CHAPTER 1

INTRODUCTION

1. Background and rationale

Chronic peptic ulcer is a common gastrointestinal disease that results in loss of work and high-costs to the medical services. The most common types are chronic gastric ulcer and chronic duodenal ulcer. Since it was reported that *Helicobacter pylori* might be an important pathogenic factor for these diseases, pharmacotherapeutic strategy has been revolutionally changed, and its standard regimen is particular antibiotics with conventional antiulcer regimens in patients with *Helicobacter pylori* (Kradjan, 2001). Although, hospitalization for chronic duodenal ulcers has declined, hospitalization for chronic gastric ulcers has remained stable. This supports the premise that other mechanisms are involved in the pathogenesis of chronic gastric ulcer (Kradjan, 2001). Recently, oxygen-derived free radical mediated oxidative damage of membrane lipid and protein and the depletion of glutathione have been reported in human gastric ulcer (Bhattacharjee, *et al.*, 2002). Therefore, the exact cause of chronic gastric ulcer remains to be elucidated for the development of effective regimens. Reflux esophagitis is another common and costly gastrointestinal disease that is increasingly recognized as a significant health problem. Acid-suppressive therapy plays an important role in medical management. Nevertheless, a considerable number of patients do not achieve complete mucosal healing or suffer from either sustained symptoms or complications (Kradjan, 2001), suggesting that the severity of reflux esophagitis cannot be fully explained by acid reflux alone and other damaging factors or impaired mucosal resistance are also involved in the pathogenesis of reflux esophagitis. Experiments on rats and studies of esophageal biopsy samples from patients have shown that mucosal damage is mediated primarily by oxygen-derived free radicals accompanied by enhanced esophageal mucosal lipid peroxidation (Wetscher, *et al.*, 1995). In addition, administration of various free radical scavengers has been found to prevent

esophageal mucosal damage induced by mixed reflux of gastroduodenal contents, whereas, acid suppression alone is not effective in either decreasing the degree of reflux esophagitis or attenuating inflammation associated oxygen derived free radical mediated nuclear factor kappa B (NF-κB) activation (Wetscher, *et al.*, 1995; Oh, *et al.*, 2001). Therefore, this disease also remains to be elucidated for development of effective regimens. Ulcerative colitis, a chronic inflammatory condition of the large bowel, is known to be an intractable disease, of which the pathogenesis remains to be clarified in detail. In recent years, the allergic mechanisms (an antibody-mediated hypersensitivity reaction) are known to be partly relevant to the disease as well as the immune dysregulation and the alteration of cytokines secretion patterns (Pallone and Monteleone, 2001). 5-Aminosalicylate-based compounds and corticosteroids, which have been shown to inhibit NF-κB activity and proinflammatory cytokines production, are the mainstay of treatment for ulcerative colitis. Nevertheless, treatment with these medications causes many undesirable side effects and increases risk of infection (Berardi, 2000). Hence, effective regimens should be investigated as well.

Curcuma longa L. (*C. longa*), which belongs to the Zingiberaceae family, is a medicinal plant that grows in tropical regions of Asia. A powder of its rhizome called turmeric is a crude drug which has been used safely for centuries as a dietary spice to give a specific flavor and yellow color in food preparation and as an aromatic stomachics and cholagogues in several traditional folklore prescriptions (Aggarwal, *et al.*, 2003). It has also been used in home remedies for the treatment of cuts wounds, bruises, and sprains (Joe, *et al.*, 2004). In Thailand, *C. longa* is one of five medicinal plants that the Ministry of Public Health declares as a fundamental drug for primary health care and develops for prescription drug production (Suppasil-Nanakorn, 1993). Presently, it is recommended by the World Health Organization and Thailand's Essential Drug List as a herbal medicine for the treatment of dyspepsia (National Drug Committee, 2000; World Health Organization, 1999).

An ethanol extract of *C. longa* rhizome has been found to inhibit acute gastric and duodenal lesions induced by pyloric ligation; hypothermic-restraint stress; and oral administration of indomethacin, 80% ethanol, reserpine, and necrotizing agents in rats

(Rafatullah, *et al.*, 1990). Nevertheless, clinical studies with turmeric powder treatment in patients with peptic ulcer have yielded conflicting results including no effect (Kositchaiwat, *et al.*, 1993; Van Dau, *et al.*, 1998), and beneficial effect (Prucksunand, *et al.*, 2001) that may be accounted by different dosages of turmeric powder and levels of active constituents in various turmeric powder sources. Previous study in acute experimental gastric lesions models has shown that curcumin (diferuloylmethane), the major polyphenolic yellowish pigment of *C. longa* rhizome, at doses less than 50 mg/kg, stimulates mucin secretion and possesses a beneficial effect in protecting the gastric mucosa against irritants such as phenylbutazone, aspirin and serotonin (Sinha, *et al.*, 1974, 1975). However, controversial data exist regarding to its antiulcerogenic activity. Curcumin does not exert any protective action in guinea pigs against histamine-induced gastric lesions. In addition, oral administration of curcumin, at doses more than 100 mg/kg over 6 days, can produce gastric ulceration in rats (Ammon and Wahl, 1991). There is also no report on its inhibitory activity on gastric acid secretion or on its curative activity in a chronic gastric ulcer model. Recently, the efficacy of curcumin against experimental colitis in murine model which resembles human Chron's disease has also been investigated. It is found that pretreatment with curcumin or mixed with the diet and given for dietary administration, exerts beneficial preventive effects in dinitrobenzene or Hapten 2,4,6--trinitrobenzene sulphonic acid-induced colitis (Salh, *et al.*, 2003; Sugimoto, *et al.* 2002; Ukil, *et al.*, 2003). However, no study on the efficacy of curcumin against experimental colitis in murine model which resembles human ulcerative colitis is available. So far, several studies have indicated that curcumin exerts antioxidant activity as a potent singlet oxygen (Das and Das, 2002) and nitric oxide radical scavenger (Sreejayan, 1997); anti-inflammatory activity as a lipoxygenase and cyclooxygenase inhibitor (Ammon, *et al.*, 1993), an inducible nitric oxide synthase (iNOS) suppressor (Chan, *et al.*, 1998; Onoda and Inano, 2000) and a potent NF-κB inhibitor (Chan, 1995; Jobin, *et al.*, 1999; Literat, *et al.*, 2001); antiallergic activity (Yano, *et al.*, 2000); and wound healing activity (Sidhu, *et al.*, 1998) *in vitro* and in standard animal models used for testing these activities. It is also known that some of curcumin, at the intestinal absorption, turns to tetrahydrocurcumin which is reported to be an active principle of curcumin for its antioxidant effect (Pan, *et al.*,

1999; Okada, *et al.*, 2001). Thus, it is necessary to elucidate the efficacy of curcumin on gastrointestinal inflammatory diseases like reflux esophagitis, gastric ulcer, and ulcerative colitis from the recent pharmacological and pharmacokinetic viewpoints. The previous proposed mechanisms for antiulcerogenic effect of curcumin such as inhibitory effect on gastric secretion, cytoprotection, antioxidant activity and inhibition of mast-cell degranulation need to be evaluated again as well. Given this, the chemical structure of curcumin will be potentially used as a model for design to approach a new natural nonsteroidal anti-inflammatory drug that has highly antiulcerogenic action. The results from animal study will also be useful for the standardization of turmeric dosage and support its clinical application for peptic ulcer treatment.

2. Review of literature

2.1 Inflammatory gastrointestinal diseases

2.1.1 *Helicobacter pylori* - negative chronic gastric ulcer

2.1.1.1 Pathophysiology

Chronic gastric ulcer can occur anywhere in the stomach but is most commonly found at the junction between the antrum and the fundus of the stomach, on the lesser curvature (figure 1). It differs from gastritis and erosions in that ulcer typically extends deeper into the muscularis mucosa. Chronic gastric ulcer can be divided into two major etiologic groups as ulcers associated with or without *Helicobacter pylori* infection. It is now generally accepted that *Helicobacter pylori* - negative chronic gastroduodenal ulcer develops when the balance between aggressive factors (primarily gastric acid and pepsin) and gastroprotective factors is lost (Kradjan, 2001). Since chronic gastric ulcer tends to have normal or low acid production, clearly factors other than acid are involved in the pathogenesis of chronic gastric ulcer. Recently, some reactive oxygen species are considered to be one of the major causation factors for mucosal lesions through oxidative damage (Bhattacharjee, *et al.*, 2002).

A. **Gastric acid and pepsin**

There are three anatomically and functionally distinct regions in the stomach (Figure 2): the cardia (containing mucus-secreting cells); the body including the fundus [containing parietal (oxyntic) cells which are responsible for hydrochloric acid and intrinsic factor secretion, and chief (zymogen) cells which are responsible for pepsiogen secretion]; and the antrum (containing G cells which are responsible for gastrin, mucus, and serotonin secretion; and D cells which are responsible for somatostatin secretion) (Henderson and Lander, 2000). Pepsinogen is converted to the proteolytic enzyme pepsin when gastric pH is below 5.0. Pepsin plays a critical role in furthering the digestive and ulcerogenic processes by disruption of the mucus bicarbonate barrier which normally protects the gastric epithelial surface and mucosa from acid (Henderson and Lander, 2000). Pepsin exhibits maximal activity at pH 2 to 3.3 and loses its activity above 5 (Henderson and Lander, 2000).

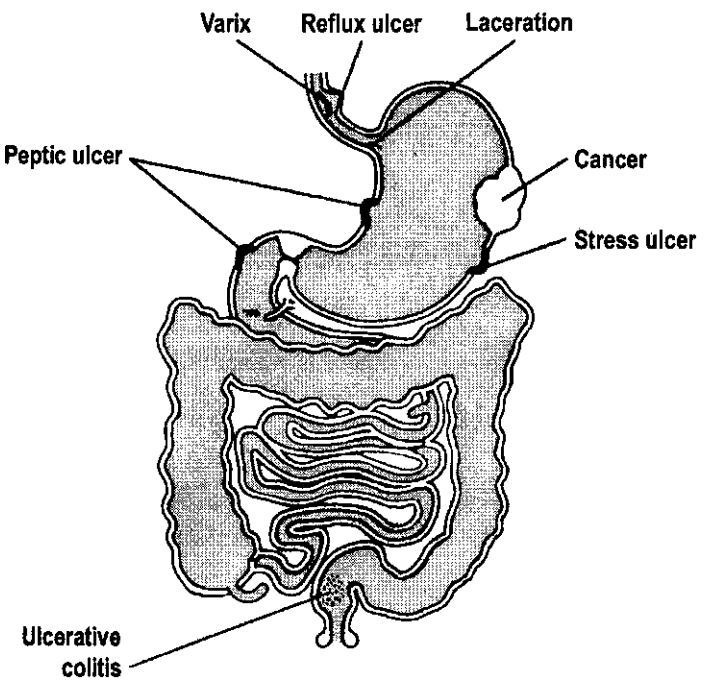


Figure 1 The common sites of upper gastrointestinal diseases

(Henderson and Lander, 2000 : 706)

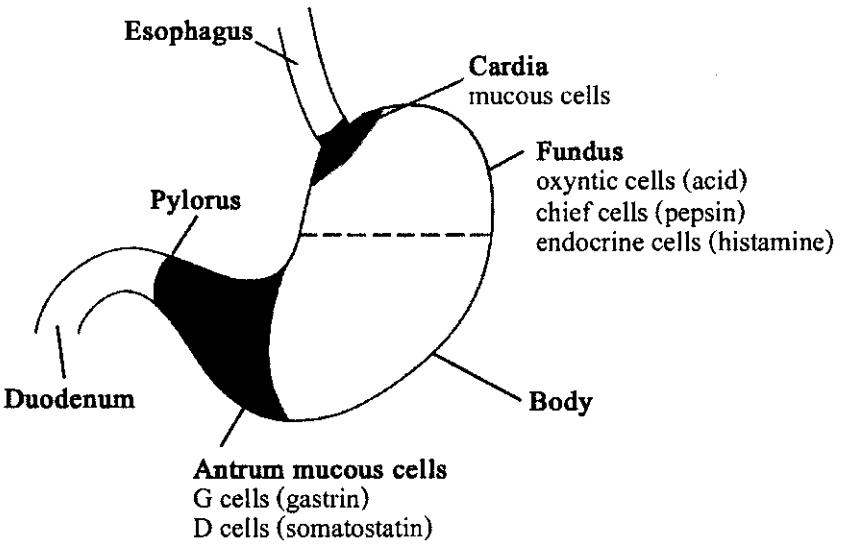


Figure 2 Gastric secretory cells (Henderson and Lander, 2000 : 700)

(1) Regulation of gastric acid and pepsin secretion

Central and peripheral factor The influence of central and peripheral stimuli on gastric acid secretion is mediated via three pathways: the neurocrine pathway, the endocrine pathway, and the paracrine pathway (Figure 3) (Hardman, *et al.*, 2001; Wolfe and Soll, 1988). The neurocrine pathway involves acetylcholine release at parietal and G cells by central mediation of local cholinergic nerves, stimulation of postganglionic vagal neurons in the stomach, and mediation through receptors in the gastric wall when the stomach is distended. The endocrine pathway involves gastrin release from G cells in the gastric antrum. The paracrine pathway involves histamine release from the enterochromaffin–like cells (ECL cells) residing in close proximility to parietal cells, and from the mast cells in the lamina propia. Acetylcholine stimulates the parietal cell directly via muscarinic M_3 receptors coupled to intracellular calcium released and calcium entry (Schubert, 2001). Acetylcholine also indirectly affects the parietal cell through the stimulation of histamine released from the ECL cells in the gastric fundus and the stimulation of gastrin release from the G cells in the gastric antrum, further increasing acid secretion (Hardman, *et al.*, 2001; Henderson and Lander, 2000). Gastrin, acting via

cholecystokinin-2 (CCK_2) receptors on ECL cells coupled to an increase in intracellular calcium, stimulates the parietal cell indirectly by inducing the release of histamine and the hypertrophy and hyperplasia of ECL cells (Schubert, 2001). Histamine plays an important role in gastric acid secretion by stimulating the parietal cell directly via H_2 receptors coupled to generation of cyclic adenosine monophosphate (cAMP) and indirectly by binding to H_3 receptors coupled to inhibition of somatostatin (potent acid secretion inhibitor) release (Schubert, 2001). The generation of changes in second messengers is caused by these three mediators after binding to receptors on the surface of the parietal cell, regulates the movement and location of the gastric proton pump or gastric hydrogen-potassium ion adenosine triphosphate (H^+K^+-ATPase) located on the membranes of the parietal cells (Hardman, *et al.*, 2001). This gastric proton pump catalyzes the exchange of luminal K^+ for cytoplasmic H^+ and is responsible for gastric luminal acidification. As the luminal hydrogen ion concentration increases too high (or the pH in the small intestine becomes too low), it inhibits further secretion of gastrin through a feedback pathway. The main inhibitor of acid secretion is somatostatin (SST), which directly inhibits acid secretion from parietal, ECL, and gastrin cells via SST_2 receptor in a paracrine matter (Athmann, *et al.*, 2000).

