, Myofibroblasts</td>
<td>cAMP increase</td>
<td>SM* relaxation</td>
</tr>
<tr>
<td>PGD₂</td>
<td>DP</td>
<td>Platelets, SM*, Neurons</td>
<td>cAMP increase</td>
<td>SM* relaxation, inhibition of neurotransmitter release, platelet aggregation</td>
</tr>
<tr>
<td>PGI₂</td>
<td>IP</td>
<td>Platelets, SM*, Neurons</td>
<td>cAMP increase</td>
<td>SM* relaxation, inhibition of platelet aggregation</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>FP</td>
<td>Kidney, SM*, Myofibroblasts</td>
<td>Ca²⁺ mobilization</td>
<td>SM* contraction</td>
</tr>
<tr>
<td>TXA₂</td>
<td>TP</td>
<td>Platelets, SM*</td>
<td>Ca²⁺ mobilization</td>
<td>SM* contraction, platelet aggregation</td>
</tr>
</tbody>
</table>

*SM; Smooth muscle

The biologic actions of PGs in the gastrointestinal tract are diverse. PGs affect virtually every component of mucosal defense (Wallace, 1992) and play a very important role in maintaining the gastroduodenal mucosal integrity and repair. In 1979
Robert et al. introduced the term of "cytoprotection" for natural prostaglandins which means the protection afforded by PGs at non-antisecretory doses to prevent the mucosal damage induced by necrotizing substances such as strong acids, base or concentrated bile and even acts in the lesions caused by boiling water. Later on PGs were also found to be involved in adaptive cytoprotection (Arakawa et al., 1990), which means exposure of mild irritants dramatically reduces the damage induced by subsequent exposure to a variety of necrotizing agent.

Gastric mucosal integrity requires continuous generation of PGE$_2$ and PGI$_2$ (Atay et al., 2000) as majority of mucosal defense mechanism are stimulated or facilitated by PGs (Wallace and Granger, 1996). Suppression of PG synthesis by NSAIDs results in increased susceptibility of mucosa to injury. When gastric mucosal PGs are compromised, exogenous noxious agents together with endogenous acid (HCl) and pepsin penetrates into the mucosa and damage the mucosal microvessels thereby reducing the blood flow. This reduction in blood flow subsequently reduces oxygen and nutrient supply, which causes release of proinflammatory and vaso-active mediators (serotonin, PAF, LTs, ET) in the gastric mucosa that in turn exaggerate ischemic necrosis (Tarnawski and Erickson, 1991). Both PGE$_2$ and PGI$_2$ reduce secretion of gastric acid by the parietal cell of stomach (Robert, 1979).

Intravenous infusions of PGE$_2$ or PGI$_2$ exert a direct vasodilator action on the gastric mucosa (Konturek et al., 1980). Increase in mucosal blood flow is extensively reported to have beneficial effect in maintaining the functional integrity of the gastric tissue (Whittle et al., 1978). PGE$_2$ is synthesized by epithelial and smooth muscle cells in the stomach, and the intra gastric administration of PGE$_2$ to the human stimulates the release of viscous mucus (Johansson and Kollberg, 1979), which plays a defensive role against mucosal injury (Allen and Garner, 1980). Other than providing these support to gastric mucosa, PGs also provide mucosal protective effect by stimulation of mucosal bicarbonate secretion, prevention of disruption of gastric mucosal barrier, acceleration of cell proliferation, promotion of formation of surface active phospholipids, maintenance of gastric mucosal sulphhydryl compounds and stabilization of cell membranes (Robert, 1979; Miller, 1983; Isenberg et al., 1985; Scheiman, 1996). PGs are also reported to suppress ROS generation carried by
neutrophils (Wogn and Freund, 1981; Gryglewski et al., 1987). PGs are also well-known proangiogenic factors that are implicated in altered vascular permeability and angiogenesis (Ziche et al., 1982). Among all these protective mechanisms supported and stimulated by PGs, maintenance of blood flow is the most accountable for repair. The defense mechanisms provided by PGs are illustrated in Table 2.8.

**Table 2.8: Defense mechanisms of prostaglandins**

<table>
<thead>
<tr>
<th>Defensive factors</th>
<th>Predominant mode of action</th>
</tr>
</thead>
</table>
| Unstirred layer of mucus and bicarbonate | • Retardation of H⁺ ion  
                                        | • Pepsin diffusion  
                                        | • Lubrication  
                                        | • Antibacterial action |
| Surface phospholipids | • Repelling of water soluble molecules  
                                      | • Protection against acid |
| Surfactant-like molecules | • Hydrophobicity of luminal surface |
| Alkaline tide | • Production of HCO₃⁻ for unstirred layer |
| Cell renewal | • Replacement of damaged cells |
| Mucosal blood flow | • Delivery of oxygen and nutrients  
                                      | • Removal of toxic metabolites |

Along with affecting the gastrointestinal tract, PGs are well-known mediators of features of inflammation—pain (color), swelling (tumor), erythema (rubor) warmth (calor) and loss of function (functio laesa). Inhibition of PG synthesis by aspirin and other NSAIDs is anti-inflammatory, analgesic and anti-pyretic. The common side effect associated with this class of drugs includes gastric ulceration, bleeding and renal dysfunction etc. Importantly both therapeutic as well as side effects of these agents are solely due to the inhibition of prostanoid synthesis. This duality of PGs as mediators of both physiological and pathological function was clarified when two different isoform of cyclooxygenase (COX) enzyme were identified (Jones et al., 1993). The
COX family into two different enzymes each of which being a key in PG synthesis has resolved the issue of PG duality.

