Chapter 2

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2.1. Introduction to Gastric Ulcers

Gastric ulcer is a serious gastrointestinal (GI) disorder, and occurs when the gastric mucosa gets damaged, leading to perforations of the stomach lining and even bleeding. Gastric mucosal injury includes (i) superficial injury limited to exfoliation of the surface epithelium; (ii) deep mucosal injury where, in addition to superficial epithelium, microvessels are also damaged and erosions are formed; and (iii) deep injury penetrating the mucosa and muscularis mucosa—an ulcer. The injury can cause a medical emergency that requires a well targeted therapeutic strategy. Typically, gastric ulcer is referred to that restricted to the stomach, while ulcers of the stomach and duodenum together are known as peptic ulcers with a lifetime prevalence of ~5-10%. Gastric ulcer is conventionally classified as acute and chronic, which are produced under different conditions.

Normally, the gastric mucosa can resist injuries by endogenous secretions [acid, pepsin and bile], and by ingested irritants [e.g. alcohol and nonsteroidal antiinflammatory drugs (NSAIDs)]. The protective ability of gastric mucosa is attributed to a number of factors that have been collectively referred to as “mucosal defense” (Wallace, 2001). Mucosal defense system consists of the endogenously released PGs, the mucosal epithelium, growth factors (EGF, VEGF, bFGF etc.), cytokines (TNF-α, IL-1 etc.), and the antioxidant systems, all of which play a crucial role in both prophylactic and healing actions (Tarnawski et al., 2001). Under various stresses, the microvascular endothelium gets damaged leading to microvascular stasis, and reduction of delivery of oxygen and nutrients, eventually producing mucosal erosions (confined to mucosa) or ulcerations (penetration to muscularis mucosa). The mucosal necrosis leads to destruction of the mucosal components, including microvessels. Healing of deep mucosal erosions requires reconstruction of the surface epithelium (re-epithelialization), glandular epithelial structures, and restoration of the lamina propria including the mucosal microvascular network, nerves and connective tissue cells.

2.2. Mucosal Defense in the G. I. Tract

*Mucosal defence* in stomach exhibits remarkable resistance to the damaging effects of acid, pepsin, ingested irritants like indomethacin and foreign particles. The *first*
level of defence consists of the factors, secreted into the lumen and include acid, mucus, bicarbonate, and antibacterial substances (e.g., immunoglobulins and lactoferrin). The epithelium also acts as a barrier to the passive diffusion of harmful substances and constitutes the second level of defence to acid-induced injury (Sanders et al., 1985). Damage to the 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. The third level of mucosal defence is represented by the microcirculation, which is significantly modulated by the nervous system as well as a number of inflammatory mediators. Diffusion of acid or toxins into the mucosa results in a sensory afferent nerve-mediated elevation of mucosal blood flow, which dilutes and/or neutralizes the acid/toxin, preventing them from accumulating in the mucosal tissue to cytotoxic concentrations.

The mucosal immune system which constitutes the fourth level of defence, consists of the various immunocytes within the lamina propria that act as sentinels. Mast cells and macrophages can sense the entry of foreign materials (e.g., antigens and endotoxin) into the mucosa and respond by releasing chemical mediators that coordinate an appropriate inflammatory response. The ultimate level of mucosal defence is called into play when an ulcer has been formed. In these circumstances, the ulcer is repaired through growth and re-development of gastric glands, growth of new blood vessels (angiogenesis), and re-innervation of the mucosa by the extrinsic and intrinsic nerves (Wallace, 2001). The resistance of the gastro-intestinal (GI) mucosa to injury ultimately depends upon the balance between these defensive and aggressive factors present in the lumen.

2.3. Regulators of Mucosal Defense

2.3.1. Prostaglandins: Prostaglandins (PGs) are 20-carbon fatty acids produced by the cyclooxygenase (COX)-catalyzed reaction of arachidonic acid (Fig. 2.1). PGs generally have short half-lives (sec to min) and act in an autocrine or paracrine manner. These appear to exert their cytoprotective action by stimulating mucus and bicarbonate secretion, maintaining mucosal blood flow, and enhancing the resistance of epithelial cells to injury induced by cytotoxins (Hawkey and Rampton, 1985). Further, PGs also
contribute to ulcer healing by inducing angiogenesis (Jones et al., 1999). There has been accumulating evidence that PG might contribute to ulcer healing by maintaining the balance between the pro-angiogenic (VEGF, FGF, and EGF) and antiangiogenic factors (endostatin) (Wallace, 2005).

They inhibit leukocyte recruitment (Asako et al., 1992a,b), which could contribute to their beneficial effects in NSAIDs-mediated inflammation in GI mucosa. PGs can suppress the generation of reactive oxygen metabolites produced by neutrophils, thereby reducing inflammation and tissue injury (Wong and Freund, 1981). One of the mechanisms through which PGs can down regulate inflammatory responses, and in doing so, reduce the severity of mucosal injury, is through modulation of the activity of immunocytes within the mucosa. For example, PGE$_2$ has been shown to be a
potent suppressor of pro-inflammatory cytokines such as IL-1 and TNF-α release (Kunkel et al., 1986) from macrophages, and reduce the production of the potent chemotaxin, leukotriene B4 from neutrophils (Haurand and Floh, 1989). The mast cell is another target for the inhibitory effects of PGs (Raud, 1990; Hogaboam et al., 1993).

2.3.2. Leukotrienes and thromboxane: The leukotrienes (LTs), comprising of the leukotriene B4 (LTB4) and the peptido-leukotrienes (LTC4, LTD4, and LTE4) are also synthesized mainly in immunocytes, epithelial cells and endothelial cells from arachidonic acid by the enzyme, 5-lipoxygenase. Of these, LTB4 is a very potent chemotaxin for neutrophils, and can promote leukocyte recruitment from the vasculature by upregulating expression of the β2 integrins (CD11/CD18) on those cells. It is believed to contribute to the pathogenesis of NSAID-induced gastric damage by promoting leukocyte adherence to the vascular endothelium (Asako et al., 1992; Hudson et al., 1993) and generating neutrophils. Elevated production of LTB4 by the human stomach has been documented in patients taking NSAIDs (Hudson et al., 1993). The peptidoleukotrienes, produced primarily in the mast cell stimulate smooth muscle contraction, increase the permeability of the vascular endothelium and promote leukocytes rolling by increasing platelet-derived P-selectin expression on these cells (Kanwar et al., 1995), thereby.

Thromboxane, which is a major arachidonic acid metabolite produced by platelet (via COX-1) is a very potent vasoconstrictor and agonist for platelet aggregation. It has been suggested to contribute to ulceration in the GI tract by altering the mucosal blood flow (Whittle et al., 1981). Further, it can also contribute to gastropathy via modulation of inflammatory responses by stimulating LTB4 release and the adherence of leukocytes to the vascular endothelium (Goldman et al., 1991).