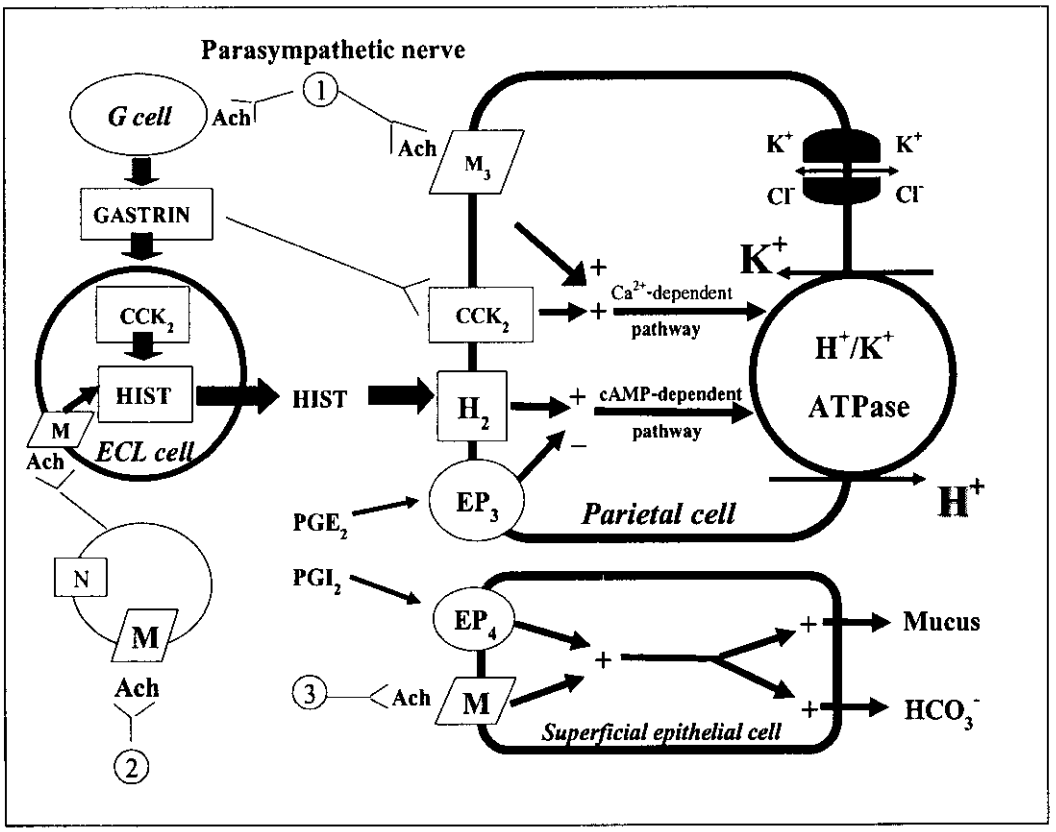


Figure 3 Physiological regulation of gastric secretions (Hardman, *et al.*, 2001 : 1006)

Abbreviation: Ach = acetylcholine, cAMP = cyclic adenosine monophosphate, CCK_2= cholecystokinin receptor, ECL cell = enterochromaffin-like cell, EP_3 = prostaglandin E_2 receptor, EP_4= prostaglandin I_2 receptor, H_2= histamine receptor, HIST = histamine, H^+K^+-ATPase = hydrogen-potassium ion adenosine triphosphate M= muscarinic receptor, N = nicotinic receptor,

(+) = stimulate, (-) = inhibit

(1) and (3) indicate possible input by cholinergic postganglionic fibers

(2) shows neural input from the vagus nerve.

Eicosanoids Eicosanoids are arachidonic acid derivatives that include prostaglandins (PGs), thromboxanes, and leukotrienes (LTs). The synthesis of PGs and thromboxanes are mediated via cyclooxygenase, whereas the synthesis of LTs is mediated via lipoxygenase as shown in Figure 4.

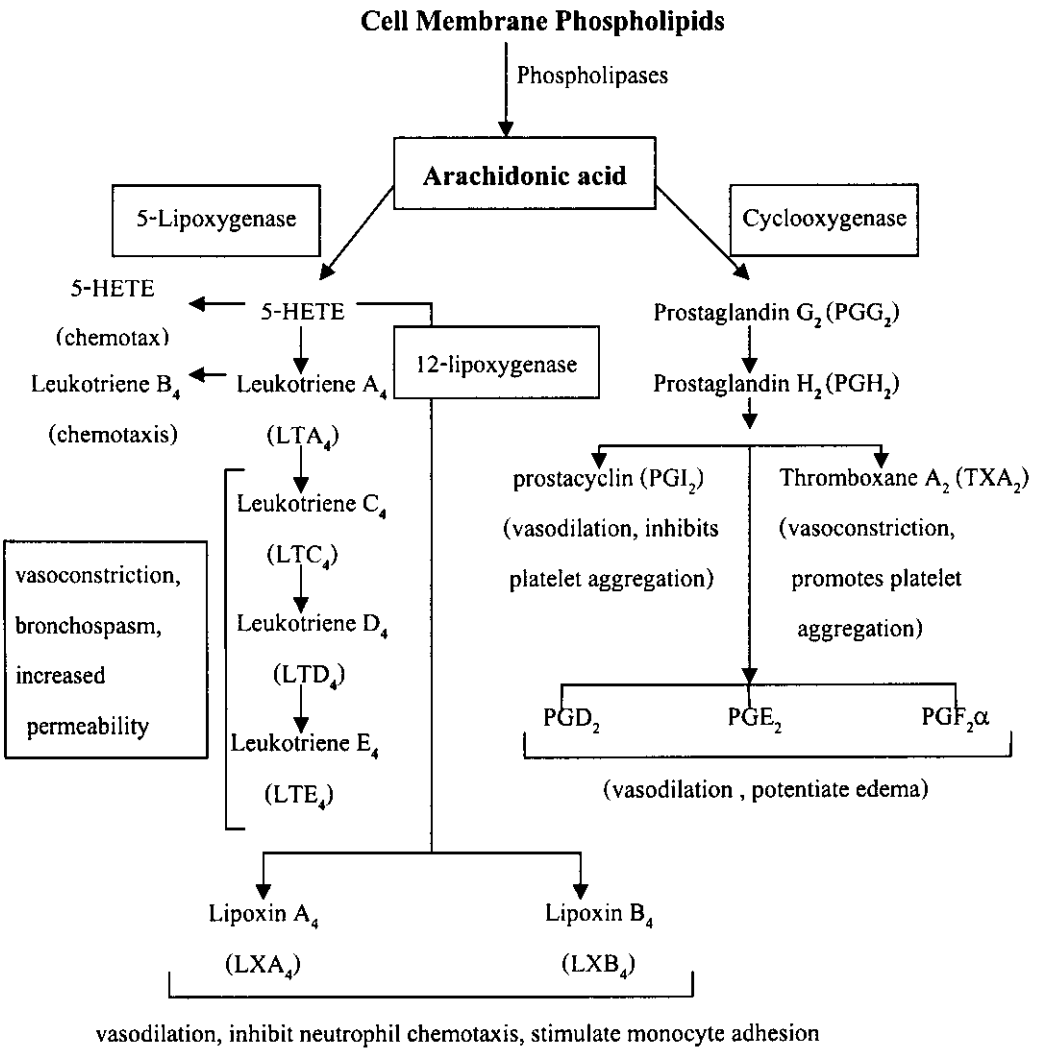


Figure 4 Generation of arachidonic acid metabolites and their roles in inflammation

(Kumar, *et al.*, 2005 : 69)

Abbreviation: HPETE = hydroperoxyeicosatetraenoic acid, HETE= hydroxyl--eicosatetraenoic acid, PG = prostaglandin

PGs are thought to play a physiological role in the regulation of acid secretion as well as the maintenance of gastric mucosal integrity. There are two isoforms of cyclooxygenase enzymes: COX-1 and COX-2. COX-1 is constitutively expressed in large quantities in normal gastrointestinal tissue (Atay, *et al.*, 2000). COX-2 expression is found to be both constitutively expressed and inducible in specific inflamed

or injured locations of the gastric mucosa by cytokines, mitogens, endotoxins, tumor promoters or growth factors (Halter, *et al.*, 2001). PGs produced in normal gastrointestinal tissue are primarily derived from COX-1 and are responsible for mucosal protection and regulation of acid secretion. Recently, it has been reported that PGs derived from COX-1 also exert an influence in regulating acid secretion even in the presence of inflammation (Barnett, *et al.*, 2000). Prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) are the major prostaglandins that inhibit acid production by binding to the EP_3 receptor on parietal cells resulting in inhibition of adenyl cyclase and decreased levels of intracellular cyclic AMP (Hardman, *et al.*, 2001).

LTs, in contrast to PGs, have been found to enhance pepsin secretion (Atay, *et al.*, 2000).

Nitric Oxide (NO) NO is a soluble gas synthesized from L-arginine, molecular oxygen, reduced nicotinamide-adenine dinucleotide phosphate (NADPH), and five cofactors [flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin (BH_4), heme and calmodulin (CaM)] via the catalytic action of nitric oxide synthase (NOS) (Figure 5).

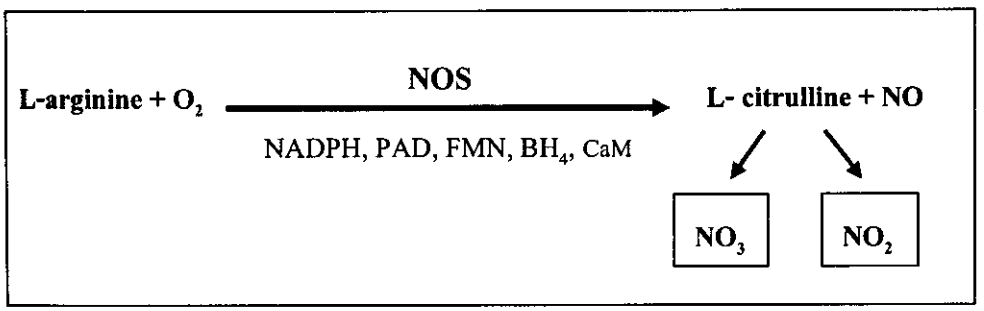


Figure 5 Synthesis reaction of nitric oxide

There are three isoforms of NOS: constitutive neuronal NOS (nNOS), constitutive endothelial NOS (eNOS), and inducible NOS (iNOS) (Martin, *et al.*, 2001).

nNOS is located in the neurons of the central and peripheral nervous system. Within the gastrointestinal tract, nNOS is found in nerves of the myenteric plexus, where NO acts as an inhibitory neurotransmitter in regulating gastrointestinal

smooth muscle relaxation, and facilitates vasoactive intestinal polypeptide (VIP) release from nerve terminals (Jin, *et al.*, 1996). Recently, nNOS expression has also been detected in the mucosa; chief, endocrine, and parietal cells, which suggests that endogenous NO produced by nNOS may participate in the regulation of gastric acid and mucus secretion (Garcia-Victoria, *et al.*, 2000; Premaratne, *et al.*, 2001). eNOS appears primarily in endothelial cells, platelets and mesangial renal cells where NO is involved in the regulation of blood flow and maintenance of vascular tone (Martin, *et al.*, 2001). It has also been found in smooth muscle cells, epithelial cells of the bronchial tree, platelets and pyramidal cells of the hippocampus. Within the gastrointestinal tract, eNOS is found in smooth muscles where it is activated by VIP and pituitary adenylate cyclase activating polypeptide, to generate NO which regulates muscle relaxation (Jin, *et al.*, 1996; Teng, *et al.*, 1996). It is also found in the interstitial cells of Cajal (located at the boundary between muscles and nerves) which are responsible for electrical slow wave activity in the gastrointestinal tract but the role of eNOS in this cell is not clear (Xue, *et al.*, 1994). Recently, it has been reported that the gastric mucosa is capable of expressing high levels of constitutive NOS which is localized in the epithelial cells as well as in the mucosal neurons or even in the gastric smooth muscle cells (Konturek and Konturek, 1995). Studies on the role of NO in the contractile activity of the gallbladder and the sphincter of Oddi also suggested that there is a key L-arginine-NO pathway generating locally NO by constitutive NOS to regulate the gallbladder relaxation and the contraction tone of sphincter of Oddi under the basal conditions (Konturek and Konturek, 1995). Both nNOS and eNOS are activated by an increase in intracellular calcium (Cotran, *et al.*, 1999) and are constitutively expressed, which indicates that their activity can be detected without exposure to inducing agents. The enzyme activity will last for a very short period of time usually in terms of second or minutes.

iNOS is expressed in macrophages, hepatocytes, smooth vascular muscle, neutrophils or endothelial cells. Within the gastrointestinal tract, iNOS can be induced in epithelial cells (Tepperman, *et al.*, 1993) and in the cells of the muscularis propia (Watson, *et al.*, 1993). It produces NO which is essential in the inflammatory process. In contrast to nNOS and eNOS, iNOS is Ca^{2+} independent and is

expressed in response to certain stimulating agents such as endotoxin or cytokines (Busse and Mulsch, 1990). Once expressed, iNOS generates large amounts of NO for many hours even without further stimulation.