2.7 Cyclooxygenase

Cyclooxygenase (COX), an important enzyme family is constituted by enzymes responsible for the synthesis of PGs from arachidonic acid, which in turn plays varied range of roles in various physiological and pathological conditions. COX also referred as prostaglandin H synthases or prostaglandin endoperoxide synthases, catalyze the rate limiting step in PG and thromboxane synthesis. COX exists in two isoforms: COX-1 and COX-2. COX-1 was purified and characterized in the 1970s and the gene of COX-1 was located in 1988 (DeWitt and Smith, 1988). The discovery and cloning of the second isoenzyme, COX-2 in 1991 by Xie et al., initiated a revolution in understanding of PGs and their function in normal physiology and disease.

Chandrasekharan et al. (2002) identified a COX-1 variant COX-3 that was sensitive to inhibition by acetylsalicylic acid (SA) and dipyrone in whole insect cells expressing the protein. Recently it has documented that COX-3 (COX-1b) does appear to be an endogenous protein in rat that is highly expressed in certain tissue such as heart, brain and kidney but it doesn't appear to do with eicosanoid metabolism. Snipes et al. (2005) have cloned and sequenced COX-1b/COX-3 mRNA from cerebral endothelial cells. They also suggested that COX-3 doesn’t have cyclooxygenase activity and confirmed that it has no effect on PG production by acetylsalicylic acid. They concluded that this protein has a completely different amino acid sequence than COX-1 and COX-2. They suggested a name COX variant protein to distinguish it from the known PG-synthesizing cyclooxygenase isoforms.

2.7.1 Molecular structure of cyclooxygenase

(i) Gene structure:

Both COX-1 and COX-2 are membrane bound proteins that reside in the endoplasmic reticulum (Spencer et al., 1998). The two enzymes are highly similar in structure and enzymatic activity. They are encoded by different genes located on different chromosomes and seem to serve different functions. The genes for COX-1
and COX-2 are located on chromosomes no 9 and 1 respectively (Kraemer et al., 1992). COX-1 mRNA is approximately 2.8 kilobases while COX-2 mRNA is 4.0 kilobases. COX-1 represents a housekeeping gene which lacks a TATA box (Kraemer et al., 1992), whereas COX-2 gene contains a TATA box and binding sites for several transcription factors including cAMP response element, IL-6 response element, CCAAT/enhancer binding proteins, nuclear factor-κB and glucocorticoid response elements (Wu, 1995). Thus, the expression of COX-2 is regulated by a broad spectrum of mediators involved in inflammation.

COX-2 is also regulated at the post-transcriptional level. A 3’-untranslated region of COX-2 mRNA has shown multiple copies of adenylate and uridylate rich elements that confer post transcriptional control of COX-2 expression by acting as an mRNA instability determinant or as a translation inhibitory element (Dixon et al., 2000).

(ii) Protein structure:

Although genes for COX-1 and COX-2 are different but the protein share approximately 60% homology at amino acid level (Xie et al., 1991). Both COX-1 and 2 catalyses the same reaction and are identical in length. The mature protein of both the enzymes contains three distinct domains. The first one is a conformation domain, which is highly similar to EGF and is termed the EGF-like domain. The function of this domain in COX enzyme is poorly understood but is thought to facilitate recruitment and interaction with other cellular proteins. The second domain contains a series of amphipathic helices, which comprise the membrane attachment site. The third domain is large globular one, which contains the COX and peroxidase activity (Miffin and Powell, 2001). The COX active site lies in this narrow hydrophobic channel framed by the membrane attachment helices. Studies have also demonstrated that the amino acid conformation for the substrate binding sites and catalytic regions are almost identical (Picot et al., 1994; Kurumbail et al., 1996).

However, there are important differences in these regions, particularly the exchange of Isoleucine in COX-1 for Valine in COX-2 at position 434 and 523 (Mitchell and Warner, 1999). These amino acids are a part of substrate binding channel and one consequence of these substitutions is that COX-2 has a wider and
more flexible channel. This wider channel (side pocket) is the basis behind the design of various drugs (COX-2 selective NSAIDs-Coxibs) specifically targeted to inhibit COX-2. The structure of COX-1 and COX-2 enzymes with their active site is shown in Figure 2.13.