2.3.3. Platelet-activating factor (PAF): PAF, derived from the membrane phospholipids by phospholipases can modulate smooth muscle tone, activate neutrophils, and act as a chemotaxin for eosinophils. It is also an extremely potent ulcerogenic factor. It stimulates leukocyte adherence to the vascular endothelium and granulocytes release in experimental endotoxic shock, hemorrhagic shock, and ischemia-reperfusion, as well as
during generation of reactive oxygen metabolites (Wallace et al., 1987, 1990; Sun et al., 1996).

2.3.4. Nitric oxide (NO): NO, first described in 1987 as "endothelium-derived relaxing factor" also exerts many important actions in the GI tract (Wallace and Miller, 2000) such as (i) controlling the muscles of the fundus, (ii) maintaining mucosal blood flow, (iii) tissue regeneration, (iv) mucus secretion, (v) reducing acid and alkaline secretion, and (vi) inhibiting leukocyte migration during inflammatory processes. The constitutive forms of NO synthase (cNOS), neuronal NOS and endothelial NOS (eNOS) contribute to these beneficial attributes of NO in the normal function of the GI tract and its suppression renders the gastric mucosa more susceptible to injury (Whittle et al., 1990). However, sustained over production of NO by the inducible NOS (iNOS) contributes to inflammation in various gastroduodenal disorders and contributes to mucosal injury and dysfunction (Kolios et al., 2004; Kaise et al., 2003; Souza et al., 2004). An increase in iNOS activity and a decrease in cNOS activity in the gastric mucosa are closely related to the development of gastric mucosal lesions (Nishida et al., 1997).

2.3.5. Inflammatory mediators: Leukocyte-endothelial cell adhesion has been implicated in the pathogenesis of a variety of diseases such as atherosclerosis, gastric ulcers, haemorrhagic shock, myocardial infarction, stroke, and malaria. The cellular and molecular basis for the recruitment of leukocytes to sites of inflammation is highly complex and multifactorial. However, data derived from both in vitro and in vivo models of leukocyte-endothelial cell adhesion have revealed the relative contributions of different leukocyte and endothelial cell adhesion molecules (CAMs) to the adhesion responses elicited by various inflammatory stimuli. Both leukocyte and endothelial CAMs participate in slowing the leukocyte as it exits the capillary and enters the postcapillary venule, which is the major site of leukocyte-endothelial cell adhesion. The initial low affinity interaction between leukocytes and venular endothelium is manifested as a rolling behaviour. Rolling leukocytes can then become firmly adherent (stationary) on the vessel wall, where trans-endothelial leukocyte migration can occur, when a chemotactic signal is generated in the perivascular compartment. Each of the three stages of leukocyte recruitment, i.e., rolling, firm adhesion (adherence) and trans-endothelial migration, involves the participation of different families of adhesion molecules.
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(Schleiffenbaum and Fehr, 1996), including the selectins, β-integrins, and supergene immunoglobulins. The selectin family of adhesion molecules tethers leukocytes to EC which initiates the rolling of leukocytes on EC.

(A) Circulating leukocyte

(B) Rolling leukocyte

(C) Stationary leukocyte

(D) Emigrating leukocyte

![Fig. 2.2. Steps in the recruitment of leukocytes in postcapillary venules. (A) illustrates that in the absence of an inflammatory stimulus, leukocytes are largely flowing in the stream of red cells with no adhesive interactions with venular endothelium. (B) illustrates the low affinity interaction between leukocytes and endothelium that is mediated by selectins and manifested as rolling. (C) activation of leukocytes and/or endothelial cells can result in stationary adhesion of leukocytes. (D) firmly adherent leukocytes can emigrate from venules into the adjacent interstitial compartment, usually along a chemotactic gradient. (Adopted from Panés J et al., 1999).](image)

Blockade of L-selectin, which is constitutively expressed on leukocytes, suppresses the rolling of neutrophils and thereby reduces inflammation (Tedder et al., 1995). The platelet-derived P-selectin is expressed on the surface of activated endothelial cells and platelets. When endothelial cells are stimulated, P-selectin is mobilized to the surface of activated endothelial cells within minutes. During inflammation, endothelial P-selectin acts to recruit leukocytes into postcapillary venules, while P-selectin promotes the aggregation of leukocytes with platelets to form thrombi. Unlike P-selectin, the expression of endothelial E-selectin on endothelial cells is entirely under transcriptional control. While E-selectin is not constitutively expressed on endothelial cells, its synthesis (and expression) can be induced by cytokines such as interleukin-1 (IL-1) and TNF-α or by endotoxins. On the other hand, heterodimeric glycoproteins, integrins are expressed on
leukocyte surface, where they can mediate leukocyte-endothelial cell adhesion. In addition, the five members of the immunoglobulin (Ig) superfamily also act as adhesion molecules; ICAM-1, ICAM-2, VCAM-1, PECAM-1 and MadCAM-1. Amongst these, endothelial activation can significantly increase ICAM-1 expression, depending on the cell type.

Data derived from both in vitro and in vivo studies have implicated a number of chemical and physical factors that can influence both the time-course and magnitude of leukocyte-endothelial cell adhesion. The principal physical influence on the adhesion process is shear stress, a force that is generated by the movement of blood in postcapillary venules. Venular wall shear stress determines the level of leukocyte rolling and firm adhesion, and dictates the contact area between rolling leukocytes and the endothelial cell surface. Reductions in venular blood flow (shear stress) facilitate leukocyte rolling and adhesion, while increases in blood flow tend to oppose leukocyte-endothelial cell adhesion. Agents, such as histamine, PAF, IL-8 and TNF-α promote leukocyte adhesion by enhancing the transcription-dependent expression of endothelial cell adhesion molecules that act, extending and increasing the leukocyte rolling (E-selectin) and adherence/emigration (ICAM-1) responses. Some of the anti-adhesive compounds (NO, PGI₂, and adenosine) are also potent vasodilators, which raises the possibility that their actions in vivo can be attributed to increases in venular shear rate. NO and glucocorticoids appear to exert at least some of their effects by inhibiting the transcription-dependent expression of endothelial cell adhesion molecules. The role of the CAMs in inflammation and the potential cellular and molecular loci that can be targeted against this is excellently reviewed (Panes et al., 1999).