NO generated in the gastrointestinal tract might influence parietal cell secretion by diffusing to and acting on adjacent neurons, and/or endocrine cells. The mechanisms by which NO regulates the activity of the parietal cell, are not completely clarified, but the available data suggest a multifactorial process. Hasebe, *et al.* (1998) has found that endogenous NO regulates gastric acid secretion via histamine release from histamine-containing cells, possibly ECL or mast cells in the gastric mucosa. Later, Hasebe, *et al.* (2001) has also found that NO functions as a mediator responsible for both stimulation and inhibition of gastric acid secretion depending on its amount. NO donor at a high dose has an inhibitory effect on gastric parietal cells, resulting in decreased acid secretion, and at a lower dose, facilitates histamine release from histamine-containing cells leading to increased acid secretion.

The secreted acid itself is not sufficient for ulcer formation, however, its corrosive property and the increase in peptic activity serve as a cofactor with other potential causes of gastric ulcer (Soll, 1998). Hence, even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost.

B. Gastroprotective factors

The ability of the mucosa to withstand damage is attributable to a complex mucosal defence system, which includes the continuous mucus-bicarbonate secretion; the maintenance of the mucosal microcirculation for the supply of oxygen, nutrients and secretagogues to the secretory cells; mucosal immune system, and rapid epithelial turnover (mucosal repair). Originally, PGs have been considered to play a major role in the activation of these defense mechanisms but, then, many other substances such as growth factors and NO have been found to induce gastroprotection.

(1) Gastric mucus

A glycoprotein mucin is synthesized in the surface and gland mucus cells under regulation by local paracrine control mechanisms within the mucus-

producing part of the mucosa and by neural and paracrine control mechanisms involving calcitonin gene-related peptide (CGRP), nNOS, PGE_2 , PGI_2 and COX (Holzer, 2001). The continuous production of this glycoprotein mucin layer serves as a physical barrier and represents the prime factor in preepithelial defense.

(2) Eicosanoids

PGs derived from COX-1 as well as COX-2 have recently been found to be important in maintaining normal gastrointestinal mucosal integrity and ulcer healing (Halter, *et al.*, 2001; Wallace, *et al.*, 2000). Inhibition of COX-1 activity will decrease gastric mucosal blood flow without affecting leukocytes adherence to the vascular endothelium of the gastric mucosal microcirculation, whereas, inhibition of COX-2 activity will increase leukocytes adherence without reducing gastric mucosal blood flow. Increase of leukocytes adherence in turn causes mucosal injury through ischaemia and release of oxygen derived free radicals and proteases. Thus, gastrointestinal mucosal damage will be developed only when the activity of both COX-1 and COX-2 is suppressed simultaneously. Both PGE_2 and PGI_2 exert a phenomenon of gastric cytoprotection by the mechanisms other than the reduction of gastric acid secretion. The mechanisms of this gastric cytoprotection involve increase of mucus and bicarbonate secretion; increase or maintenance of mucosal blood flow; enhancement of epithelial cell renewal by protection of proliferative zone cells, stabilization of mucosal cytomembranes, increase the expression of growth factors, and inhibition of mucosal cell apoptosis (Atay, *et al.*, 2000). The ability of PGE_2 to stimulate mucous secretion is mediated by prostaglandin EP_4 receptor (Hassan, 1996; Holzer, 2000). It has also been suggested that the adaptive cytoprotection effect of mild irritants in reducing the damage induced by subsequent exposure to a variety of necrotizing agents, is mediated through enhanced formation of endogenous PGs. The underlying mechanism differs depending on the period after the irritation. The early phase is mediated mainly by PGs derived from COX-1, while the later phase is mediated by PGs derived from both COX-1 and COX-2 (Yamamoto, *et al.*, 1999). Furthermore, PGE_2 not PGI_2, are found to play an important role in adaptive cytoprotection (Takeuchi, *et al.*, 2001).

LTs, in contrast to PGs, have been found to impair gastric mucosal integrity and exacerbate the damaging effects of noxious agents by reducing mucosal blood flow and interfering with gastric emptying. In addition, LTB_4 is the major chemotactic factor for leukocytes adherence, whereas LTC_4 is one of the most potent vasoconstrictors (Atay, *et al.*, 2000).

(3) NO

NO produced by constitutive NOS in the gastric mucosa is also an important mediator of gastric mucosal defense. It exerts cytoprotective effects through mechanisms similar to those of the PGs in the stimulation of mucus and bicarbonate secretion and maintenance of mucosal blood flow. The mucosal protecting action of NO also results from its antioxidant activity (Wallace and Miller, 2000). NO produced by iNOS exerts protective action as a pro-inflammatory mediator in modulating a defensive inflammatory response. It is also evident in a number of studies that iNOS/NO could contribute to mucosal protection in the later phase of adaptive cytoprotection (Tepperman and Soper, 1994; Yamamoto, *et al.*, 1999).

A considerable body of evidence has indicated that PGs act in a cooperative manner with NO to increase the gastric mucosal intregity. In circumstances in which the production of one of these mediators is suppressed, there are compensatory increases in the production of the other. COX-1 and cNOS/NO catalyze the synthesis of PGI_2, whereas COX-2 and iNOS/NO catalyze the synthesis of PGE_2 (Martin, *et al.*, 2001). In addition, PGs and NO can modulate the expression of enzymes responsible for the synthesis of one another (Martin, *et al.*, 2001; Salvemini, *et al.*, 1993, 1996; Swierkosz, *et al.*, 1995).

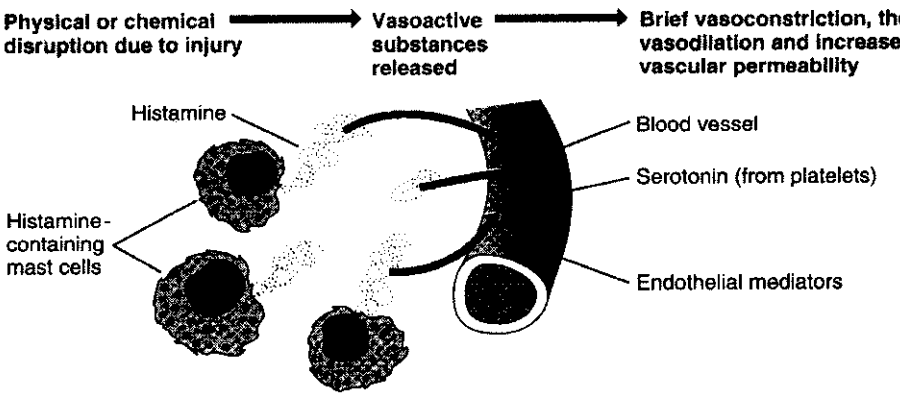
C. Mucosal immune system

The human system has three lines of interdependent defense against injury and invasion (Hansen, 1998). The first line of tissue defense consists of the physical barrier created by the epithelial cell of the intact skin and mucous membrane; the chemical barrier of the surface pH (usually acidic); the biochemical defense of normal flora organisms; and mechanical removal of smooth muscle contraction and ciliary action. The

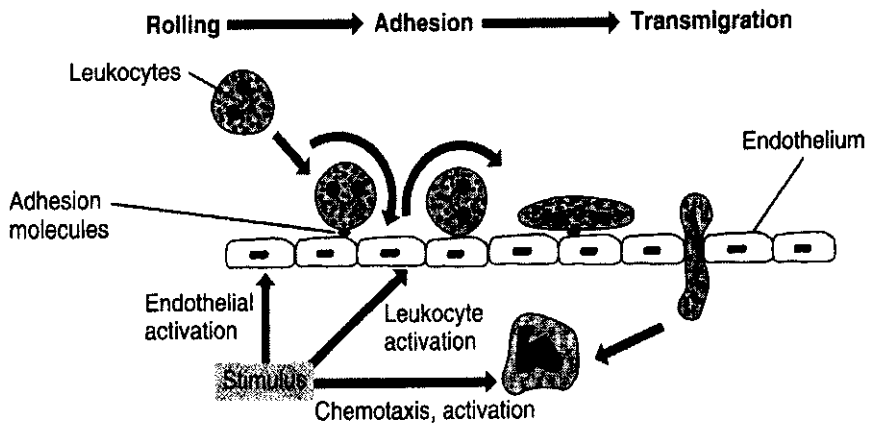
second line of tissue defense, formed when the first line of defense is breached, occurs as an inflammatory response. The third line of tissue defense results from the specific immune responses mounted by specific proteins (antibodies or immunoglobulin) and activated lymphocytes (cytotoxic T lymphocytes) that target invading microorganisms and genetically altered self antigens produced by neoplastic cells.

Inflammation is a local protective response occurring in the vascularized connective tissue, including blood vessels, plasma, circulating cells, and cellular and extracellular constituents of connective tissue. The circulating cells are white blood cells or leukocytes (neutrophils, monocytes, eosinophils, lymphocytes and basophils), and platelets. The connective tissue cells are mast cells found at mucosal surfaces and in the connective tissues surrounding blood vessels; connective tissue fibroblasts; and macrophages derived from blood monocytes. These cells, when exposed to foreign matter or antigens to which the organism has previously been sensitized, are activated and released soluble mediators and cytokines that alter mucosal blood flow and vascular permeability (Figure 6). Many of the inflammatory mediators and cytokines (Table 1 and 2) exert chemotactic effects on leukocytes, resulting in their recruitment into the injured area where they are activated for their phagocytotic function to clear the injured area in preparation for wound healing. Among the leukocytes related to the inflammatory and immune response, neutrophils are essential phagocytic cells during acute inflammatory response, whereas lymphocytes and macrophages are the principal mediators of chronic inflammatory and immune responses. The ultimate step in phargocytosis by neutrophils and macrophages is killing and degradation. This can occur by oxygen-independent or oxygen dependent mechanisms. The oxygen-independent mechanisms involve the action of substances in leukocyte granules which include bactericidal permeability increasing protein, lysozyme, lactoferrin (an iron binding protein), major basic protein (cytotoxic to many parasites), and defensin (cytotoxic to microbes and mammalian cells). The oxygen-dependent mechanisms stimulate a burst in oxygen consumption, glycogenolysis, glucose oxidation, and production of reactive oxygen metabolites as shown in Figure 7. The interaction of pro-inflammatory agents with specific receptors on the phagocyte plasma membrane results in the activation of NADPH oxidase function which lead to the

production and release of a large amount of superoxide anion (O_2^-). Some intracellular oxidases (such as xanthine oxidase, NADPH cytochrome p450 reductase, lipoxygenase, and prostaglandin synthase) can also generate O_2^- as a consequence of their activity. O_2^- is then converted into hydrogen peroxide (H_2O_2) which is an effective antimicrobial agent. H_2O_2 can further be catalyzed by iron or copper ion through Fenton reaction to form the potent cytotoxic hydroxyl radical (OH^-). In addition, enzyme myeloperoxidase (MPO) contained in the granule of neutrophils which, in the presence of a halide, converts H_2O_2 to hypochlorous (HOCl) which is 100-1,000 times more cytotoxic than either O_2^- or H_2O_2 and can further to interact with O_2^- to produce OH^- through non-Fenton related interaction. The actions of these reactive oxygen species can be manifested as impaired endothelium-dependent vasodilation, activation of nuclear transcription factors, production of inflammatory cytokines, enhanced recruitment and activation of leukocytes, accelerated apoptosis, and parenchymal cell necrosis. Reactive oxygen species may also mediate epithelial and mucosal injury indirectly by altering the protease/antiprotease balance that normally exists within the gastrointestinal interstitium. Furthermore, iNOS production of NO by activated macrophages also possesses antimicrobial activity and can interact with reactive oxygen species to form other potent cytotoxic metabolites such as peroxynitrite ($OONO^-$), S-nitrosothiols (RSNO), and nitrogen dioxide (NO_2^-). These reactive nitrogen species amplify the cytotoxic effect by inhibition of SH-dependent enzymes, induction of oxidative stress on DNA strand, and inhibition of enzymes involved in DNA repair. (Cho, 2001; Cotran, *et al.*, 1999; Hansen, 1998; Krieglstein, *et al.*, 2001; Martin, *et al.*, 2001).