Figure 2.13: Molecular structure of COX-1 and COX-2

There are also differences in the amino acid sequence in the N and C terminus of these enzymes. Small differences in the structure of COX-1 and COX-2 have brought important pharmacological and biological differences. Apart from gastrointestinal tract and inflammation, both of the COX isoforms are also reported to be involved in various other physiological as well as pathological conditions where they play crucial roles. Different properties and the functions in different systems of COX-1 and 2 are summarized in Table-2.9.

Various authors have performed different experiments to better characterize the physiological and pathological functions of each isoform in-vivo by utilizing gene targeted to generate mice deficient in either COX-1 or COX-2 (Langenbach et al., 1995; Morham et al., 1995; Loftin et al., 2001 and 2002). Studies performed in mice deficient in either COX-1 or COX-2 showed that these isoforms are not only involved in physiological but also in developmental process. Table 2.10 represents the characteristics of mice deficient in either COX-1 or COX-2.
### Review of Literature

Table 2.9: Different properties and functions of COX-1 and COX-2 enzyme

<table>
<thead>
<tr>
<th>Properties</th>
<th>COX-1</th>
<th>COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>2.8 Kb</td>
<td>4.0 Kb</td>
</tr>
<tr>
<td>mRNA stability</td>
<td>Stable</td>
<td>Unstable</td>
</tr>
<tr>
<td>Amino acids</td>
<td>576</td>
<td>581</td>
</tr>
<tr>
<td>Subcellular localization</td>
<td>Endoplasmic reticulum and</td>
<td>Mainly nuclear membrane</td>
</tr>
<tr>
<td></td>
<td>nuclear membrane</td>
<td></td>
</tr>
<tr>
<td>Aspirin treatment</td>
<td>No metabolite</td>
<td>15 (R) hydroperoxy-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>eicosa-tetra-enoic acid</td>
</tr>
<tr>
<td>Substrate</td>
<td>Mainly arachidonic acid</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>Arachidonic acid utilized</td>
<td>Mainly exogenous</td>
<td>Exogenous and</td>
</tr>
<tr>
<td>Glucocorticoid treatment</td>
<td>No effect</td>
<td>endogenous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibited</td>
</tr>
</tbody>
</table>

#### Functions

| Inflammation                    | Involved in inflammatory  | In initial stage act as pro-  |
|                                 | hyperalgesia               | inflammatory and in          |
|                                 |                            | resolution stage acts as     |
|                                 |                            | anti-inflammatory            |
| Renal development and function  | Maintenance of physiological| Involved in development      |
| Female reproduction             | process                    | of kidney                    |
|                                 | Required for fertility and | Important for ovulation,     |
|                                 | fetal development, Initiation of labor | implantation and          |
|                                 |                            | decidualization              |
| Cardiovascular system           | Involved in regulatory     | Involved in regulatory       |
|                                 | homeostasis                | homeostasis, anti-           |
|                                 |                            | atherigenic                 |
| Tumorigenesis                   | Involved in angiogenesis, growth of tumor | Induction of angiogenic factor, anti-apoptotic, development of malignancy |
| Fever                           | Mediate                    | Mainly involved              |
Table 2.10: Characteristics of mice deficient in either COX-1 or COX-2

<table>
<thead>
<tr>
<th>Physiological/pathological process</th>
<th>COX-1 (−/−) mice</th>
<th>COX-2 (−/−) mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal mortality</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Adult mortality</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Postnatal kidney development</td>
<td>Normal</td>
<td>Impaired</td>
</tr>
<tr>
<td>Ovulation</td>
<td>Normal</td>
<td>Impaired</td>
</tr>
<tr>
<td>Implantation</td>
<td>Normal</td>
<td>Impaired</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td>Impaired</td>
<td>Normal</td>
</tr>
<tr>
<td>Tumor development</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Altered</td>
<td>Altered</td>
</tr>
<tr>
<td>Peritonitis incidence</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Constitutive PG synthesis</td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>Inducible PG synthesis</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Autoimmune arthritis</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Bone resorbtion</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Intestinal stem cell survival</td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>Colonic inflammation</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Induced cerebral blood flow</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Resting cerebral blood flow</td>
<td>Decreased</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ischemic brain injury</td>
<td>Unknown</td>
<td>Decreased</td>
</tr>
<tr>
<td>Ischemia/reperfusion injury</td>
<td>Increased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

2.8 NSAIDs and COX-2 isozyme

NSAIDs are currently used as a first line therapeutics in the treatment of osteoarthritis (OA), rheumatoid arthritis (RA), systemic lupus erythematosis (SLE) and other inflammatory syndromes. With the discovery of two different forms of COX isozymes, selective inhibitors of COX-2 are developed which not only provide anti-inflammatory and analgesic action but are also devoid of any gastrointestinal side effects. Even before the discovery of COX-2 isoform, pharmaceutical companies made three NSAIDs (nimesulide, etodolac and meloxicam) with fewer side effects. However after cloning of COX-2, inhibitors were designed with even greater
selectivity for COX-2 (coxibs). It was proposed that selective inhibition of COX-2 isotype with coxibs would achieve anti-inflammatory and analgesic effects comparable to those of the conventional NSAIDs, without causing damage to gastrointestinal tract both in animals (Schmaassmann et al., 1998) and humans (Simon et al., 1999). In 1998, U.S. Food and Drug Administration (FDA) approved first COX-2 inhibitor-celecoxib, which is used to treat RA and OA. In 1999 rofecoxib, second COX-2 inhibitor became available and is used to treat OA. Second generation valdecoxib are now also available in market to treat RA and OA.