2.4. Genesis of Stomach Ulcer

Gastric ulcer is a complex pluricausal disease and is known to develop due to imbalance between aggressive and protective factors (Glavin and Szabo, 1992), as discussed above. Several endogenous and exogenous factors are responsible for gastric ulceration. These include *Helicobacter pylori* infection, increased production of gastric acids, pepsin and stomach juices, certain types of medicines, notably NSAIDs, and even personal factors such as consumption of tobacco, alcohol and caffeine, as well as emotional and physical stresses. The pathogenesis is caused both by topical (Graham et
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...inhibition of COX isozymes, and the NF-κB-mediated modulation of the levels of adhesion molecules and pro-inflammatory cytokines and chemokines (Tak and Firestein, 2001). Further, factors such as production of free radicals, inhibition of cell proliferation, and infiltration of inflammatory cells are also involved in the pathogenesis of ulcer. Many of the ulcerogens generate excessive ROS, which disrupts the balance between the defensive and offensive factors such as levels of cytokines, prostaglandins (PGs) and enzymes (COXs, NOS etc.), pro- and anti-angiogenic as well as tissue growth parameters. Some of the agents/factors responsible for stomach ulceration are listed below:

a) Infection by *H. pylori* – *H. pylori* is a spiral-shaped bacterium that lives in the stomach and duodenum, despite the highly acidic environment. It is believed to be transmitted orally by means of fecal matter ingested in tainted food or water, or by oral contact from bacteria transmitted into the mouth by belching or gastric reflex. The bacterium takes advantage of the stomach's own mucus for its protection. The urease present in it converts the stomach acid to bicarbonate and ammonia, which protects the bacteria by their acid-neutralizing capability. The body's innate immunity system responds to the presence of *H. pylori* and sends infection-fighting cells to that area. However, the stomach lining prevents the neutrophils from reaching the *H. pylori*-infected place easily. Inflammation in the stomach tissue occurs as the neutrophils bursts and superoxide radicals on the stomach wall are released, resulting in tissue damage. The nutrients sent by the immune system to help the neutrophils, are utilized by *H. pylori*. Even if *H. pylori* itself does not cause a stomach ulcer, it causes the inflammation in the stomach lining, and accounts for about 70% gastric ulceration cases (Chung *et al.*, 1998).

b) Anti-inflammatory medicines – Given that the present investigations are pertaining to the gastropathy of the NSAIDs, especially indomethacin, a more detailed account of them is provided latter.

c) Other rare causes such as some virus infections.

d) Crohn's disease.
2.4.1. NSAID induced gastropathy and mucosal injury: The term “NSAID gastropathy” encompasses a wide array of acute and chronic GI mucosal lesions and symptoms associated with use of NSAIDs (Fiorucci et al., 2001). The point prevalence of NSAID induced ulcers is 10–30% while 15–35% of all peptic ulcer complications are caused by NSAIDs (Kourounakis et al., 2000). The NSAID-induced ulcers usually occur in the stomach (gastric ulcer) or proximal small intestine (duodenal ulcer).

2.4.1.1 Mechanism of action of NSAIDs in the GI tract: This effect of the NSAIDs on the gastro-duodenal barrier is manifested by three principle ways. At the epithelial level, they reduce the synthesis and the secretion of mucus and make the proteolytic action of pepsins easier. This changes the viscosity and electric capacity of the mucus, favoring the back diffusion of ions. At the intraepithelial level, they cause epithelial denudation due to direct cellular damage, since ionized NSAIDs stay trapped inside the epithelium. At the sub-epithelial level, it leads to thrombosis in the microcirculation, and vasoconstriction of the arterioles of the sub-mucosa. The fact that intravenous or rectal administration of NSAIDs can cause gastrointestinal lesions confirms the hypothesis that they must have some action on a systemic level on the mucosal barrier. Studies have confirmed that all the NSAIDs do not have the same capacity to induce lesions. Drugs such as piroxicam, indomethacin, aspirin or azapropazone are in the high risk NSAID group, while ketoprofen, sulindac or sodium diclofenac contribute to medium risk. On the other hand, nabumetone, ibuprofen or naproxen, are the low risk NSAIDs (Garcia and Jick, 1994; Savage et al., 1993). A mechanistic approach of drug development might provide new NSAIDs that are clinically effective and safer to use. Currently, efforts are on to develop highly selective COX-2 inhibitors, since they are believed to provide anti-inflammatory and analgesic effects, without gastro-toxicity. Although some of these have been a tremendous commercial success, there remain questions about the central tenets of the “COX-2 hypothesis” (Wallace, 1999). This is with regard to the fact that subtle stimulation like NSAID induced irritation can quickly up-regulate COX-2 in the gastric mucosa (Kargman et al., 1996). Indeed, a series of NO-releasing NSAIDs has been developed that do not cause GI damage due to the controlled production of NO (Wallace et al., 1994; Reuter et al., 1994) and even accelerate gastric ulcer healing (Elliott et al., 1995).
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2.4.1.2 Medicinal attributes of indomethacin: Indomethacin \([1-(4\text{-chlorobenzoyl})-5\text{-methoxy}-2\text{-methyl}-1\text{-H\text{-}indole\text{-}3\text{-acetic\ acid}}]\) is a non-steroidal drug for long-term use in rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. It can cross the blood-brain barrier and placenta, and gets distributed in the body by enterohepatic circulation, while it is eliminated via renal excretion, metabolism, and biliary excretion, with a mean half-life of about 4.5 h. At a typical therapeutic regimen of 25 or 50 mg (three times a day), the steady-state plasma concentrations of indomethacin are an average 1.4 times those following the first dose. About 99% of indomethacin is bound to protein in plasma over the expected range of therapeutic plasma concentrations. It is effective in moderate to severe rheumatoid arthritis including acute flares of chronic disease, moderate to severe ankylosing spondylitis, moderate to severe osteoarthritis, acute painful shoulder (bursitis and/or tendinitis), and in acute gouty arthritis. However, it should not be used in patients with history of acute asthmatic attacks, urticaria, or rhinitis, proctitis or recent rectal bleeding. Indomethacin has also been reported to decrease the tubular secretion of methotrexate and to potentiate its toxicity.

![Chemical structure of indomethacin](image)

2.4.1.3 Effects of indomethacin on different organs:

- **Gastrointestinal:** Single or multiple ulcerations, including perforation and haemorrhage of the oesophagus, stomach, duodenum or small and large intestine, have been reported to occur with indomethacin. Fatalities have been reported in some instances. Rarely, intestinal ulceration has been associated with stenosis and obstruction. Gastrointestinal bleeding without obvious ulcer formation and perforation of pre-existing sigmoid lesions (diverticulum, carcinoma, etc.) has occurred. Increased abdominal pain in ulcerative colitis patients or the development of ulcerative colitis and regional ileitis are also reported in rare cases. The gastrointestinal effects may be reduced by using it after meals, or with antacids. Symptomatic upper GI ulcers, gross bleeding or perforation
appear to occur in approximately 1% of patients treated for 3-6 months, and in about 2-
4% of patients treated for one year.

Renal: As with other NSAIDs, long term administration of indomethacin to
animals have resulted in renal papillary necrosis and other abnormal renal pathology. In
humans, there have been reports of acute interstitial nephritis with haematuria,
proteinuria, and occasionally nephritic syndrome. A second form of toxicity is seen in
patients with prerenal and renal conditions leading to a reduction in renal blood flow or
volume, where the renal PGs have a supportive role in the maintenance of renal
perfusion.