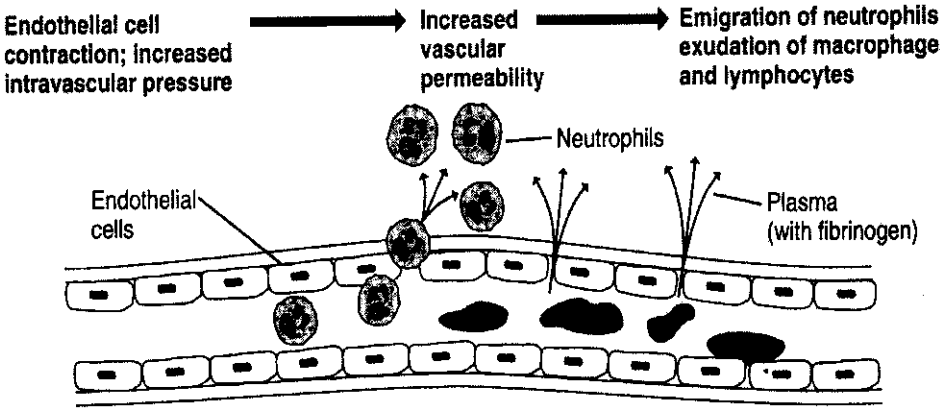


A. Vasomotor response Cellular injury triggers a brief vasospasm, followed by vasodilation and increased capillary permeability in the area of injury permeability in the area of injury.

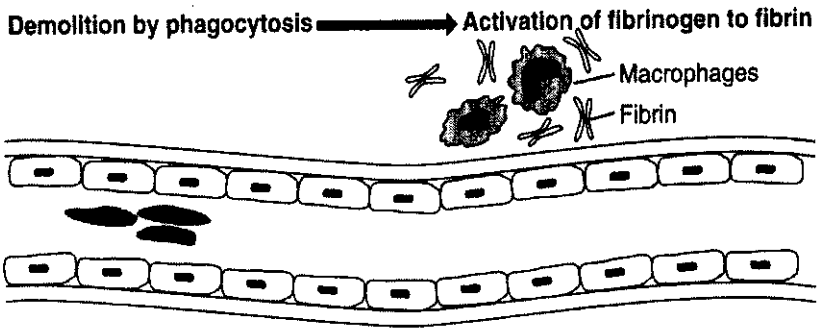


B. Adhesion and Chemotaxis Local mediators attract leukocytes (neutrophils) to the area by chemotaxis. Adhesion molecules expressed on neutrophils and endothelial cells slow the neutrophils, inducing rolling and adhesion of these cells to the endothelium

Figure 6 Phase of inflammation (Hansen, 1998 : 273)



C. **Exudation** Neutrophils, and later macrophages, exude into the injured tissues, where they engulf and digest injurious agents



D. **Fibrin barrier formation** Phagocytosis clears the area in preparation for wound healing. Fibrinogen exudes into the area and is activated to fibrin. Fibrin forms a barrier around the area of injury as well as a matrix for deposition of new tissue during wound healing.

Figure 6 Phase of inflammation (continued)

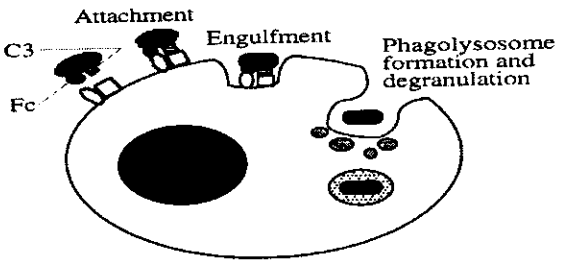
Table 1 Summary of mediators of inflammation response

Mediator	Source	Actions		
		Vascular leakage	Chemotaxis	Other
Histamine & serotonin	Mast cells, platelets	+	-	-
Bradykinin	Plasma substrate	+	-	Pain
Complemet C3a	Plasma protein via liver	+	-	Opsonic fragment (C3b)
Complemet C5a	Macrophages	+	+	Leukocyte adhesion, activation
Prostaglandins	Mast cells, from membrane phospholipids	Potentiate other mediators	-	Vasodilation, pain, fever
Leukotriene B_4	Leukocytes	-	+	Leukocyte adhesion, activation
Leukotriene C_4 ,D_4 ,E_4	Leukocytes, mast cells	+	-	Bronchoconstriction, vasoconstriction
Oxygen metabolites	Leukocytes	+	-	Endothelial damage, tissue damage
Platelet activating factor	Leukocytes, mast cells	+	+	Bronchoconstriction, leukocyte priming
Interleukin-1 and tumor necrosis factor	Macrophages, others	-	+	Acute phase reactions, endothelial activation
Chemokines	Leukocytes, others	-	+	Leukocyte activation
NO/iNOS	Macrophages, endothelium	+	+	Vasodilation, cytotoxicity

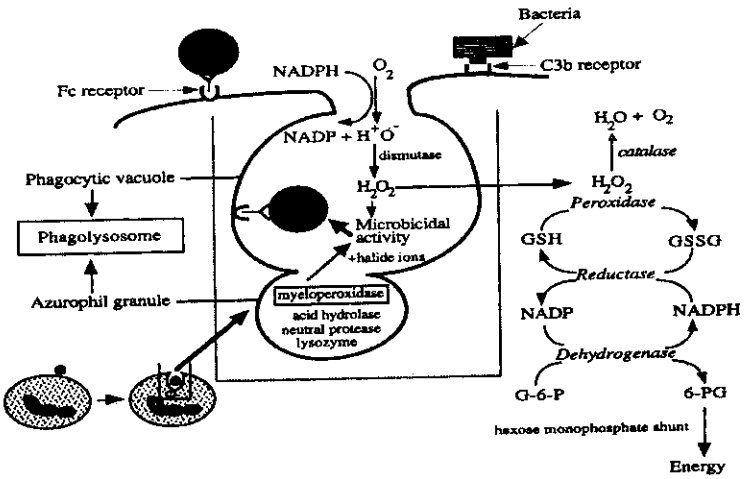
Table 2 Cytokines: Types, sources and effect in regulation of inflammation

(Hansen, 1998 : 269)

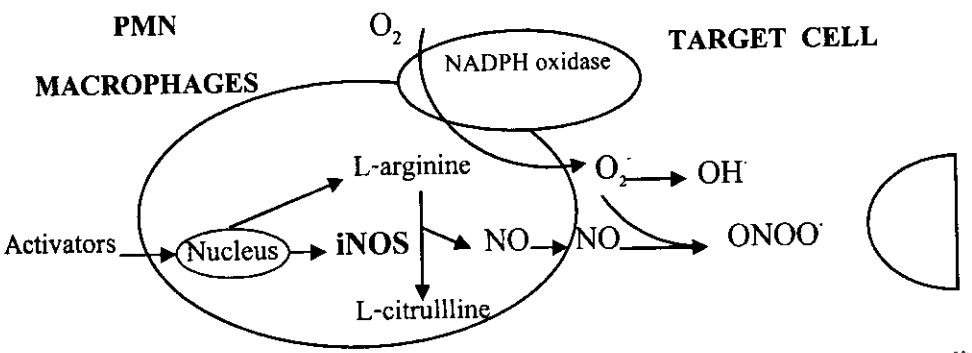
Mediator	Source	Effect
Interleukins (IL)		
IL-1	Macrophages, lymphocytes, neutrophils, epithelial cells, fibroblasts	Regulation of hematopoiesis, pyrogenic activity, tissue catabolism, stimulation of PGs and adhesion molecule synthesis, activation of neutrophils
IL-3	T lymphocytes, monocytes	Colony-stimulating factor
IL-4	T lymphocytes	Colony-stimulating factor
IL-5	T lymphocytes	Eosinophil differentiation, Monocyte-macrophage differentiation
IL-6	Monocytes, endothelial cell ,fibroblasts, T lymphocytes	Colony-stimulating factor, antiviral effects
IL-8	Monocytes, neutrophils, endothelial cells, virally infected fibroblasts	Activation of neutrophils, chemotactic factor
IL-9	T lymphocytes	Colony-stimulating factor, mast cells stimulation
IL-10	Lymphocytes	Immune response regulation, mast cells stimulation
IL-13	T lymphocytes	Inhibition of pro-inflammatory interleukins
Tumor necrosis factor	T lymphocytes, mast cells, macrophages,	Pyrogenic factor, phagocyte activation
Transforming growth factor	Macrophages	Hematopoietic regulation, stimulation of endothelial and fibroblast growth
Transforming growth factor-β	Platelets	Tissue remodeling during wound healing, limitation of inflammatory response
Platelet-derived growth factor	Platelets, macrophages, endothelial cells	Stimulation of wound healing
Basic fibroblast growth factor	Endothelial cells and others	Stimulation of blood vessel growth, stimulation of mitosis
Interferons	Leukocytes, fibroblasts, activated T lymphocytes	Antiviral effects, phagocyte activation
Colony- stimulating factors	Monocyte-macrophages, endothelial cells, fibroblasts	Activation of neutrophils, monocyte-macrophages, eosinophils, basophils



A. Phargocytosis of a particle involves attachment and binding of Fc (fragment of immunoglobulin G) and C3b (opsonin fragment of complement C3) to receptors on the leukocyte membrane, engulfment, and fusion of granules with phagocytic vacuoles, followed by degranulation.



B. Summary of oxygen-dependent bactericidal mechanisms within the phagocytic vacuole



C. The polymorphonuclear (PMN) leukocytes and macrophages –derived superoxide radicals ($O_2^{\cdot-}$) in inflammatory processes, react quickly with iNOS-derived NO to form peroxinitrites ($ONOO^-$).

Figure 7 Phargocytosis and oxygen-dependent bactericidal mechanism within the phagocytic vacuole (Cotran, *et al.*, 1999: 69; Martin, *et al.*, 2001: 888)

(1) Regulation of immune response

Neuronal and endocrine systems have minimal influence on immune or inflammation response. Several cytokines, PGs and NO, appear to play crucial roles in regulating the immune response as well as in modulating gastric mucosal defence.

Cytokines Cytokines are the proteins produced and secreted by many cell types (leukocytes, monocytes-macrophages, platelets and epithelial cells) during immune and inflammatory responses. Cytokines mediate their effects by binding to specific receptors on target cells. They can influence the synthesis or action of other cytokines and are multifunctional in that an individual cytokine may have both positive and negative regulatory actions. These products have additional effects that play important roles in both acute and chronic inflammation. At the initiation of inflammation, NF-κB is one of the transcription factors that is activated. NF-κB appears to be a regulator of the inducible expression of many cytokine genes in lymphocytes, monocytes and epithelial cells in the gut. The inappropriate regulation of NF-κB and its dependent genes have been associated with various pathological conditions. NF-κB proteins reside in the cytoplasm in an inactive state. Following cytokine stimulation, the endogenous inhibitor of NF-κB (I-κB), is phosphorylated and NF-κB is released, translocates to the nuclease, and activates transcription of multiple κB-dependent genes, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, and other chemokines; iNOS; and COX-2 (Figure 8) (Barne and Karin, 1997; Pavlick, *et al.*, 2002). Whereas NF-κB up-regulates TNF-α production, TNF-α activates NF-κB reciprocally. TNF-α, IL-1β, IL-6, IL-8 and IL-12 are the major pro-inflammatory cytokines that mediate many of the physiologic effects of inflammation (Hansen, 1998). TNF-α can activate resident macrophage and promote the release of other pro-inflammatory mediators including nitric oxide, prostacyclin and platelet-activating factor. Additionally, TNF-α induces expression of adhesion molecules on vascular endothelium and favoring the influx of new inflammatory cells into the mucosa. IL-1 stimulates the production of inflammatory eicosanoids (PGE_2 and LTB_4) (Cominelli, 1989) and the production of interleukin-8 (Baggiolini, 1989). TNF-α and IL-1β potentiate the production of each other and also act together to mediate the endothelial and neutrophil activation, the release of lipid mediators, and the synthesis and proliferation of fibroblasts

(Cotran, *et al.*, 1999; Dinarello, 1996). IL-8 is an inflammatory cytokine with neutrophil-chemoattractant and neutrophil stimulating properties which stimulate the release of enzymes and reactive oxygen species from neutrophils leading to tissue damage and an increase in capillary permeability (Hansen, 1998). Both TNF-α and IL-1β also play a key role in causing recurrence of healed gastric ulcer (Arakawa, *et al.*, 1998; Watanabe, *et al.*, 1997). In contrast, circulating interleukin-1 receptor (IL-1ra), IL-4, IL-10, and IL-11 secreted from lymphocytes including transforming growth factor-β (TGF-β) secreted from platelets, are anti-inflammatory cytokines (Figure 9) (Hansen, 1998; William, 2001). It has also been found that TGF-β plays important effect in tissue remodeling during wound healing as well (Cotran, *et al.*, 1999).