Although coxibs are very effective in patients with RA and OA but recent studies by Cheng and Harris, (2004) has shown coxibs to exhibit significant toxicity in the renal and cardiovascular systems. The cardiovascular toxicity of rofecoxib was the reason for its withdrawal from world makers on September 30, 2004. Reports of increased cardiovascular complications in trials of rofecoxib, celecoxib, valdecoxib, etoricoxib and paracoxib support the notion that this toxicity is a feature of the entire class of selective COX-2 inhibitors (Mukherjee et al., 2001; Mamdani et al., 2004).

2.9 Classical COX hypothesis

COX-1 and COX-2 generate a different pattern and different amount of eicosanoids, hence activation of COX-1 and COX-2 results in different biological responses. Differences in tissue distribution and regulation of expression of two isoform are considered crucial for the physiological role, beneficial and adverse effects of COX inhibitors. The generally held concept (Classical hypothesis) states that COX-1 (constitutive enzyme) is expressed constitutively in most tissue, at fairly constant level including gastrointestinal mucosa (Langenbach et al., 1995) whereas the other form COX-2 is undetectable under normal/ basal conditions in most tissues but it can be rapidly induced by variety of proinflammatory and mitogenic stimuli, including lipopolysaccharides, cytokines (IL-1β, tumor necrosis factor), endotoxins, growth factors and tumor promoters (Habib et al., 1993; Lyons-Giordano et al., 1993; Hempel et al., 1994) in response to tissue injury, hence the enzyme is also named as inducible enzyme. Based on their expression in most tissue and cells, it was thought that COX-1 produced PGs, that are called as good prostaglandins which
performs physiological functions or housekeeping function such as regulation of acid secretion, maintenance of mucosal integrity, local regulation of blood flow, motor functions and control of homeostasis by modulation of platelet aggregation. On the other hand, the inducible COX-2 isoform, on induction by various stimuli, catalyzes production of PGs, which play a major role in pathological conditions and hence are called as bad prostaglandins. These COX-2 derived PGs are mainly involved in inflammatory reactions and are responsible for inflammatory signs like fever, pain, capillary edema and vasodilation. As a direct consequence, the specific inhibition of COX-2 was expected to cause significant anti-inflammatory relief without interfering with COX-1 produced PG mediated physiological processes on gastrointestinal mucosa (Katori and Majiima, 2000).

However, this classical concept was challenged by the detection of COX-2 in several tissues and organs under normal conditions (Harris et al., 1994; Breder et al., 1995; Walenga et al., 1996) and suggested that this classical hypothesis is over simplistic. The situation is more complex than initially anticipated. COX-2 plays much wider physiological role in the development and function of certain organs more than playing its role in inflammation and mediating pain.

2.10 Shift from classical COX hypothesis; wider biological role of COX-2

The classical COX hypothesis stating the role of COX-2 in inflammation was overruled by the fact that COX-2 appears in different tissues even under normal conditions and it performs different physiological and pathological roles like COX-1 enzyme. The constitutive expression of COX-2 was found in brain (Lukiw and Bazan, 1997), kidney (Harris, 1996) and pancreatic islet (Robertson, 1998). Not only constitutive expression but COX-2 deficient mice showed defect in renal function (Morham et al., 1995), female reproductive physiology (Lim et al., 1997) and regulation of bone resorption (Raisz, 1999), which points towards a physiological involvement of COX-2 in development and function of certain organs. There is considerable evidence that PGs derived from COX-1 contribute to the generation of features of inflammation, particularly pain (Wallace and Ma, 2001). Several selective inhibitors of COX-2 are also found to significantly reduce carrageenan-induced
Review of Literature

inflammation in rats only when given at which the drugs also suppressed COX-1 (Wallace et al., 1998). COX-2 deficient mice has shown to have an impaired ability to down regulate inflammatory responses (Wallace et al., 1998) and studies in a rat pleurisy model found that COX-2 derived PGs particularly PGD₂ and its metabolite are important in turning off inflammatory responses mainly by inducing apoptosis of infiltrated neutrophils and macrophages (Gilroy et al., 1999, 2003). A comparison of selectivity of various NSAIDs for the COX isoenzymes in-vitro suggested that the ulcerogenicity of the drugs correlates with the selectivity for COX-1 but not for COX-2. Both in experimental animals and humans, COX-2 inhibitors did not cause measurable inhibition of gastric PG formation. This is in contrast to standard NSAIDs that potently suppress gastric PGs. Taken together, these finding led to concept that NSAIDs induced gastrointestinal side effects are due to blockade of COX-1 and inhibition of COX-2 doesn't contribute much to the gastric mucosal damage (Peskar, 2001).