Ocular: Corneal deposits and retinal disturbances have been observed in some
patients, receiving prolonged indomethacin therapy. Blurred vision may be a significant
symptom and warrants a thorough ophthalmological examination at periodic intervals.

Central nervous system: Indomethacin may aggravate depression or other
psychiatric disturbances, epilepsy, and Parkinsonism, and should be used with
considerable caution in patients with these conditions.

2.4.2. Oxidative stress in NSAID-induced gastropathy: Free radicals are reactive
biochemical intermediates which have been increasingly implicated over the years in the
pathogenesis of various human diseases. The reactive oxygen and nitrogen species
(ROS/RNS) is a collective term to include the radical (O$_2^*$, *OH, RO$_2^*$, ONO$_2^*$ etc.) and
non radical (hydrogen peroxide, hypochlorous acid, ozone, singlet oxygen) derivatives of
oxygen and nitrogen. Oxidative stress refers to a situation of serious imbalance between
production of ROS/RNS and antioxidant defence. This is manifested from diminished
antioxidants, mutations affecting antioxidant defence enzymes, or increased production
of ROS/RNS and is induced by various endogenous and exogenous factors.

In principle, oxidative stress can result from diminished antioxidants, mutations
affecting antioxidant defence enzymes, or increased production of ROS/RNS, caused by
both endogenous and exogenous factors. This leads to extensive damage to key
biomacromolecules leading to various diseases including gastric ulceration. It is now
established that neutrophil infiltration, generation of ROS (Yoshikawa et al., 1993),
cytokine imbalance, and initiation of lipid peroxidation play significant roles in the
pathogenesis of peptic ulcer. In particular, the indomethacin-induced gastropathy is
known to lead to excessive lipid peroxidation and $H_2O_2$ accumulation that might cause increased glutathione consumption (Avila et al., 1996; Bertrand et al., 1999).

Even drugs like omeprazole (Omez), which, until a few years ago, were thought to be acting via acid inhibitory mechanism, are now reported to exert their anti-ulcer effect via the antioxidant action (Biswa et al., 2003). Recent evidences now point towards the fact that the underlying cause of various ulcer disorders is due to an imbalance between free radicals and biological antioxidant machinery. Even in *H. pylori*-related gastritis, the involvement of iNOS and nitrotyrosine has been suggested. It has been shown that pre-treatment with DL-cysteine, methyl metionine sulfonium bromide, dimethyl sulfoxide or allopurinol produce dose dependent protection against ulceration. The superoxide radical plays an important role in the formation of gastric lesions induced by ischemia and hydrochloric acid. In the process of protecting the GI surface epithelium against oxidants, produced within the lumen the gastric mucin gets depolymerized leading to break-down of the protective barrier. Peptic ulceration is associated with high circulatory concentration of free radical activity and reduced plasma antioxidant concentrations. Patients with peptic ulcer disease have a higher level of malondialdehyde (MDA) in blood platelets, whereas the SOD activity is significantly decreased in comparison with the controls.

Likewise, oxygen derived free radicals are also directly implicated in the mechanism of duodenal ulcer relapse, and their removal decreases the recurrence of these ulcerations. Smokers and social drinkers who have endoscopically proven acute duodenal ulcers, when treated with cimetidine and allopurinol/DMSO, showed significantly lower relapse rate than cimetidine alone. Under normal conditions, the built-in antioxidant defence (discussed below) comprising of small molecules as well as enzymes and other proteins is sufficient to prevent most of the potential physiological damages. However, under conditions such as inflammation, illness and ageing the oxidative stress is manifested due to the reduction in the production of the cellular antioxidants as well as their destruction by the excessive amount of ROS/RNS. External administration of suitable antioxidants is mooted to improve the conditions.

2.4.3. Delayed gastric ulcer healing by NSAIDs and regulation by growth factors: Besides causing gastric ulceration (Hawkey, 1990), common NSAIDs often delay healing
of existing ulcers (Lancaster-Smith et al., 1991; Halter et al., 2001; Baatar et al., 2002). The inhibitory action of NSAIDs on angiogenesis is also evident from the studies on cancer-related angiogenesis with experimental animals. Clinical and experimental data also demonstrate that both COX nonselective and COX-2-selective NSAIDs delay gastric and oesophageal ulcer healing, in part by inhibiting angiogenesis in granulation tissue (Halter et al., 2001; Baatar et al., 2002). Indomethacin has been reported to completely block the angiogenic response in acutely injured gastric mucosa. Based on the experimental data and the literature, the mechanisms by which NSAIDs inhibit angiogenesis appear to be multifactorial and likely include local changes in angiogenic growth factor expression, alteration in key regulators and mediators of VEGF, increased endothelial cell apoptosis, inhibition of endothelial cell migration, recruitment of inflammatory cells and platelets, and/or thromboxane A2 mediated effects as well as activation of endostatin (Ma et al., 2002). It has been observed that inhibition of angiogenesis by NSAIDs involves interference with MAP kinase activity and its nuclear translocation (Jones et al., 1999). Interestingly, the regulation of endothelial cell proliferation by NSAIDs involves cell cycle proteins and interference of retinoblastoma protein phosphorylation. NSAIDs also inhibit activation of Egr-1 in microvascular endothelial cells (Stula et al., 2000; Vidal et al., 2000).

Following superficial mucosal injury, the continuity of the surface epithelium is promptly reestablished by the process referred to as epithelial restitution. The healing process involves migration of the epithelial cells from the gastric pits and upper regions of the glands bordering injury to cover the denuded mucosal surface, re-epithelialization, formation of new blood vessel (angiogenesis), and matrix deposition, all ultimately leading to scar formation. Angiogenesis is a pivotal process in gastric ulcer healing, and is modulated by stimulatory and inhibitory growth factors.

A number of signaling peptides viz. growth factors and cytokines are involved in the regulation of various physiological responses. Numerous cell types including macrophages, neutrophils, lymphocytes and fibroblasts produce these growth factors. Proangiogenic factors include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), platelet-derived endothelial growth factor (PDEGF), and epidermal growth factor (EGF). Angiostatin and endostatin
are among the most potent antiangiogenic factors (Distler \textit{et al.}, 2002). Several potent angiogenic stimulators including VEGF (Maloney \textit{et al.}, 1998), PDEGF (Miyazono and Heldin, 1989), EGF (Hwang \textit{et al.}, 1992), and PDGF (Linder \textit{et al.}, 1979) are stored in platelets that stimulate endothelial cell proliferation and capillary-like formation in vitro (Pipili-Synetos \textit{et al.}, 1998).