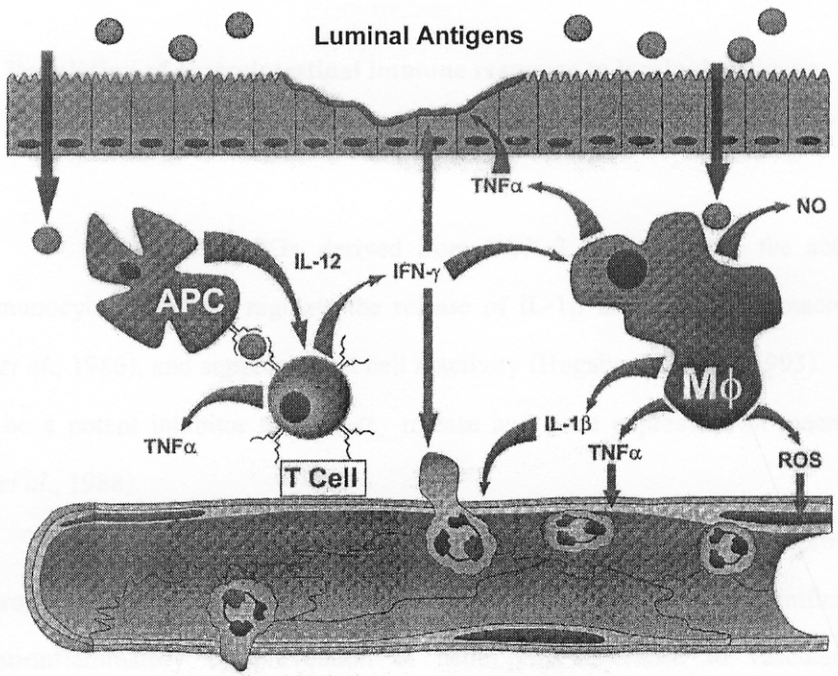


Figure 8 Cell-mediated immune responses in the gastrointestine and colon

(Pavlick, *et al.*, 2002: 313)

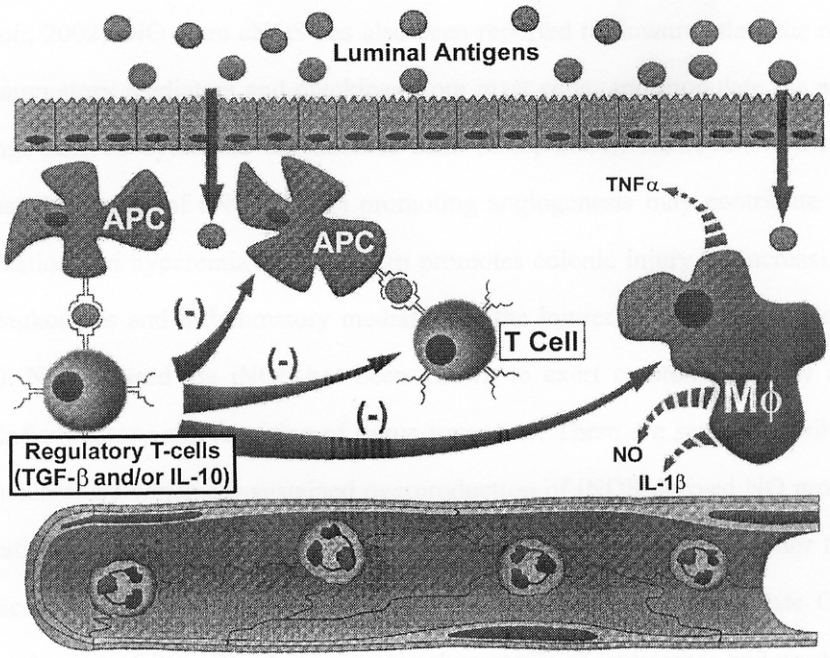


Figure 9 Regulation of gastrointestinal immune response to luminal antigens

(Pavlick, *et al.*, 2002: 314)

PGs PGs, derived from COX-2, can modulate the activity of many immunocytes. PGs can regulate the release of IL-1β and IL-8 from macrophages (Kunkel, *et al.*, 1986), and suppress mast cell reactivity (Hogaboam, *et al.*, 1993). PGE_2 is found to be a potent inhibitor of TNF-α release and gene expression in macrophages (Kunkel, *et al.*, 1988).

NO NO possesses both anti-inflammatory as well as pro-inflam--matory properties. The continuous liberation of NO by eNOS plays a significant role as an anti-inflammatory by prevention of neutrophil adherance to vascular walls, maintenance of cellular integrity, and maintenance of mucosal blood flow (Ma and Wallace, 2000; Martin, *et al.*, 2001). The depletion of NO increases oxidative stress within mast cells and endothelium and together these events promote neutrophil adhesion within the vasculature (Niu, *et al.*, 1996). The antiadhesion property of NO may relate to two mechanisms. The first mechanism relates to its ability to induce the expression and stabilize

IκB, thereby maintaining NF-κB in its inactive form in the cytoplasm (Peng, *et al.*, 1995). The second mechanism relates to its ability to rapidly interact with and decompose $O_2^{\cdot}$ (Pavlick, *et al.*, 2002). NO from cNOS has also been reported to downregulate the release of some inflammatory mediators and cytokines from mast cells, and modulate the actions of macrophage-derived cytokines on mucosal cells (Cho, 2001). However, it has been suggested that the ability of eNOS/NO in promoting angiogenesis may contribute to the neovascularization and hyperemia which in turn promotes colonic injury by increasing the delivery of leukocytes and inflammatory mediators to the injured colonic tissue (Pavlick, *et al.*, 2002). NO released via iNOS has been known to exert cytotoxic activity and is essential in inflammatory and repairing of tissue processes. There are several possibilities for the mechanisms by which the sustained overproduction of iNOS-derived NO promotes gut inflammation (Pavlick, *et al.*, 2002). First, it has been implicated as a mediator for the enhanced microvascular permeability induced by IL-2. Second, it can modulate COX-2 dependent production of PGs. Third, it promotes chemotaxis of leukocytes and production of pro-inflammatory mediators and the last mechanism includes its ability to rapidly react with leukocyte-derived $O_2^{\cdot}$ to form $OONO^{\cdot}$. Nevertheless, iNOS-derived NO seems to be a critical protective response to injury in acute intestinal inflammation possibly by downregulation of endothelial cell adhesion molecule expression and reduction of leukocytic infiltration (Kawachi, *et al.*, 1999; McCafferty, *et al.*, 1997). Additionally, the lack of iNOS does not influence the onset or severity of experimental chronic colitis (McCafferty, *et al.*, 1997). Due to the dual nature of nitric oxide, which is on the one hand, cytotoxic and on the other hand, cytostatic and thus potentially protective, both nitric oxide donors and inhibitors of nitric oxide synthase protect against some forms of injury. NO is therefore likely to have multifaceted role in inflammatory reactions, ranging from the enhancement of vasodilation and the formation of edema, through modulation of sensory nerve endings and leukocyte activity, to tissue cytotoxicity. These actions greatly depend on the type of isozyme involved, the source NO derived, the total amount and duration of NO produced, and the circumstances where NO is acting (Cho, 2001; Yamamoto, 1999).

Loss of control of the inflammatory response can lead to an inappropriate recruitment of phagocytes into the mucosa and, in turn, to mucosal injury.

The metabolic and membrane pertubations that occur in leukocytes during chemotaxis, activation, and phagocytosis result in the release of substances not only within the phagolysosome, but also potentially into the extracellular space. The most important of these substances in leukocytes is lysosomal enzymes which are present in the granules; reactive oxygen and nitrogen metabolites from the activation of the respiratory (oxidative) burst; and products of lipid mediator metabolism. These substances are powerful mediators of endothelial injury and tissue damage and amplify the effects of the initial inflammatory stimulus. Thus, if persistent, the leukocyte infiltration itself may represent persistent mucosal abnormalities reflecting the poor quality of ulcer healing (Arakawa, *et al.*, 1998). Likewise, the suppression of the inflammatory reaction by inhibition of pro-inflammatory cytokines generation supports gastric mucosal defense and promotes the onset of ulcer healing (Shimizu, *et al.*, 2000).

D. Nonenzymatic and enzymatic oxidant defenses

Normally, most tissues possess sufficient amounts of protective nonenzymatic and enzymatic oxidant defenses to decompose excess amounts of oxygen and nitrogen-derived radicals that escape into the surrounding environment thereby limiting tissue damage. Examples of nonenzymatic oxidant defenses are naturally occurring antioxidants [such as ascorbate, β-carotene, α-tocopherol, and reduced glutathione (GSH)] which either block the initiation of free radical formation or inactivate or scavenge free radicals and terminate radical damage, and transport proteins (such as transferring, ferritin, lactoferrin, and ceruloplasmin) which bind iron and copper ion, thereby minimizing $OH^{\cdot}$ formation. A series of enzymatic oxidant defenses to detoxify reactive oxygen and nitrogen species are superoxide dismutase (SOD) which converts $O_2^{\cdot}$ to H_2O_2; catalase which decomposes H_2O_2 to water, and glutathione peroxidase which catalyzes free radical breakdown (Figure 10). Thus, an imbalance between oxidant defenses and pro-oxidative forces will create oxidative stress which leads to chronic gastrointestinal inflammatory diseases. (Cotran, *et al.*, 1999).

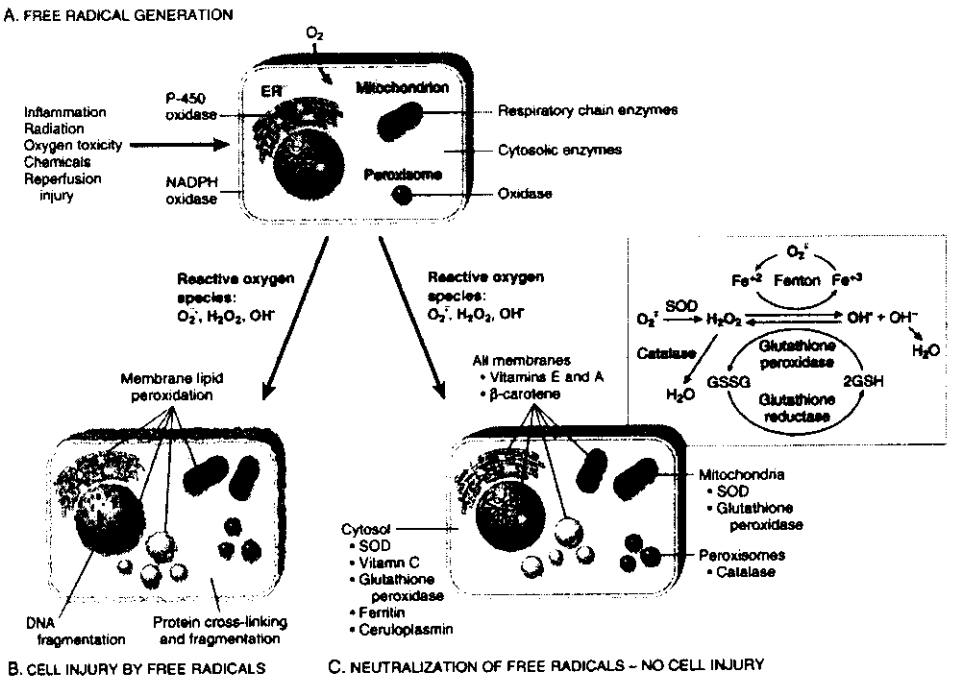


Figure 10 Neutralization of free radicals (Kumar, *et al.*, 2005 : 17)

E. Mucosal repair

Mucosal repair after injury is related to epithelial cell restitution, growth and acute wound healing. The onset of repair is accelerated by early removal of injurious factors such as acid secretion and oxygen free radicals; or the suppression of inflammation through inhibition of inflammatory cytokines formation and neutrophil infiltration (Holzer, 2001). In addition, mucosal repair is also facilitated by stimulation of COX-2 to enhance epithelial cell proliferation and increase expression of growth factors; epidermal growth factor and transforming growth factor-α which facilitate predominantly the recovery of the epithelium; trefoil factor family peptides which are critical mediators of gastrointestinal mucosal restitution; basic fibroblast growth factor and vascular endothelial growth factor which promote restoration of the connective and microvasculature tissue; and eNOS which plays a role in the angiogenesis and repair of the ulcerated gastric mucosa (Halter, *et al.*, 2001; Holzer, 2000; Ma and Wallace, 2000).

2.1.1.2 Drug therapy

A common practice to control *Helicobacter pylori* - negative gastric ulcer is oriented primarily toward medications that neutralize gastric acid (antacids) or inhibit gastric acid secretion (H_2 receptor antagonists, proton pump inhibitors) or protect the gastric mucosa from the effects of acid (sucralfate). The therapeutic choice depends on patient-specific criteria and preferences of both clinicians and patients. Proton pump inhibitors may be slightly more effective than H_2-receptor antagonists but are generally reserved for refractory gastric ulcer. (Henderson and Lander, 2000; Kradjan, 2001)

A. Antacids

Antacids relieve epigastric pain and promote healing of ulcer by providing a cytoprotective effect, neutralizing gastric acid which also inhibits the action of pepsin when the gastric pH increases above 4, and stimulating restitution of the gastric mucosa (Hollander and Tarnawski, 1989; Walt and Langman, 1991). Magnesium and aluminum hydroxide are the most commonly used antacid. The regimen most commonly used is 30 ml 1 hour and 3 hours after a meal and at bedtime. The liquid formation has a more rapid acid-neutralizing action than the tablets (Kradjan, 2001). Although this intensive antacid regimen therapy has been shown to be less expensive than and as effective as the H_2 receptor antagonist and sucralfate, it is no longer common to use as sole therapy. This results from the inconvenience of the regimens used, palatability and adverse effects (Henderson and Lander, 2000). At present, antacids are widely used on an as-needed basis for symptom control (Kradjan, 2001).