At least one-half of the world population has ongoing gastric inflammation, attributable to colonization of stomach by H. pylori. This infection is associated with significantly elevated expression of COX-2 in the stomach (Tatsuguchi et al., 2000). In a study of Takahashi et al. (2000), treatment with COX-2 selective inhibitors in patients with H. pylori associated gastritis resulted in significant damage, which showed that there is remarkable contribution of COX-2 in mucosal defense in H. pylori associated inflammation. All these finding support the fact that COX-2 is not a mediator of inflammation; in fact COX-1 is the enzyme primarily responsible for inflammatory characters.

The second area where the classical COX hypothesis was challenged is with respect to the contribution of COX-2 to mucosal defense. Although it was initially thought that COX-2 is not expressed in the gastrointestinal tract but now there is considerable evidence of expression of COX-2 in normal gastric mucosa of rats, rabbits and humans (Iseki, 1995; Zimmermann et al., 1998). The authors speculated that COX-2 might be an important enzyme generating vasodilatory and cytoprotective prostanoids in the gastric mucosa, whereas in other study on rats made clear that selective inhibition of COX-1 or COX-2 didn't elicit gastric or intestinal damage;
rather inhibition of both the enzymes are required for NSAIDs induced damage (Tanaka et al., 2002). It can therefore be concluded that PGs derived from both COX-1 and COX-2 contribute to mucosal defense. This work was further confirmed by Gretzer et al. (2001) who showed that cotreatment of rats with COX-1 inhibitor and COX-2 selective inhibitor, rofecoxib, induced severe gastric lesions. The authors also showed that specific inhibition of both COX isoenzymes is necessary to weaken the resistance of the gastric mucosa. This proposal was in line with finding of Wallace et al. (2000) that observed an induction of gastric mucosal injury after combined treatment with SC-560 (COX-1 inhibitor) and the COX-2 inhibitor celecoxib but no effect with SC-560 alone.

Wallace et al. (2000) has also shown that there is good evidence that the two isoform of COX may influence different components of mucosal defense: suppression of COX-1 accounts for the reduction in mucosal blood flow, while suppression of COX-2 accounts for the increase in leukocyte adherence to vascular endothelium that is observed following NSAID administration. Both COX-1 and COX-2 selective inhibitors when administered together caused increase in neutrophil adherence and reduced gastric blood flow which have been implicated as mechanism in NSAID induced gastrototoxicity. This study made clear that both these effects mediated by specific inhibition of COX isoform are necessary for sufficient interference with mucosal defense to cause ulceration. The finding that neither isolated inhibition of COX-1 nor COX-2 is ulcerogenic was in keeping with observation made in COX knockout mice. COX-1 deficient mice didn’t show spontaneous gastric pathology, similarly no lesion was found in COX-2 deficient mice.

Another circumstance in which COX-2 appears to play a role in mucosal resistant to injury is when the stomach is subjected to a brief period of ischemia followed by reperfusion (Brzozowski et al., 1999). Ischemia-reperfusion leads to marked upregulation of COX-2 mRNA expression in the stomach whereas mRNA level of COX-1 was not affected. Treatment with a selective COX-2 inhibitor prior to the period of ischemia resulted in a significant worsening of gastric injury (Maricic et al., 1999). Similarly, inhibition of the upregulation of COX-2 expression, through prior administration of glucocorticoid, resulted in a significant exacerbation of
ischemia-reperfusion induced gastric damage. As ischemia-reperfusion is primarily a neutrophil driven response (Hernandez et al., 1987), it is possible that the ability of selective COX-2 inhibitors to increase leukocyte adherence to the vascular endothelium (Muscara et al., 2000) contributed to the production of injury in the stomach.

Emerging evidence indicates that COX-2 plays an expanded role in modulating resistance to luminal irritants when other mediators of mucosal defense are pharmacologically or genetically depressed. Studies in rat have demonstrated that when nitric oxide synthesis is inhibited, administration of selective COX-2 inhibitors results in significant gastric damage (Ehrlich et al., 2004). Sensory afferent nerves also contribute significantly to the ability of gastrointestinal mucosa to resist injury, mainly by regulating mucosal blood flow. When sensory afferent nerves are chemically ablated, administration of selective COX-2 inhibitors results in formation of hemorrhagic lesions (Ehrlich et al., 2004). Thus at molecular level, mucosal resistance to luminal irritants involves significant cross talk among COX-2 derived signals and other endogenous pathways.

2.11 COX-2 and ulcer healing

Studies in the mid 1990s explored the role of COX-2 in the resolution of experimental colitis (Reuter et al., 1996), which proposed that this enzyme played a key role in ulcer healing. COX-2 expression in normal mucosa is very low, however Mizuno et al. (1997) demonstrated that a COX-2 mRNA and protein was strongly expressed at the margins of experimental ulcers in mice with a three fold increase in PG generation but not in the adjacent non-ulcerated mucosa, whereas no change in COX-1 expression was observed. Administration of selective COX-2 inhibitor delayed healing of experimentally induced acetic acid induced ulcers.