![Diagram of ulcer healing](image)

\textbf{Fig. 2.3. Schematic representation various growth factors, and regulatory molecules in ulcer healing.}

All these growth factors have different specificity, time of action and potency. Typically, the relative potency of these is as follows: EGF > bFGF > PDGF (epithelial cell proliferation and migration), bFGF > PDGF (fibroblast proliferation) and VEGF > bFGF >> PDGF >> EGF (angiogenesis) (Das \textit{et al.}, 1992; Deuel and Kawahara, 1992). Interestingly, stimulation of vascular factors is found to be sufficient for ulcer healing because epithelial cells apparently spontaneously proliferate and migrate over a dense granulation tissue to complete the healing process (Tarnawski, 2005).

Under normal physiological conditions, the regulated process of angiogenic response is not activated, and the endothelial cells within microvessels remain quiescent (resting phenotype), their turnover being low with a life span of several years (Folkman
and D’Amore, 1996). In certain situations, for example, healing of wounds and focal tissue necrosis such as erosions and ulcers, the resting phenotype is changed to an angiogenic phenotype (Constant et al., 1996; Carmeliet, 2003; Jain, 2003). As a result, the endothelial cells from preserved microvessels at the wound edge respond to angiogenic stimuli and form microvascular tubes which ultimately reestablish a microvascular network. In vivo and in vitro studies have demonstrated that angiogenesis occurs through: i) release/production of angiogenic growth factors such as VEGF and bFGF during tissue injury and hypoxia, ii) focal degradation of basement membranes and the extracellular matrix by proteases, iii) migration of endothelial cells into extravascular space and matrix invasion, iv) endothelial cell proliferation, tube formation and lumen reconstruction, v) anastomoses of adjacent tubes to form a vascular network, and vi) attachment of supportive cells (e.g., pericytes) and microvessel stabilization.

2.4.4. Other mediators of angiogenesis:

(a) Prostaglandins: The PG-mediated angiogenesis (Cheng et al., 1998) involves stimulation by PGs of angiogenic growth factor production. The increased PG levels, generated by activated COX-2 during tissue injury healing or cancer growth can facilitate angiogenesis by stimulating VEGF synthesis. PGE\(_2\) induces VEGF mRNA and protein expression in normal gastric microvascular endothelial cells via ERK2 and JNK kinase signaling pathway. The effect of PGs on angiogenesis is possibly amplified via feedback mechanisms, since VEGF, in turn, activates COX-2 expression via an autocrine and/or paracrine mechanism (Tamura et al., 2002). Co-localization of COX-2 and VEGF expression in areas undergoing angiogenesis supports this contention (Gallo et al., 2001).

(b) ROS: Interestingly, ROS have been found to stimulate angiogenic response in the ischemic reperfused hearts (Maulik and Das, 2002). It thus appears that after causing injury to the cells, ROS promptly initiate the tissue repair process by triggering angiogenic response. Hence, appropriate manipulation of ROS production may prove to be of medical relevance.

(c) Nitrogen metabolizing enzyme: The L-arginine metabolizing enzymes, arginase and NOS play vital roles in wound healing. As discussed earlier, NO, produced by the NOS pathway can be both beneficial and toxic to the GI tract, depending on its cellular concentration that depends on the involvement of eNOS or iNOS in its generation
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(Nishida et al., 1997a). On the other hand, metabolism of L-arginine via arginase increases the level of polyamines, which play a significant role in wound healing (Fig. 2.4). Two distinct isoforms of arginase have been identified in mammals: arginase I, a cytosolic enzyme, mainly expressed in the liver, and arginase II, a mitochondrial enzyme, which is mainly expressed in extrahepatic tissues (Wu and Morris, 1998). Gut plays a major role in whole body amino acid metabolism, particularly arginine homeostasis (Grimble, 2005). Since NOS and arginase compete for arginine, extra hepatic arginase activity has been implicated in the regulation of NO synthesis by limiting the availability of intracellular L-arginine for NOS (Wu and Morris, 1998; Boucher et al., 1999).

![Arginine metabolism and cell growth regulation.](image)

Both arginase isoforms are constitutively expressed in the airways, particularly in the bronchial epithelium and in fibroblasts from peribronchial connective tissue. Spermine synthesized by the arginase-ornithine decarboxylase pathway has been shown to inhibit iNOS translation, and NO production, thereby inhibiting the proinflammatory gene expression (Bussiere et al., 2005).

(d) Cytokines: Cytokines play a central role in the regulation of the mucosal immune system, and therefore are extremely important in mucosal defence. One of the key factors in the GI pathogenesis, irritable bowel disease (IBD) is a disturbed balance between the production of pro- and anti-inflammatory cytokines. Even the cross-talk amongst
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NOS/NO and arginase/polyamine is guided by the cytokine profile of the host (Jenkinson et al., 1996; Satriano, 2003). The cytokines, which have been most extensively characterized in the context of mucosal inflammatory diseases are IL-1β and TNF-α, both of which are regarded as proinflammatory cytokines and IL-10, an anti-inflammatory cytokine.

IL-1β and TNF-α are released as an early inflammatory reaction contributing to systemic responses to inflammation or infection such as the acute phase response, effects on appetite, and the generation of fever (Dinarello, 1988). IL-1 is produced by various types of cells, including monocytes, macrophages, neutrophils, endothelial cells, and fibroblasts (Dinarello, 1988). Elevated levels of IL-1 have been found in plasma and tissue of IBD patients, as well as in several experimental models of colitis (Beck and Wallace, 1997). With respect to the upper GI tract, IL-1 has been shown to increase the resistance to injury and reduce the severity of gastroduodenal damage in several models (Wallace et al., 1992; Shibasaki et al., 1992). Although not fully understood, the mechanisms responsible for the protective actions of IL-1 may be through a paradoxical inhibitory action on leukocyte adherence or anti-secretory action. However, it can also induce expressions of COX-2 (Wu et al., 1991) and iNOS (Palmer et al., 1993). Thus, IL-1 may reduce or enhance gastroduodenal injury by stimulating PGs and NO release.

TNF-α is a key contributor to many forms of gastric mucosal injury, including that associated with *H. pylori* infection and the use of NSAIDs. NSAIDs have been shown to elevate the release of TNF-α markedly, and inhibition of TNF-α synthesis results in attenuation of the damaging effects in the rat stomach (Santucci et al., 1994; Appleyard et al., 1996). PGs are potent inhibitors of TNF-α release from the macrophage (Kunkel et al., 1988) and the mast cell (Hogaboam et al., 1993). This may partly account for the healing effect of the PGs against the NSAID-induced gastric injury.

IL-10, produced mainly by the Th2 lymphocytes plays a central role in down regulating the inflammatory cascade by depressing the production of a number of pro-inflammatory cytokines (Stordeur and Goldman, 1998; Moore et al., 1993) and enhancing production of other anti-inflammatory cytokines (Casatell et al., 1994). Imbalance between IL-10 level and other anti-inflammatory cytokines is observed during IBD, ulcerative colitis, Crohn’s disease and chronic enterocolitis.
2.5. Symptoms of Gastric Ulcer

The most common symptom of gastric ulcer is a gnawing or burning pain in the upper abdomen between the sternum (breastbone) and the navel. The pain often occurs between meals, and in the early hours of the morning, usually lasting for a few minutes to a few hours. Less common ulcer symptoms may include: belching, nausea, vomiting, poor appetite, loss of weight, tiredness and weakness. The symptoms of stomach and duodenal ulcers may resemble other medical conditions or problems.