The adverse effects of antacids are diarrhea or constipation, hypermagnesemia and hyperalbuminemia (in the presence of renal failure), phosphorus depletion which may caused osteoporosis in long-term use (Henderson and Lander, 2000).

Antacids, by alteration of gastric pH, can interfere with the absorption of drugs that require an acidic environment for dissolution and absorption (Kradjan, 2001). Antacids can also cause drug interaction by chelation of the drug or enhancing renal tubular reabsorption through an increase in urine pH (Henderson and Lander, 2000).

B. H_2 receptor antagonists

H_2 receptor antagonists (such as cimetidine, ranitidine and famotidine),

competitively and reversibly bind to the H_2 receptor of the parietal cells, causing a dose-dependent inhibition of gastric acid secretion (Henderson and Lander, 2000). All H_2 receptor antagonists available in the market are equally effective in the healing of gastric ulcer, well tolerated and can be administered in divided daily doses or daily as a single dose with similar rates of success in ulcer treatment (Kradjan, 2001). H_2 receptor antagonists are approximately equal in efficacy, but the H_2 receptor antagonists are much easier to use than the complicated daily-dosing regimens associated with both antacids and sucralfate (Kradjan, 2001).

In general, H_2 receptor antagonists are remarkably safe and well tolerated when used in recommended doses for treatment of gastric ulcer (Berardi, 1999). Gastrointestinal disturbances occur most frequently. Central nervous system effects, particularly drowsiness and headache, occur most often and have been reported with all of the H_2 receptor antagonists (Berardi, 1999). Cimetidine has antiandrogenic effects and its use has been associated with the development of gynecomastia and impotence (Kradjan, 2001). Hepatotoxicity has occasionally been observed in patients taking ranitidine (Grant, 1989). The elderly patients and those with altered renal or hepatic function are at greater risk for developing adverse effects.

Several drugs interact with the H_2 receptor antagonists, in particular cimetidine. Cimetidine binds to the cytochrome P450 mixed-function oxidase enzyme system and inhibits the biotransformation of several drugs by the liver (Kradjan, 2001). Ranitidine binds less intensely to the cytochrome P450 whereas famotidine does not bind appreciably to the cytochrome P450 system (Henderson and Lander, 2000). Cimetidine and ranitidine inhibit the renal tubular secretion of cationic compounds (Kradjan, 2001). Therapeutic doses of cimetidine and ranitidine enhance the absorption of ethanol following ingestion of moderate amounts of alcohol (Kradjan, 2001). All of the H_2 receptor antagonists can potentially affect the absorption and reduce the bioavailability of some drugs by altering the gastric pH (Kradjan, 2001).

C. Proton pump inhibitors

Proton pump inhibitors (omeprazole, lansoprazole, pantoprazole and rabeprazole) are highly specific dose-dependent inhibitors of basal and stimulated gastric

acid secretion (Soll, 1998). Under acidic condition in the parietal cell, the parent compound is protonated and converted to active metabolites which react covalently with H^+K^+-ATPase. A sulfhydryl bond is formed that noncompetitively and irreversibly inhibits the activity of enzymes. A full restoration of acid secretion after discontinuing the proton pump inhibitors takes about 3 to 5 days (Berardi, 1999). Therefore, they cause almost total shutdown of acid release (Kradjan, 2001). Proton pump inhibitors relieve symptoms and heal ulcer more quickly than H_2 receptor antagonists, however, the absolute healing rates for drugs in both groups are comparable after completion of the requisite course of therapy (Kradjan, 2001). All of proton pump inhibitors provide similar healing rates and symptomatic relief (Berardi, 1999).

The adverse effects of these agents are relatively negligible and comparable to those of H_2 receptor antagonists (Kradjan, 2001).

Omeprazole selectively binds to P450 oxidative enzymes and therefore potentially interferes with hepatic drug metabolism whereas lansoprazole, pantoprazole and rabeprazole do not appear to interact with hepatic P450 enzymes (Berardi, 1999).

D. Sucralfate

Sucralfate is an aluminum salt of a sulfated disaccharide. When exposed to gastric acid, sucralfate forms a viscous adhesive that binds electrostatically to positively charged protein molecules in the ulcer crater, forming a protective barrier that inhibits back diffusion of hydrogen ions (McCarthy, 1991; Soll, 1998). It protects ulcerated tissue from aggressive factors such as pepsin, acid, and bile salts. It may have other protective actions on the mucosa that are possibly mediated by prostaglandin and gastric bicarbonate secretion (Shorrock and Rees, 1989). It does not have an important effect on acid secretion (Berardi, 1999). Sucralfate also appears to be as safe and effective as H_2 receptor antagonists in the treatment of gastric ulcer (McCarthy, 1991). However, US Food and Drug Administration (FDA) does not approve its use for gastric ulcer.

Because sucralfate is minimally absorbed, its adverse effects are usually minor and occur in less than 5% of patients. Constipation is most common whereas nausea, indigestion, dry mouth, dizziness, and a metallic taste occur infrequently (Kradjan, 2001).

The concomitant use of sucralfate with fluoroquinolones, phenytoin, digoxin, theophylline, quinidine, amitryptyline, warfarin, ketoconazole and L-thyroxine may reduce their bioavailability and effectiveness (Berardi, 1999).

E. Prostaglandins

Misoprostol, a synthetic PGE_1 analog, moderately inhibits acid secretion and enhances mucosal defense (Soll, 1998). It heals ulcer comparable to conventional H_2 receptor antagonists or sucralfate regimens. However, it is not recommended for the treatment of gastric ulcer in the United States (Berardi, 1999).

The most common adverse effects are crampy abdominal pain and diarrhea. It is uterotrophic and can produce contraction that may endanger pregnancy (Berardi, 1999).

2.1.2 Reflux esophagitis

Reflux esophagitis refers to the inflammation of the esophagus resulting from repeated exposure for prolonged periods of time to refluxed gastric and/or duodenal contents from the stomach into the esophagus (Williams, 1999). Nearly 50% of the patients have complications of the disease, including esophagitis, stricture, or Barrett's esophagus. Patients who present with erosive esophagitis predictably follow a course of relapsing disease requiring more intensive treatment with acid-suppressive therapy followed by long-term maintenance therapy (Williams, 1999). It has the potential to lead to serious consequences, including cancer of the esophagus. (Henderson and Lander, 2000; Kradjan, 2001)

2.1.2.1 Pathophysiology

The development of reflux esophagitis is associated with a disruption of the balance between aggressive factors (gastric acid, pepsin, bile salts and pancreatic secretions) and defensive mechanisms which involve the lower gastroesophageal sphincter closure; esophageal peristalsis; and the mucosal resistance resulting from esophageal mucus, epithelial cell turnover, mucosal blood flow, tissue prostaglandins and the acid-base status of the tissue (Kradjan, 2001; Williams, 1999). When the mucosa is repeatedly exposed to the refluxate or if there is a defect in the normal mucosal defenses, hydrogen

ions will diffuse into the mucosa, leading to the cellular acidification and necrosis that ultimately cause esophagitis (Fennerty, *et al.*, 1996). In an animal model, it was found that if the pH of refluxate is less than 2, pepsin is activated and esophagitis may develope secondary to protein denaturation (Williams, 1999). A mixed reflux of gastroduodenal contents has been reported to inflict more damage than when gastric juice is refluxed alone (Kauer, *et al.*, 1995), and maximal epithelial injury occurs during exposure to bile salts combined with acid and pepsin (Lillimoe, *et al.*, 1983). However, the fact that the degree of damage does not completely correlate with the amount of refluxed materials, suggests other contributing mechanisms in reflux esophagitis including impaired mucosal resistance (Lanas, *et al.*, 1997; Orlando, 1994). More recent studies in rats as well as in esophageal biopsy samples of patients with reflux esophagitis have shown that mucosal damage in reflux esophagitis is mediated primarily by oxygen derived free radicals accompanied by enhanced esophageal mucosal lipid peroxidation (Wetscher, *et al.*, 1995; 1997). In addition, administration of various free radical scavengers has also been found to prevent esophageal mucosal damage whereas antisecretory treatment alone with ranitidine is not effective in either decreasing the degree of reflux esophagitis or attenuating inflammation associated oxygen derived free radical mediated NFκB activation (Oh, *et al.*, 2001; Wetscher, *et al.*, 1995c). They suggest that antioxidant treatment should be considered as supplementary therapy in the prevention or treatment of reflux esophagitis with acid suppression.

2.1.2.2 Drug therapy

A. H_2 receptor antagonists

H_2 receptor antagonists in full-sized divided daily doses are the mainstay of treatment for mild to moderate reflux esophagitis (Kradjan, 2001). The severity of the disease, the dose of the drug and the duration of therapy affect the response to H_2 receptor antagonists. The more severe the esophageal damage, the poorer the response to H_2 receptor antagonists (Williams, 1999).

B. Proton pump inhibitors

Proton pump inhibitors heal esophageal ulcers quicker and more effectively than H_2 receptor antagonists (Kradjan, 2001). They are the drug of choice in patients with moderate to severe reflux esophagitis (Rai and Orlando, 2001). They are also

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effective in treating reflux esophagitis refractory to H_2 receptor antagonists or in severe cases (Rai and Orlando, 2001; Sontag, *et al.*, 1989). However, in patients with complicated symptoms, relapse is common and long-term maintenance therapy is generally indicated (Williams, 1999).

C. Prokinetic agents

Prokinetic agents such as metoclopramide, domperidone and cisapride, stimulate the motility of the upper GI tract without affecting gastric acid secretion and increase gastric peristalsis which leads to accelerated gastric emptying. It is effective in improving defects related to esophagogastric motility such as decreased lower esophageal sphincter pressure, decreased esophageal clearance, or decreased gastric emptying. It can be used as an adjunct to acid-suppressing therapy if one of these motility problems is noted in a patient or in the patient who fails to high-dose proton pump inhibitor therapy (Williams, 1999).

2.1.3 Ulcerative colitis

Inflammatory bowel diseases in humans, including ulcerative colitis and Crohn's disease, are chronic idiopathic inflammatory disorders of the ileum and/or colon that are characterized by rectal bleeding, severe diarrhea, abdominal pain, and weight loss. Ulcerative colitis manifests primarily as a continuous inflammation involving mostly the mucosal and the submucosal layer, but not the deeper longitudinal muscular layer or serosa of the intestinal wall (Berardi, 2000).

2.1.3.1 Pathophysiology

The etiology of ulcerative colitis remains unknown and is likely multifactorial. The lesion seems to be related to an antibody-mediated hypersensitivity reaction and a dysregulated immune response increasing the synthesis of pro-inflammatory cytokines (especially IL-1, IL-6 and TNF-α), and the influx of nonspecific inflammatory cells (macrophage and neutrophil) into the inflamed mucosa (Dinarello, 1996; Pallone and Monteleone, 2001; Podolsky, 2002). These infiltrating nonspecific inflammatory cells may be related to ischemic injury involving the release of reactive oxygen species which damage epithelial cells and disrupt the integrity of the epithelial barrier (Berardi, 2000).

Among the inflammatory cytokines, IL-1 and TNF-α have broad spectrum of biologic effects relevant to ulcerative colitis (Dinarello and Wolff, 1993; Fiocchi, 1998). It can also contribute to intestinal damage by directly altering the integrity of epithelial membrane. Both TNF-α and IL-1β stimulate gut fibroblasts to synthesize matrix metalloproteinases without altering production of tissue inhibitors of matrix metalloproteinases (MacDonald, *et al.*, 1999). Matrix metalloproteinases are a family of endopeptidases that degrade most components of the extracellular matrix. They are secreted as proenzymes such as collagenase and stromelysin which have proteolytic activity resulting in tissue injury with destruction of the mucosa. Furthermore, cytokines could indirectly produce changes in the gut by enhancing stromal cell production of epithelial growth factors especially keratinocyte growth factor (KGF) which is responsible for the crypt cell hyperplasia (MacDonald, *et al.*, 1999). Thus, both inflammation and epithelial repair are controlled by the same regulatory molecules. The mucosal and serum prostaglandin concentrations are also found to be elevated. However, there is evidence against their role as mediators of inflammation because non-steroidal anti-inflammatory drugs (NSAIDs) have failed to provide clinical improvement (Berardi, 2000). It has also been indicated that iNOS/NO together with peroxynitrite play a significant role in the inflammatory respose in the colon as well (Rachmilewitz, *et al.*, 1993). Recently, it is becoming increasingly appreciated that IL-10 and TGF-β play important roles in regulating tissue inflammation and injury in different models of colitis since they can downregulate Th1 and macrophage-derived cytokine synthesis (Powrie, 1995).