In another study, Schmassmann et al. (1998) showed that in chronic gastric cryoulcers of rats, COX-2 immunoreactivity was negligible in the normal gastric wall but after ulceration it occurred in abundance in the cytoplasm of monocytes, macrophages, fibroblasts and endothelial cells in region of maximal repair activity below and between the regenerative glands. Time course studies revealed that the COX-2 immunoreactivity gets rapidly increased during the initial phases of ulceration
reached maximum during healing phase and then declined. On the contrary rapid COX-1 immunoreactivity was detected in normal cells that decreased after gastric ulceration in the ulcerated mucosa but reappeared in the regenerative epithelial cells. Other investigators subsequently confirmed the finding using various rodent models of gastric ulcer (Schmassmann et al., 1998; Shigeta et al., 1998; Godessart et al., 1999; Brozozowski et al., 2001; Ma et al., 2002) and concluded that COX-2 plays a very divergent and effective role in the repair mechanism of gastric ulcer healing. This interesting observation suggested that PGs formed by COX-2 may be involved in healing in gastrointestinal mucosa and interference with this pathway by a selective COX-2 inhibitor may impair ulcer healing. COX-2 is rapidly induced during gastrointestinal ulcerative processes where it generates large amounts of PGs, which contribute to the gastric ulcer healing process.

Initially, it was believed that COX-2 affects ulcer healing purely through the production of PGs, but recent studies have strongly suggested a much broader mechanism because inhibitors of COX-2 cause retardation of ulcer healing through both PG dependent and PG independent pathways. Hirose et al. (2002) has shown that a selective COX-2 inhibitor (JT522) could inhibit proliferation of gastric epithelial cells, but there was dissociation between the effect of this drug on proliferation and its effect on PGE2 synthesis. He suggested that COX-2 inhibitors produce some of its detrimental effects on epithelial proliferation through a PG independent mechanism. Brzozowski et al. (2001) arrived a similar conclusion from in vivo studies in a rat gastric ulcer model. They compared the effects of several NSAIDs and selective COX inhibitors and found that after seven days of treatment, conventional NSAID and COX-1 selective inhibitor-resveratrol delayed ulcer healing, reduced blood flow at ulcer margin and markedly suppressed PGE2 formation in both ulcerated and non-ulcerated tissue. In contrast, selective COX-2 inhibitor rofecoxib delayed ulcer healing, caused only a moderate decrease in PGE2 synthesis in the non-ulcerated tissue (which was more profound in ulcer margin) and significantly reduced gastric blood flow. This observation suggested that these drugs inhibit the healing process in part through PG-independent actions.
There is very strong evidence to suggest that one of the most important targets for selective COX-2 inhibitors in terms of retardation of ulcer healing is the process of angiogenesis. Angiogenesis is a crucial component of the ulcer healing process and has been shown to be inhibited by direct effects of COX-2 inhibitors on the endothelium (Jones et al., 1999) and indirectly, through effect on circulating levels of pro and anti-angiogenic factors (Ma et al., 2002). Jones et al. (1999) demonstrated that there is a role of both COX-1 and COX-2 in regulating angiogenesis at the level of endothelial cell, and it involves inhibition of mitogen activated protein (MAP) kinase activity and interference with ERK nuclear translocation. They further demonstrated that these effects are having PG dependent as well as PG independent components. Guo et al. (2002) also confirmed the study by observing anti-angiogenic effect of selective COX-2 inhibitor-rovocoxib.

The inhibition of ulcer healing associated with inhibition of COX-2 activity may be in part related to effects on serum levels of growth factors that regulate angiogenesis. Growth factors released from platelets and contained within serum can profoundly affect ulcer healing (Ma et al., 2001). When gastric ulcer are induced in rats, a shift in the serum and platelet level of growth factors occurs such that the balance between the pro and anti-angiogenic factors is tilted in favour of promotion of angiogenesis, thereby assisting ulcer healing (Ma et al., 2001). Another finding of Ma et al. (2002) further lifted the concept of COX-2 mediated angiogenesis, when rats with pre-established gastric ulcer were treated with a selective COX-2 inhibitor (celecoxib) or a conventional NSAID (flurbiprofen), the balance of pro-and anti-angiogenic factors in serum was altered in the opposite direction, favoring inhibition of angiogenesis. The authors of the study also observed and other authors have reviewed that both celecoxib and flurbiprofen significantly inhibited ulcer repair in their study (Wallace and Devchand, 2005).

The duration of up regulation of COX-2 in-vivo is found to be dependent on the severity of the induced lesions and the duration of healing. The increase in immuneactivity in the region of maximal repair activity at the ulcer margin showed close correlation and the same time sequence as the increase in cell proliferation. In contrast, COX-1 immunoreactivity, which was strongly present in normal mucosa and
is localized to the mucous neck cells, was low in the early phase of ulcer healing and progressively reappeared over the 21 day observation period (Schmassmann et al., 1998). These findings also provide support for the important role of COX-2 in the ulcer healing.