2.6. Diagnosis of Gastric Ulcer

A number of options are available for diagnosing ulcers and for testing of the *H. pylori* bacterium. These include:

a) upper GI (gastrointestinal) series (also called barium swallow)- Here the patient is asked to swallow barium (a metallic, chalky liquid) so that it can coat the inside of organs. X-ray imaging subsequently helps in evaluating the digestive organs.

b) esophagogastroduodenoscopy (EGD or upper endoscopy.) - An EGD is a procedure that allows the physician to examine the inside of the esophagus, stomach, and duodenum. The endoscope allows the physician to view the inside of this area of the body, as well as to insert instruments through the scope for the removal of a sample of tissue for biopsy (if necessary).

c) blood, breath, and stomach tissue tests – This performed to detect the presence of *H. pylori*.

2.7. Therapy of Gastric Ulcer

Specific treatment for stomach and duodenal ulcers is decided by the physician on the basis of patient’s age, overall health, medical history, extent of the pathogenesis, tolerance for medications, procedures, or therapies, and expectations or preference. Besides, personal factors such as smoking, caffeine, alcohol, stress, secretion of acid and pepsin etc. are suspected to play a role in the development of stomach or duodenal ulcers. Hence, the simplest treatment involving lifestyle changes viz. abstinence from smoking, alcohol and stressful profession works in many cases.

Earlier, the major approach towards therapy was targeted to reduce the secretion of gastric acids, which were considered as the sole cause of ulcer formation. Now, the
treatment modality has changed to potentiation of the mucosal defense along with reduction of acid secretion (Wallace, 2005). The treatment of peptic ulcer is often designed with single or combination drugs. The medical support is designed either singly or in combination, by blocking the receptor sites for example with a H₂ receptor antagonist, inhibition of intracellular mechanism involving calcium and/or CAMP, protection of gastric mucosa (cytoprotective function) from chemically induced injury, H⁺K⁺-ATPase, inhibition of terminal step of acid secretion, and eradication of H. pylori infection. For very severe conditions, standard open surgery is the only method available at present. These include: (i) vagotomy - a procedure that involves cutting parts of the vagus nerve to interrupt messages sent through it, therefore, reducing acid secretion; (ii) antrectomy - an operation to remove the lower part of the stomach (antrum), which produces a hormone that stimulates the stomach to secrete digestive juices. A vagotomy is usually done in conjunction with an antrectomy; (iii) pyloroplasty - a surgical procedure that may be performed along with a vagotomy, in which the opening into the duodenum and small intestine (pylorus) are enlarged, enabling contents to pass more freely from the stomach.

The most commonly used drugs include antibiotics to kill H. pylori, acid blockers (cimetidine, ranitidine, or famotidine), proton pump inhibitors (omeprazole), and tissue lining protecting agents (sucralfate, bismuth). These drugs have decreased the morbidity rates, but produce many adverse effects including relapse of the disease, and are often expensive for the poor populations. A brief account of these is presented below.

Cimetidine: Cimetidine was the very first drug specifically designed to block the acid producing cells in the stomach. The location of its action within the cell is the histamine 2 site. Hence this, and other related drugs are called histamine 2 receptor antagonists (H₂RA). The drug proved to be remarkably effective in healing and preventing the recurrence of ulcers, while being fairly safe for long-term use. Since the generic form is the least expensive way of controlling stomach acid, many physicians recommend this preparation for various stomach acid conditions. Its major side effects include hallucinations or mental confusion (more common in the elderly persons), unusual fatigue, fever, sore throat, shortness of breath, and abnormal skin bruising. Breast
swelling or tenderness in men, headache, rash, diarrhoea, achy joints, dizziness, muscular pain, reduced sexual potency, and reduced sperm count are the minor side effects.

*Misoprostol:* Misoprostol, a synthetic PGE\(_2\) analogue exerts therapeutic effect both by enhancing mucosal defensive properties and inhibiting gastric-acid secretion (Wolfe and Soll, 1988). Its efficacy in preventing particularly the NSAID-induced gastroduodenal mucosal injury remains unclear. The side effects observed are abortion, premature birth, or birth defects as well as diarrhea, headache, stomach pain, upset stomach, gas, vomiting, constipation, indigestion well as minor effects.

*Omeprazole:* Omeprazole (Omez), is a class of drugs called proton pump inhibitors (PPI), which blocks the production of stomach acid. The PPIs are used for the treatment of conditions such as ulcers, gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome as well as for eradicating *H. pylori* infection, in combination with antibiotics. Omez can potentially increase the concentrations in blood of diazepam, warfarin, and phenytoin by decreasing their elimination through liver. Its major side effects include nervousness, abnormal heartbeat, muscle pain, weakness, leg cramps and water retention and severe skin rash. The structural formula of Omez is shown below.

![Structural formula of Omez](image)

*Sucralfate:* It is a basic aluminum salt of sucrose octasulfate and is effective in the treatment of both NSAID-related and unrelated duodenal ulcers. Sucralfate works by forming a "barrier" or "coating" over the ulcer for the stomach acid, allowing it to heal. It is equally effective as the H\(_2\)-receptor antagonists in healing non-NSAID-related gastric ulcers. It interacts with cimetidine, ciprofloxacin, digoxin, enoxacin, ketoconazole, norfloxacin, phenytoin, blood thinners as well as antacids. Its main side effects include backache, skin rash, hives, or itching.
2.8. Possible Therapy against NSAID-induced Gastropathy

Prevention of NSAID induced gastropathy has remained a major challenge till now. To this end, various approaches have been adopted by the pharmaceutical industries. These include:

i) Use of prodrugs like sulindac, nabumetone etc., which on metabolism by hydrolytic or redox reactions provide the drug with anti-inflammatory action, but without gastro-toxicity. One such formula includes acetylsalicylic acid, citric acid and bicarbonate, which on reacting with water, produces sodium acetylsalicylate, sodium citrate and CO₂. This gives rise to a solution with a pH between 5.5 and 6.5, which is less irritant for the mucosa. However, many of these formulations are associated with adverse effects, like haemorrhage.

ii) Use of NO releasing NSAIDs has also been proposed, but was less successful. A combination of the above two strategies has also been tried wherein ibuprofen is used in association with either L-arginine or L-lysine. Besides helping the drug absorption, the amino acid acts as a buffers, raising the pH in the area near to the drug, while acting as a precursor of NO.

iii) Given that only the (S)-enantiomers of arylpropionic acid derivates like ibuprofen, naproxen, flurbiprofen, ketorolac or ketoprofen block PG synthesis, use of their active enantiomers was also mooted to offset their secondary effects. However, in vivo racemization of these drugs remains a major problem in this approach.

iv) Retarded-release galenic forms based on the microencapsulation of the drug in matrixes of resins, wax, plastic or polymers (such as piroxicam with betacyclodextrine) can somewhat diminish the direct toxic effect. However, adequate plasmatic levels of these are difficult to obtain.

v) Administering the drugs parentally eliminates the topical effect, therefore reducing the incidence of superficial lesions in the gastroduodenal mucosa and possibly the dyspeptic symptoms in patients. However, with this strategy, neither PG synthesis nor the systemic effects are impeded. There is also no decrease in the incidence of clinically significant adverse effects, such as the appearance of bleeding or perforations (Rodríguez-Téllez et al., 2001).
vi) In future, the treatment may possibly include drugs with selective effects on adhesion molecules or on phenomena involved in NSAID-induced lesions such as rolling or leukocyte activation.