The ability of the epithelium to withstand injury is mediated by protective factors such as secretory immunoglobulin A (IgA), mucus, trefoil peptides, secretory leukocyte proteinase inhibitor (SLPI), heat shock proteins and growth factors. Trefoil peptides are known to be critical mediators of gastrointestinal mucosal restitution but the mechanisms regulating their expression have not been understood (Stenson, 2001). SLPI is a secreted proteinase that inhibits enzymes with serine proteinase activity including digestive enzymes and leukocyte enzymes. SLPI is increased by TNF-α and IL-1. SLPI itself does not affect the epithelial barrier but protects the epithelium from neutrophil induced injury by inhibiting neutrophil elastase (Si-Tahar, *et al.*, 2000). Heat shock proteins

are a family of both constitutively expressed and inducible proteins. Inducible heat shock proteins have been shown to increase the survival of intestinal epithelial cells faced with oxidant or heat-induced stress while heat shock protein 72 protects intestinal epithelial barrier function from oxidant stress (Musch, *et al.*, 1999; Stenson, 2001). Therefore, trefoil peptides, SLPI and heat shock proteins play the most important role in protecting the integrity of the epithelial barrier. Other agents, growth factors, regulate epithelial cell proliferation and differentiation after injury including other events relevant to mucosal repair such as angiogenesis, extracellular matrix remodeling and fibroblast activation (Beck and Podolsky, 1999).

2.1.3.2 Drug therapy

A. Aminosalicylates

Aminosalicylate preparations remain the mainstay of initial treatment for mild-moderate ulcerative colitis. Topical preparation has been shown to be advantageous as first-line therapy for distal ulcerative colitis (Marshall and Irvine, 1995, 1997).

Sulfasalazine has been used for many years to treat ulcerative colitis. Sulfasalazine is cleaved by gut bacteria in the colon to sulfapyridine (which is mostly reabsorbed and excreted in the urine) and mesalamine (5-ASA) (which mostly remains in the colon and is excreted in the stool). The mechanism of action of sulfasalazine is related to only the mesalamine component whereas sulfapyridine component is believed to be responsible for many of the adverse reactions to sulfasalazine. Mesalamine alone can be used and oral derivatives of mesalamine were also developed with a lower frequency of adverse effects. However, at present, sulfasalazine is still used in preference to oral mesalamine derivatives, mainly because it costs much less. The precise mechanism of action of mesalamine is not well understood but is generally thought to be a topical effect (Van, *et al.*, 1980). Mesalamine may inhibit macrophage production of cyclooxygenase, thromboxane synthetase, platelet activating factor synthetase and IL-1 (DiPiro and Schade, 1999). An alternative theory suggests that mesalamine acts as a superoxide-free radical scavenger (DiPiro and Schade, 1999).

Dose-related side effects of sulfasalazine include gastrointestinal

disturbance such as nausea, vomiting, diarrhea or anorexia. These adverse reactions tend to occur more commonly on initiation of therapy and decrease in frequency as therapy is continued. Idiosyncratic reactions are rash, fever or hepatotoxicity most commonly, as well as relatively uncommon but serious reactions such as bone marrow suppression, thrombocytopenia, pancreatitis and hepatitis (DiPiro and Schade, 1999).

B. Corticosteroids

Corticosteroids and adrenocorticotropic hormone (ACTH) have been widely used for the treatment of ulcerative colitis. The mechanism of action or the site of action of corticosteroids is not known but is believed to involve modulation of the immune system and inhibition of cytokines and mediators production (DiPiro and Schade, 1999).

The well-appreciated adverse effects of corticosteroids are hyperglycemia, hypertension, osteoporosis, acne, fluid retention, electrolyte disturbances, myopathies, muscle wasting, increased appetite, psychosis and reduced resistance to infection including adenocortical suppression.

C. Immunosuppressives

Immunosuppressives such as azathioprine and 6-mercaptopurine (a metabolite of azathioprine) are effective for long-term treatment of ulcerative colitis for patients who are refractory to steroids and are usually used in conjunction with mesalamine derivatives and/or steroids whereas cyclosporine in a continuous intravenous infusion has been of short-term benefit in treatment of acute, severe ulcerative colitis (DiPiro and Schade, 1999).

Azathioprine and 6-mercaptopurine cause bone marrow suppression, lymphoma, skin cancer, pancreatitis and nephrotoxicity. Cyclosporine possesses risk of nephrotoxicity and neurotoxicity. (DiPiro and Schade, 1999).

5-Aminosalicylate-based compounds remain the mainstays of treatment for mild to moderate ulcerative colitis (Berardi, 2000). Corticosteroids are used when 5-aminosalicylate-based compounds are inadequate, while immunosuppressive and immunomodulatory agents are generally appropriate for patients in whom the dose of corticosteroids cannot be tapered or discontinued (Berardi, 2000).

2.2 Curcumin

2.2.1 Medicinal background

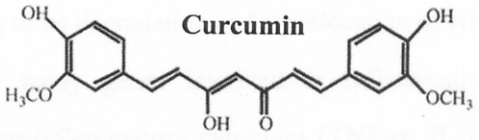
C. longa Linn. or *Curcuma domestica* (*C. domestica*) Val. or so-called "Khamin Chan", a member of the ginger family (Zingiberaceae), is widely distributed throughout the tropical and subtropical regions of the world mainly in Asian countries. It is a perennial herb that measures up to 1 meter high with a short stem and tufted leaves (Figure 11A). Its rhizomes are oblong, ovate, pyriform and often short-branched. As a yellow to yellowish-brown powder, called turmeric, it has been in extensively continuous use for imparting color and flavor in both vegetarian and non-vegetarian food preparations as well as for medicinal purposes. Indigenous systems of medicine, including the Chinese and Ayurvedic (the Indian traditional medicine) systems, have widely used turmeric for centuries in the treatment of many inflammatory conditions and diseases. In old Hindu texts, it is ascribed for its aromatic, stimulant and carminative properties including the treatment of sprains and swelling caused by injury (Ammon and Wahl, 1991). Current traditional Indian medicine claims the use of its powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (Jain and DeFilipps, 1991). Traditional medicine in China uses turmeric in diseases which are associated with abdominal pains and icterus (Ammon and Wahl, 1991). In the Nepali medicinal books, turmeric is considered to be stimulating, carminative, purifying, anti-inflammatory and anthelmintic when use internally. Externally, the mixing of rhizome powder with alum is also applied as a paste to wounds, bruises, inflamed joints and sprains (Eigner and Scholz, 1999). In the United States, turmeric is an approved food additive and is commercially available at low cost (Grant and Schneider, 2000). In Thailand, it is recommended by World Health Organization and Thailand's Essential Drug List as a herbal medicine for the treatment of dyspepsia (National Drug Committee, 2000; World Health Organization, 1999).

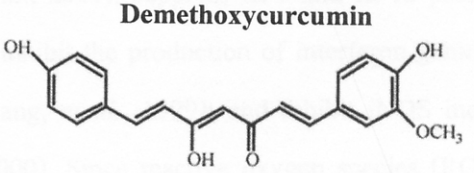
2.2.2 Chemistry

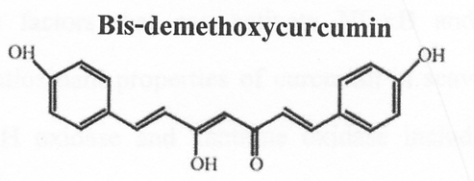
Analytical studies have so far revealed that turmeric contains curcuminoids

and volatile oils. In Thailand, the curcuminoid content of various turmeric rhizomes cultivated in Phichit, Trang and Tak experimental fields is about 0.48-16.46 %w/w (Tewtrakul, 1993). Curcumin [diferuloylmethane or 1,7-bis-(4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione; Figure 11B] is the main constituent of curcuminoids fraction (0.38-7.54 %w/w). The others are the derivatives of demethoxycurcumin, bis-demethoxy--curcumin (Tewtrakul, 1993). Curcumin has a molecular weight of 368.37 and a melting point of 183 ^{0}C. It is relatively insoluble in water, but dissolves in ethanol, alkali, acetone, acetic acid, dimethylsulphoxide, and chloroform (Araujo and Leon, 2001). It is unstable at basic pH, and degrades within 30 minutes to trans-6-(4'-hydroxy-3'-methoxyphenyl) -2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin (Wang, *et al.,* 1997). However, it is more stable in cell culture medium containing 10% fetal calf serum, human blood, antioxidants such as ascorbic acid, N-acetylcysteine or glutathione (Wang, *et al.,* 1997). Samples containing curcumin should be protected from light because of its light sensitivity (Tonnesen, *et al.,* 1986).

A







B

Figure 11 The plant *C. longa* Linn. (A) from which curcumin is derived and its structure (B)

2.2.3 Pharmacology of curcumin

2.2.3.1 Anti-inflammatory activity

A systematic investigation on the anti-inflammatory activity of turmeric has indicated curcumin to be the major constituent responsible for the anti-inflam--matory activity of the extracts. The potency of curcumin (intraperitoneal administration) is approximately equal to phenylbutazone (reference drug) in the carrageenin-induced rat paw edema test, but it is only half as active in the chronic experiments when given orally (Srimal and Dhawan, 1973, 1985). The oral effective dose needed for 50% inhibition of paw edema is 100.2 mg/kg in mice and 48.0 mg/kg in rats, respectively. As illuatrated in Figure 12, the primary target of curcumin induced anti-inflammatory action could be the plasma membrane where the activity of protein kinase C (PKC) (Liu, *et al.*, 1993) and epidermal growth factor (EGF)-receptor tyrosine kinase (Korutla, *et al.*, 1995) are first inhibited. PKC-mediated nuclear protein factors such as IκB kinase (IKK) and NF-κB are then inhibited through various signal transduction pathways (Jobin, *et al.*, 1999; Pan, *et al.*, 2000). As an inactive form, NF-κB is bound to an inhibitor IκB and resides in the cytoplasm. Activation of NF-κB is induced by a cascade of events leading to the activation of IKK, which phosphorylates IκB, leading to its degradation and translocation of NF-κB to the nucleus. Since NF-κB up-regulates many genes involved in inflammation and immunity, curcumin is found to inhibit pro-inflammatory cytokines (TNF-α, IL-1β, and IL-8) production (Chan, 1995; Literat, *et al.*, 2001); suppress IL-6 and IL-12 production (Bharti, *et al.*, 2003; Kang, *et al.*, 1999); inhibit the production of interferon gamma and Th1 cytokine profile in $CD4^+$ T cells (Kang, *et al.*, 1999); and inhibit iNOS induction (Chan, *et al.*, 1998; Onoda and Inano, 2000). Since reactive oxygen species (ROS) are considered to be endogenous mitogenic factors that can activate NF-κB and other transcription factors in the nucleus, the antioxidant properties of curcumin in scavenging ROS produced from both NADPH/NADH oxidase and xanthine oxidase including its ability to scavenge nitric oxide radicals are also contributed to its anti-inflammatory action. In addition, curcumin is found to be a potent inhibitor of all branches of the arachidonic acid cascade by inhibition of both lipoxygenase and cyclooxygenase enzyme activities (Ammon, *et al.*, 1993). Curcumin also lowers the release of proteolytic enzymes such as

collagenase, elastase, and hyaluronidase from activated macrophages (Joe and Lokesh, 2000) including several matrix metalloproteinases (Onodera, *et al.*, 2000). Furthermore, it has been reported that curcumin exhibits potent suppressive activities on allergy type I and IV (Yano, *et al.*, 2000).

A double blind crossover clinical trial reported the significant efficacy of curcumin in improvement of symptoms in 18 patients with definite rheumatoid arthritis when administerd in the doses of 1200 mg daily orally for 2 weeks (Deodhar, *et al.*, 1980). A separate double-blind trial found that oral administration of 400 mg curcumin three time a day over 6 days was superior to placebo or phenylbutazone (reference drug) for alleviating post-surgical inflammation (Satoskar, *et al.*, 1986).