2.12 Treatment strategies for gastric ulcer

The treatment goals for peptic ulcers are to relieve symptoms, promote ulcer healing and prevent ulcer recurrence and complications. There are so many classes of drugs currently used to combat acid-peptic disorders. The current treatment strategies include proton pump inhibitor (omeprazole, lansoprazole and rabeprazole), histamine receptor antagonist (cimetidine, ranitidine), cytoprotection agent (sulfate and misoprostol), antacids, therapies for *H pylori* and other miscellaneous drugs.

1. Acid neutralizing/inhibitory drugs

This class of drugs mainly acts through potent inhibition of acid secretion or neutralization. This mainly includes H₂ receptor antagonists, proton pump inhibitor and antacids

a) **H₂ receptor antagonists**: The description of selective H₂ receptor was the landmark in the history of pharmacology and set the stage for the modern approach of the treatment of acid-peptic diseases. Four different H₂ receptor antagonists are available (cimetidine, ranitidine, famotidine and nizatidine). All the four drugs are available in dosage form for oral administration, intravenous and intramuscular preparation.

H₂ receptor antagonists inhibit acid production by reversibly competing with histamine for binding to H₂ receptors on the basolateral membranes of parietal cells. The most prominent effects of these drugs are on the basal acid secretion. Significant inhibition is reported in stimulated acid production (feeding, gastrin, hypoglycemia or vagal stimulation). These agents are thus particularly effective in suppressing nocturnal acid secretion, which reflects mainly basal parietal cell activity. This fact has clinical relevance in duodenal ulcer healing as they suppress nocturnal acid secretion.

The acid inhibitory effect obtained with high doses of H₂ receptor antagonists is built up rapidly but has the tendency to fade. The term tolerance has been applied to characterize this phenomenon. The reason for tolerance effect of H₂ antagonists is still
not entirely understood. An up-regulation of alternative receptors on parietal cells (ACH and gastrin receptor) may be involved (Sachs, 1999). Another disadvantage of ranitidine therapy is that it is associated with marked rebound hypersecretion of acid (El-Omar et al., 1996). Drug interactions in this class are mainly observed in cimetidine. Cimetidine inhibits cytochrome P450 and therefore alters the metabolism and increase the level of drugs that are substrate for cytochrome P450. Such drugs include warfarin, phenytoin, quinidine, tricyclic antidepressant, metronidazole, calcium channel blockers. Special care should be taken with concomitant use of other drugs with cimetidine (Hoogerwerf and Pasricha, 2001). The overall incidence of adverse effects of H₂ receptor antagonist is low (<3%). Side effects usually are minor and include diarrhea, headache, drowsiness, fatigue, muscular pain and constipation. Other less common effects include those effecting central nervous systems (confusion, delirium, hallucinations, slurred speech and headaches).

H₂ receptor antagonists are reported to be used in acute stress ulcers and erosions, Zollinger-Ellison (ZE) syndrome, gastro esophageal reflux diseases (GERD) and in healing of both duodenal and gastric ulcers. Maintenance therapy with H₂ receptor blockers, given at bed time, is effective in preventing symptomatic relapses in patients with duodenal ulcers (Feldman and Burton, 1990).

b) Proton pump inhibitors: The most effective suppressors of gastric acid secretion undoubtedly are the gastric H⁺K⁺ATPase/proton pump inhibitors (PPIs). They are the most effective drugs used in anti-ulcer therapy and have found worldwide popularity over the past decade (Hoogerwerf and Pasricha, 2001). Currently, there are several different PPIs available for the clinical use: Omeprazole, lansoprazole, rabeprozole and pantoprazole. They are α-pyrindymethylsulfinyl benzimidazole with different substitutions on the pyridines or the benzimidazole groups.

These agents are “prodrugs” requiring activation in an acid environment. They are supplied to body in the form of enteric-coated capsules or tablets that pass through the stomach intact and are absorbed in the proximal small bowel. Once absorbed, all PPIs have a relatively short plasma half life (about one to two hours). Their duration of action is much longer because of their unique mechanism of action. These agents enter the parietal cells from the blood and because of their weak basic nature,
accumulate in the acidic secretory canaliculi of the parietal cell, where they are activated by a proton-catalysed process that results in the formation of a thiophilic sulfonamide or sulfenic acid. This activated form reacts by covalent binding from the extracellular domain of H⁺K⁺-ATPase. Binding of cysteine 813, in particular, is essential for inhibition of acid production, which is irreversible for that pump molecule (Richardson et al., 1998).

PPIs inhibit the activity of some hepatic cytochrome P450 enzymes and therefore may decrease the clearance of benzodiazepines, warfarin, phenytoin and many other drugs (Hoogerwerf and Pasricha, 2001). Omeprazole can increase carbazepine concentration (Yates, 2005). PPIs are generally well tolerated and the frequency of adverse effect is ~5% (Vanderhoff and Tahboub, 2002). The most common adverse effects are headache, diarrhea, abdominal pain and nausea. Very rarely omeprazole shows allergic reaction, itching, dizziness, muscle and joint pain, blurred vision and dry mouth. Chronic treatment with omeprazole decreases the absorption of vitamin B₁₂. Long term omeprazole users have shown to suffer from hypergastrinemia, which can promote growth of different kind of tumors in the stomach (Decktor et al., 1988). PPIs can also cause achlorhydria in chronic users, which can increase bacterial infections with microbes as this condition allows the microbes to enter and grow.