2.9. Limitations of the Gastric Ulcer Drugs

In spite of good prognosis, the recurrence of ulcer remains a problem with many of these drugs. For example, although the healing rates with H$_2$ antagonists and PPIs are about 80–100% after a 4 to 8-week therapy, the recurrence of ulcer within a year after stopping the treatment is between 40–80% in most of the studies (Brooks, 1985).

In view of these, extensive work is being carried on developing suitable formulations that ameliorate the toxicity of the NSAIDs without compromising their desired action.

2.10. New Anti-inflammatory Agents as a Solution to NSAIDs-induced Gastropathy

An alternative strategy to limit the risk of GI damage induced by NSAIDs is to enhance the protective mechanisms of the gastric mucosa. This can be pursued by association of conventional NSAIDs with antisecretory and/or protective drugs or by generating hybrid NSAID molecules, chemically-modified with groups capable of releasing protective mediators. The increased knowledge of the key role of nitric oxide (NO) in gastric mucosal defence and inflammatory property of COX-2 has generated. NO-donor NSAIDs as well as selective COX-2 inhibitors (Coruzzi et al., 2007). The limitations of the NO-donor NSAIDs have already been mentioned. Regarding the selective COX-2 inhibitors, several of them reduce pain and inflammation only at doses where selectivity is lost (Wallace et al., 1998a,b). Also, PGs alone have been shown to inhibit inflammation in other animal models of arthritis (Zurier and Quagliata 1971). Moreover, studies have shown that cyclooxygenase-2 produces prostaglandins that exert anti-inflammatory actions and that play an important role in the healing of gastric ulcers (Shigeta et al., 1998; Brzozowski et al., 2001). Thus, development of new NSAIDs is desirable not only to prevent gastric ulceration but also because inflammation is the genesis of many diseases leading to trauma and multiple organ dysfunction.

Macrophages play a central role in inflammation and the regulation of the immune response. Lipopolysaccharide (LPS), a component of the cell wall of Gram-
negative bacteria, activates macrophages to produce pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and secondary mediators, such as leukotrienes and PGs, which act in an autocrine or paracrine manner to induce and amplify the host response. A major source of TNF-α, IL-1β and PGE2 during sepsis is the macrophages. These substances are important regulators of both innate and adaptive immunity. However, their uncontrolled expression can cause acute or chronic inflammatory syndromes. An acute inflammatory syndrome induced by these mediators is the septic shock syndrome, which is characterized by fever, hypotension, disseminated intravascular coagulation and multiple organ failure (Parillo, 1993).

2.10.1. **LPS signaling pathways**: LPS is one of the best studied immunostimulatory components of bacteria and can induce systemic inflammation and sepsis (Beutler *et al.*, 2003). LPS signaling is initiated by an interaction between LPS and LPS-binding protein, allowing binding to CD14 and association with another cell membrane receptor, which contains an intracellular signalling domain. This other receptor has recently been determined to be Toll-like receptor (TLR) 4, and significant work has been done to define this complex (Aderem and Ulevitch, 2000; Golenbock and Fenton, 2001; Imler and Hoffmann, 2001). The currently favoured model is outlined in Fig. 2.6. Toll-like receptors (TLRs) comprise a family of transmembrane proteins that recognize and bind different microbial components termed pathogen-associated molecular patterns (PAMPs) and trigger the expression of proinflammatory cytokines as well as the functional maturation of antigen presenting cells of the innate immune system (Akira *et al.*, 2006). The TLR family now consists of 10 members (TLR1–TLR10). Each TLR has been shown to recognize specific components of pathogens, thus demonstrating that the mammalian immune system detects invasion by pathogens via the recognition of microbial components by TLRs (Fig. 2.5).
Fig. 2.5. TLRs and their ligands. TLR1–TLR7 and TLR9 have been characterized to recognize microbial components. TLR2 is essential for the recognition of microbial lipopeptides. TLR1 and TLR6 associate with TLR2, and discriminate subtle differences between triacyl- and diacyl lipopeptides, respectively. TLR4 recognizes LPS. TLR9 is the CpG DNA receptor, whereas TLR3 is implicated in the recognition of viral dsRNA. TLR5 is a receptor for flagellin. (Adopted from Takeda K et al., 2004).

Upon LPS recognition, TLR4 undergoes oligomerization and recruits its downstream adaptors through interactions with the cytoplasmic toll-interleukin-1 receptor (TIR) domains. MyD88, a TIR domain-containing adaptor was suggested to be critical in the interleukin-1 receptor (IL-1R) signaling pathway. Recent accumulating evidence indicates that TLR signaling pathways consist, at least, of a MyD88-dependent pathway that is common to all TLRs, and a MyD88-independent (TRIF-dependent) pathway that is peculiar to the TLR3 and TLR4 signaling (Akira et al., 2001). The MyD88-dependent pathway is responsible for proinflammatory cytokines expressions, while the MyD88-independent pathway mediates the induction of Type I interferons and interferon-inducible genes.

2.10.1.1 MyD88 dependent pathway: In addition to the TIR domain, MyD88 also contains a death domain (DD), which can recruit other death domains containing molecules through homotypic interactions. Upon LPS stimulation, MyD88 recruits and activates a death domain-containing kinase, IRAK-4 which plays an important role in mRNA stability of TNF-α. IRAK-4 is responsible for the subsequent recruitment, activation and degradation of IRAK-1. Another adaptor protein, TNF receptor-associated factor (TRAF)-6 is critical for the MyD88-dependent pathway downstream of IRAK-4.
and IRAK1. TRAF6 forms a complex with UBC13 and UEV1A, and activates TAK1. It then activates downstream IKK and MAPK pathways (Sato et al., 2005).

Fig. 2.6. TLR signaling. MyD88 is an essential TIR domain-containing adaptor for the induction of inflammatory cytokines via all the TLRs. TIRAP/Mal is a second TIR domain-containing adaptor that specifically mediates the MyD88-dependent pathway via TLR2 and TLR4. In the TLR4- and TLR3-mediated signaling pathways, a MyD88-independent pathway exists that leads to activation of IRF-3 via TBK1 and IKKe/IKK/. The TIR domain-containing adaptor TRIF mediates this MyD88-independent pathway. (Adopted from Takeda K et al., 2004).