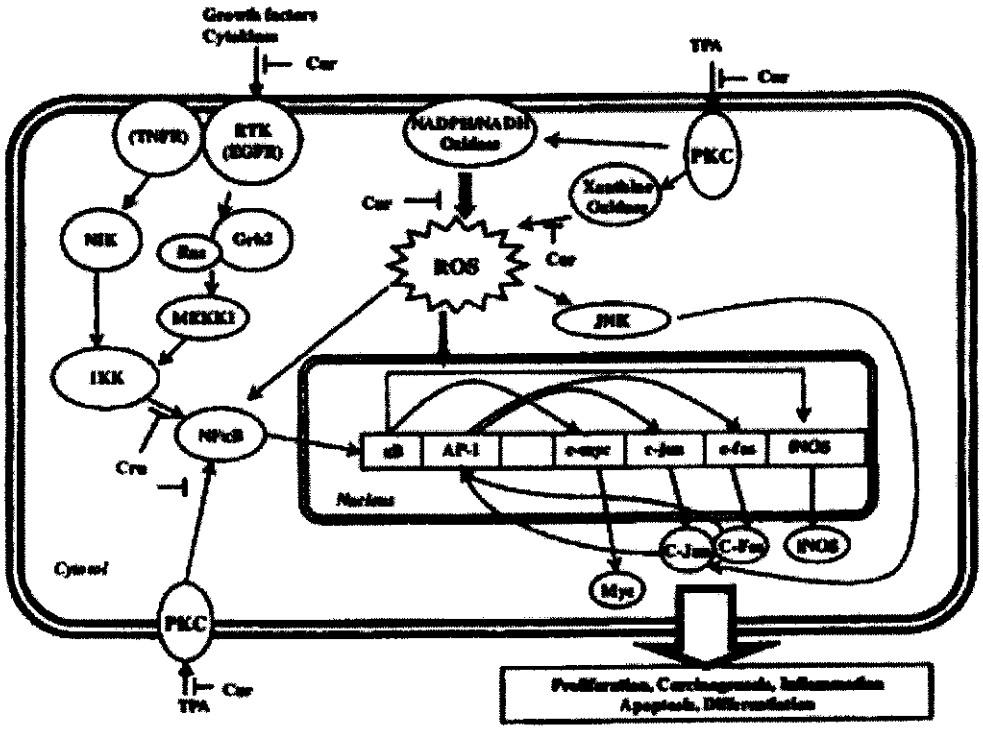


Figure 12 Possible mechanism of action of curcumin for the inhibition of inflammation and carcinogenesis at molecular target Curcumin (Cur) has been demonstrated to block several sites of these multiple signal transduction pathways as indicated by the blockade symbol (⊥) (Lin, *et al.*, 2001: 64)

Abbreviation: EGFR = epidermal growth factor receptor, ERK = extracellular response kinase, IKK = IκB kinase, JNK = c-jun N-terminal kinase, MAPK = mitogen-activated protein kinase, $MEKK_1$ = MAPK/ERK kinase kinase, MKK_4 = MAPK kinase, NIK = NFκB, PKC = protein kinase C, TNFR = tumor necrosis factor receptor, TPA = tumor promoter 12-O-tetra--decanoylphorbol-131acetate, ROS = reactive oxygen species

2.2.3.2 Antioxidative activity

The antioxidative property of curcumin has also been studied in various models by several authors (Araujo and Leon, 2001). The results demonstrated that curcumin is a good inhibitor of lipid peroxidation in rat liver microsomes, erythrocyte membranes, brain homogenates, and hemoglobin. Curcumin, at the high concentration

(270 μM), is a good scavenger of superoxide anions and hydroxyl radicals which are important to the initiation of lipid peroxidation (Kunchandy and Rao, 1990). Recent study indicates that curcumin, at very low concentration (2.75 uM) is an effective singlet oxygen quencher in aqueous systems (Das and Das, 2002). Furthermore, curcumin is found to be a potent inhibitor of reactive oxygen-generating enzymes such as lipoxygenase/cyclooxy--genase, xanthine dehydrogenase/oxidase, and inducible nitric oxide synthase (Lin and Lin-Shiau, 2001). Interestingly, curcumin also enhances the activities of other antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase (Reddy and Lokesh, 1994). It also shoud be noted that curcumin may possess pro-oxidant activity as well as antioxidant effects, dependent on dose and chemical environment (Ahsan, *et al.*, 1999). Recently it has been reported that the metabolite compound of curcumin, tetrahydrocurcumin, seems to be an active principle of curcumin for its antioxidant effect (Okada, *et al.*, 2001)

2.2.3.3 Wound healing property

Oral administration of curcumin (40 mg/kg daily for 7-11 days) significantly enhanced the cutaneous wound healing in rats and guinea pigs (Sidhu, *et al.*, 1998). Biopsies of the wound show reepithelialization of the epidermis; migration of myofibroblasts and fibroblasts into the wound bed; extensive neovascularization of the dermis; and greater collagen deposition. Moreover, polymerase chain analysis and in situ hybridization also shows an increase of TGF-β_1 which may play an important role in the enhancement of wound healing by curcumin (Sidhu, *et al.*, 1998). Similarly, topical application of curcumin is reported to accelerate healing of cutaneous wound with an enhancement of TGF-β_1 expression in dexamethasone-impaired cutaneous healing in a full thickness punch wound model in rats (Mani, *et al.*, 2002).

2.2.3.4 Effects on the gastrointestinal system

The non-specific antispasmodic activity of sodium curcuminate has been reported on the isolated guinea pig ileum against several spasmogens such as nicotine, acetylcholine, barium chloride, histamine and 5-hydroxytryptamine (Ammon and Wahl, 1991). Addition of curcumin to chickpea diet can decrease the amount of gas formation by the effect which seems not to be due to antibacterial activity. Besides, curcumin has been found to possess a protective effect on the liver (Chuang, *et al.*, 2000;

Kiso, *et al.*, 1983); a stimulation of bile secretion in rats (Deters, *et al.*, 2000); and a positive contraction effect on the human gall bladder (Rasyid and Lelo, 1999). In addition, it has been found that rats maintained on diets containing 0.5% curcumin enhance pancrealytic digestive enzymes activities (Platel and Srinivasan, 2000)

Neither turmeric nor curcumin has been extensively studied for its antiulcerogenic activity against peptic ulcer. It has been found that curcumin at a dose less than 50 mg/kg stimulates mucin secretion in guinea pig and possesses beneficial effects in protecting the gastric mucosa against irritants such as phenylbutazone, aspirin and serotonin (Sinha, *et al.*, 1975). Rafatullah, *et al.* (1990) has reported the significant anti-ulcerogenic activity of an ethanol extract of *C. longa* rhizome in rats subjected to pyloric ligation, hypothermic-restraint stress, indomethacin and reserpine administration. It has also been found that the extract not only increases the gastric wall mucus significantly but also restores the non-protein sulfhydryl content in the glandular stomachs of the rats. Similar results are also found by a group of Thai researchers. Prucksunand, *et al.* (1997), has reported such a cytoprotective effect of crude turmeric powder suspension against hydrochloric acid induced gastric necrosis in rats when orally pretreated with the suspension. Furthermore, curcumin has shown an inhibitory effect on NFκB activation and also IL-8 induction by *Helicobacter pylori* (Munzenmaier, *et al.*, 1997). Nevertheless, controversial data exist regarding to its antiulcerogenic activity. Prasad, *et al.* (1976) has reported that the administration of curcumin at a dose more than 100 mg/kg over 6 days can produce gastric ulceration in rats. Pretreatment with metiamide (an H_2 receptor antagonist) shows almost complete protection against curcumin-induced gastric ulcer and blocking of curcumin-induced decrease in mucin secretion whereas adrenergic, cholinergic or H_1 receptor antagonists provides partial protection against the development of gastric ulcer (Gupta, *et al.*, 1980).

Recently, the efficacy of curcumin against experimental colitis in murine model which resembles human Chron's disease has also been investigated. It is found that pretreatment with curcumin or mixed with the diet and given for dietary administration, exerts beneficial preventive effects in dinitrobenzene or Hapten 2,4,6- -trinitrobenzene sulphonic acid-induced colitis (Salh, *et al.*, 2003; Sugimoto, *et al.* 2002;

Ukil, *et al.*, 2003). However, no study on the efficacy of curcumin against experimental colitis in murine model which resembles human ulcerative colitis is available.

Only turmeric has been studied in clinical trials. One clinical double blind study in patients with gastric ulcers has shown turmeric to be less effective than antacids when prescribed at the dose 250 mg three times before meals and at bedtime for six weeks (Kositchaiwat, *et al.*, 1993). Also, another clinical study in patients with duodenal ulcer has not shown turmeric to be superior to placebo when prescribed at the dose 6 g/d continuously for 8 weeks (Van Dau, *et al.*, 1998). However, results from the recent clinical trial of turmeric on healing of peptic ulcer in 333 patients with ulcer located in the duodenal bulb and gastric (angular) has shown that 76 % of the patients had no ulcer after 12 weeks of receiving turmeric orally at the dose 600 mg five times daily one half to an hour before meals, at 16.00 hour, and at bedtime (Prucksunand, *et al.*, 2001).

2.2.3.5 Anti-tumor activity

Many reports from studies with cancer cell lines, animal tumor cells and several animal models, have indicated that turmeric as well as curcumin could prevent carcinogenesis (Ammon and Wahl, 1991; Araujo and Leon, 2001; Lin and Lin-Shiau, 2001; Soudamini and Kuttan, 1989). Huang, *et al.* (1988), studying a potential anti-tumor activity of curcumin on tumor promotion in mouse skin by 12-O-tetradecanoyl-13-acetate, found that curcumin could inhibit the epidermal ornithine decarboxylase and DNA synthesis with efficacy superior to chlorogenic acid, caffeic acid or ferulic acid. It has been documented that turmeric has a chemopreventive effect on benzo(a)pyrene induced forestomach and 7, 12-dimethylbenz(a)anthracene induced skin tumors in mice (Azuine and Bhide, 1992). Dietary curcumin exhibited preventive activity against carcinogenesis in forestomach, duodenum and colon (Huang, *et al.*, 1994; Rao, *et al.*, 1995). A more recent study has found that curcumin can block certain cyclosporine-resistant pathways of T-cell proliferation and may be a potential adjuvant immunosuppressive agent for the treatment of cancer (Ranjan, *et al.*, 1998). It is indicated that anticarcinogenic effects of curcumin includes antioxidant property; anti-inflammatory property; enhancement of the activities of Phase-2 detoxification enzymes of xenobiotic metabolism (such as glutathione transferase, epoxide hydrolase and NADPH: quinine reductase), while inhibiting procarcinogen

activating Phase-1 enzymes [such as cytochrome p450 1A1 (Ciolino, *et al.*, 1998); and inhibition of angiogenesis (Thaloor, *et al.*, 1998) and cell-cell adhesion (Abe, *et al.*, 1999; Chan, 1995; Gupta and Ghosh, 1999; Jaiswal, *et al.*, 2002]. Additionally, curcumin is reported to cause apoptosis in various cancer cell lines and animal tumor cells (Jiang, *et al.*, 1996). Sufficient data currently exist to advocate phase II clinical evaluation of oral curcumin in patients with invasive malignancy or pre-invasive lesions of the gastrointestinal tract, particularly the colon and rectum (Sharma, *et al.*, 2005).

2.2.3.6 Effects on microorganisms

Curcumin possesses bacteriostatic activity against *Staphylo--coccus aureus* and has an excellent anti-protozoal activity against promastigotes (extracellular) and amastigotes (intracellular) forms of *Leishmania amazonensis* (Araujo, *et al.*, 1998, 1999). It is also found to have antiviral activity, being a HIV-1 integrase inhibitor (Mazumber, *et al.*, 1995). Later, it is claimed to have anti–HIV-1 and HIV-2 activities in a recent patent application (Eigner and Scholz, 1999).

2.2.3.7 Other activities

Curcumin, at a dose of 7.5 mg/kg intravenously, produces a sharp and transient hypotensive effect in dogs which is resistant to blockade by atropine, histamine H_1 antagonist and beta-adrenergic antagonists and also produces a depressant effect on isolated guinea pig heart (Ammon and Wahl, 1991). It is stated that curcumin at doses between 25 and 100 mg/kg (intraperitoneal) inhibits collagen and adrenaline induced platelet aggregation *in vitro* as well as *ex vivo* but does not affect prostacyclin (PGI_2) synthesis by rat thoracic aorta (Ammon and Wahl, 1991). It is, therefore, conceivable that curcumin may have an anti-thromboxane A_2 activity. Curcumin is also found to preferentially inhibit the platelet-activating factor and arachidonic acid (Shah, *et al.*, 1999). Recently, it is found that curcumin can reduce oxidative damage and amyloid pathology in an alzheimer transgenic mouse model (Lim, *et al.*, 2001)

2.2.4 Pharmacokinetic property of curcumin

Ravindranath and Chandrasekhara (1980) have reported that the bioavailability of curcumin is about 60-65% after oral administration of 400 mg to rats by

determination of the amount excreted by the faeces and no urinary excretion of curcumin is observed. The amount of curcumin remaining in the colon is about 38% of the total dose when observed at 24 hours after administration. Later, Pan, *et al.*, (1998) has reported that the plasma concentration of curcumin in mice when administered orally (1.0 g/kg), can be detected after 15 minutes, reaches the maximum concentration (0.22 μg/ml) at 1 hour, and declines to below the detection limit within 6 hours. On the contrary, plasma concentration of curcumin when administered intraperitoneally is much higher than after oral administration, reaches the maximum concentration (2.25 μg/ml) at 15 minutes, and declines rapidly within the first 1 hour. The concentration of curcumin after intraperitoneal administration 1 hour is highest in the intestine (117 μg/g). Curcumin is extensively metabolized in the liver by microsomal enzyme reactions (reduction and glucuronidation) and is rapidly eliminated in the bile. The major metabolites of curcumin *in vivo* are curcumin-glucuronoside, dihydrocurcumin-glucuronoside, tetrahydrocurcumin-glucuro--noside, and tetrahydrocurcumin. The data from Phase I clinical studies performed with curcumin also demonstrate that curcumin has low oral bioavailability in humans and may undergo intestinal metabolism (Sharma, *et al.*, 2005).

2.2.5 Toxicity studies

Systemic preclinical studies funded by the Prevention Division of the US National Cancer Institute have confirmed a lack of significant toxicity even given at oral doses up to 3.5 g/kg for 3 months in rats, dogs or monkeys (NCI, DCPC, 1996). Oral administration of curcumin (1.2-2.1 g) daily to patients with rheumatoid arthritis for 2-6 weeks (Deodhar, *et al.*, 1980) or administration of high doses of curcumin up to 8 g daily in patients with pre-invasive malignant or high risk pre-malignant conditions for 3 months (Cheng, *et al.*, 2001), does not result in any reported adverse effects or toxicity.

3. Objectives

3.1 To evaluate the effect of curcumin on reflux esophagitis; gastric lesions or gastric ulcer; and ulcerative colitis.

3.2 To investigate the mechanisms of action if curcumin is experimentally effective against any of rat models for these three gastrointestinal inflammatory diseases