PPIs are mainly used principally to promote healing of gastric and duodenal ulcers and also in treatment of acid related conditions such as ulcers, GERD, NSAIDs induced gastropathy and ZE syndrome. PPIs are also used in combination with antibiotics for eradication of *H. pylori*.

c) **Antacids:** Antacids are used usually for symptomatic relief from pain now-a-days however in the past antacids were the only drugs available to treat peptic ulcers.

Antacids that are weak bases react with the HCl of the stomach and produces salt and water. This raises the pH of the gastric content, which inactivates pepsin. Alkalization of gastric contents increases gastric motility, through the action of gastrin. The most commonly used agents are mixture of aluminum hydroxide and magnesium hydroxide. Combination of aluminum and magnesium hydroxide provide a relatively fast and sustained neutralizing capacity.
2. **Cytoprotective agents**

Cytoprotective/ cell protective agents help in protection of the lining of stomach. Sucralfate and misoprostol are the two important cytoprotective agents. These agents are second line medication and are not first choice for treating ulcers.

Sucralfate, a basic aluminum salt of sulfated sucrose act by forming a physical barrier to attack by acid, bile salts and pepsin across the mucosa. It doesn’t work by inhibiting acid but forms a protective layer over a gastric ulcer to shield it against acid so that healing can occur. It also binds to the site of active ulceration. Sucralfate may act by several mechanisms, as they may bind with growth factors, enhance PG synthesis, stimulate mucus and bicarbonate secretion and enhance mucosal defenses and repair.

Misoprostol, a synthetic PG analog, is another cytoprotective agent, which is clinically widely used drug against NSAID induced gastric ulcer. Misoprostol moderately blocks acid release into the stomach and helps in maintaining the integrity of the stomach. Its therapeutic effects are mediated through enhancement of mucosal defenses and repair. PG analogs enhances mucus bicarbonate secretion, stimulate mucosal blood flow and decreases mucosal cell turnover. Misoprostol is approved by FDA for use in preventing mucosal injury caused by NSAIDs.

Sucralfate should not be given to the patients with renal failure, who are at the risk of aluminum over load. Since sucralfate forms a viscous layer in the stomach, it may inhibit absorption and bioavailability of other drugs such as phenytoin, digoxin, cimetidine, ketoconazole and fluoroquinolone antibiotics (Hoogerwerf and Pasricha, 2001). The most commonly reported side effect with sucralfate use is constipation (2%). Small amount of aluminum gets absorbed with the use of sucralfate, so it should not be used in patients with renal failure. Misoprostol shows diarrhea, with or without abdominal pain and cramps which occurs in up to 30% of people. Misoprostol is contraindicated during pregnancy, since it can cause abortion by increasing uterine contractility.

3 **H pylori therapy**

*H pylori* is associated with majority of gastric as well duodenal ulcers. Eradication of *H pylori* leads to a better treatment for peptic ulcer. *H pylori* related
Review of Literature

peptic ulcers are treated with drugs that kill the bacteria, reduces stomach acid, and protect the stomach lining. Eradication of \textit{H pylori} includes double, triple and quadruple therapies or combination products (which consist of multiple drugs combined into one package). Antibiotics are used to kill the bacteria, while those two types of acid-suppressing drugs are mainly used: \textit{H}$_2$ blockers and PPI. The most effective therapy for \textit{H pylori} is the three-drug regimens, since cure rates are typically higher with these therapies (83.3\%) (Chang \textit{et al.} 2005). Two-drug regimens tend to have a lower cure rate and four-drug regimens, while very effective, are more complicated to take. Therefore, a three-drug regimen or possibly a combination product is recommended as the most effective means of treating ulcers caused by \textit{H pylori}.

4 Miscellaneous drugs

Certain other drugs such as anticholinergic drugs are also use to reduce the acid secretion. They can reduce the basal acid production by 40-50\%. They are classically thought to be antagonists of M$_1$ cholinergic receptor and may act to suppress neural stimulation of acid secretion.

Rebamipide, an amino acid analog of 2 (1H)-quinolinone, is a novel mucosal protective and ulcer healing drug developed in Japan (Yamashaki \textit{et al.}, 1987; Ogino \textit{et al.}, 1992). It is a cytoprotective agent, which is also used as an anti-ulcer agent. Clinical and experimental data indicates that it accelerates ulcer healing, improves the quality of ulcer scar and prevents ulcer recurrence. It mainly exerts its effect by increasing PG generation in the gastric mucosa as well as by scavenging ROS. Carbenoxolone, a component of licorice root and a derivative of glycerrhizic acid, has been also used as an anti-ulcer agent in Europe.