2.10.1.2 MyD88 independent pathway: TRIF (TIR domain-containing adaptor inducing IFN-β) is an important TIR-containing adaptor protein that mediates MyD88-independent signaling. Studies using TRIF-deficient macrophages demonstrate that TRIF plays a key role in the activation of transcription factor IRF3, and the late-phase activation of NF-κB and MAPK. In addition, the deletion of both MyD88 and TRIF leads to severely impaired NF-κB and MAPK activation (Yamamoto et al., 2003). The C-terminal region of TRIF, which contains a Rip homotypic interaction motif (RHIM), mediates the interaction with
RIP1, an important component of TNFα-mediated NF-κB activation. Recent studies suggest that TRIF recruits TRAF3 to activate IRF3. TRAF3 can associate with TANK, TBK1 and IKKi to mediate downstream signaling. TBK1 and IKKi are important for the dimerization and translocation of IRF3 (Fitzgerald et al., 2003). IRF3, together with NF-κB, activates the transcription of target genes, such as Type I interferons. IKKα, IKKβ and IKKγ form a complex and phosphorylate IκB proteins. This phosphorylation leads to the degradation of IκB proteins and the subsequent translocation of the transcription factor NF-κB, which controls the expression of proinflammatory cytokines, in addition to other immune related genes.

2.10.2. NF-κB and the Mitogen-activated Protein Kinase (MAPK) signaling pathways:

A universal outcome of engaging all TLRs is the activation of the Rel NF-κB and the MAPK signaling pathways. NF-κB plays a central role in the regulation of many immune and inflammatory processes including sepsis (Tak and Firestein, 2001). It is a prototype inducible heterodimer transcription factor comprised of the RelA (p65) and NF-κB1 (p50) subunits. This variant is the most potent gene transactivator among the NF-κB family and is the major NF-κB protein found in the nucleus of cytokine-stimulated IECs (Jobin et al., 1999; Jobin and Sartor, 2002). NF-κB generally exists in an inactive form in the cytosol, bound to distinct inhibitory IκB subunits. The activation of NF-κB is induced by a wide variety of agents, including phorbol esters, IL-1, TNF-α, LPS, dsRNA, cAMP, bacteria, and viral transactivators, ICAM-1, VCAM-1, T cell receptor-α (TCR-α), and MHC class II molecules, other pro-inflammatory molecules, growth factors, bioactive lipids, reactive oxygen intermediates and the short-lived free radical, nitric oxide (NO) (Barnes and Karin, 1997). This involves phosphorylation, ubiquitination, and subsequent proteolytic degradation of IκB through activation of the IκB kinase (IKK) (Scheidereit, 2006). The liberated NF-κB translocates into nuclei and binds to κB motif in the promoters of target genes, leading to the induction of many inflammation-associated genes such as cytokines viz., TNF-α, interleukins (IL-1β and IL-6), enzymes such as iNOS and COX-2 (Arend and Dayer, 1995; Jung et al., 2008), other pro-inflammatory molecules, growth factors, bioactive lipids, reactive oxygen intermediates and NO.
Fig. 2.7. Schematic Diagram of NF-κB Activation. Activation of NF-κB involves the phosphorylation and subsequent proteolytic degradation of the inhibitory protein IκB by specific IκB kinases. The free NF-κB (a heterodimer of p50 and p65) then passes into the nucleus, where it binds to κB sites in the promoter regions of genes for inflammatory proteins such as cytokines, enzymes, and adhesion molecules. P - denotes protein, and mRNA - messenger RNA.

The activation of mitogen-activated protein (MAP) kinases and IκB kinases (IKKs) is often instrumental in activation of the transcription factors such as AP-1 (Brenner et al., 1989) and NF-κB (Baeuerle et al., 1996). Activated MAPK then regulates the activities of transcription factors or kinases further downstream by phosphorylation, and thereby controls diverse cellular processes including cell growth, proliferation, differentiation, survival, innate immunity, gene expression and development. MAPKs transmit signals in the form of sequential phosphorylation events. The phospho-relay system is composed of three kinases: a MAPK kinase kinase (MAPKKK), a MAPK
kinase (MAPKK) and a MAPK. Phosphorylation of the MAPKs occurs on a conserved dual-phosphorylation domain (Thr-Xxx-Tyr) leads to activation of the protein (Fig. 2.8) and the subsequent formation of dimers which translocate into the nucleus to activate downstream targets.

![Diagram of MAPK pathways](image)

**Fig. 2.8.** A simplified diagram showing mitogen- and stress-induced activation of mitogen-activated protein kinase (MAPK) pathways. The general architecture is shown in a box and the corresponding components of each of three main MAPK pathways are shown.

In mammalian systems, at least six independent MAPK signaling units appear to function (Widmann et al., 1999); among them, the biochemical properties of three MAPKs: ERKs, JNKs and the p38 MAPKs, have been characterized in some detail. Unlike the ERK signaling pathway, the JNK and p38 pathways are not preferentially activated by mitogens, but by inflammatory cytokines such as TNF-α and IL-1β and a diverse array of cellular stresses. Activated JNKs increase activity and stability of c-jun as a transcription factor and both JNK activation and c-Jun phosphorylation regulate cell growth, whereas sustained JNK and c-Jun activation following stress induces cell apoptosis. Although many JNK/p38-activating stimuli are pro-apoptotic, the biological
outcome of JNK/p38 activation is highly divergent and appears to be largely dependent on cell type or cellular context. Alternatively, the divergence of upstream kinases may be utilized by cells to discriminate and differentially respond to a wide variety of extracellular stimuli such as growth factors, cytokines and physicochemical stressors.

2.11. Medicinal attributes of the plants related to the present studies

Following provides a brief account of the plants used in the present investigations.

2.11.1 Picrorrhiza kurroa: *P. kurroa* (fam. Scrophulariaceae) is a small hairy perennial herb distributed in the alpine Himalayan tract and in some tropical parts of India. *P. kurroa* forms a major ingredient of many indigenous medical preparations especially useful for the treatment of diseases of liver such as hepatitis (Mittal *et al.*, 1978; Ansari *et al.*, 1988), jaundice (Handa *et al.*, 1986) as well as anaemia (Antarkar *et al.*, 1988) and asthma (Langer *et al.*, 1981). Other traditional uses include dyspepsia (similar to gentian in its bitter quality), bilious fever, chronic dysentery, and scorpion sting. The roots and rhizomes are the part of the plant used medicinally (Banerjee *et al.*, 2009).

The most important active constituents of the roots and rhizomes of *P. kurroa* are the iridoid glycosides, picrosides I, II, III, and kutkoside, known collectively as kutkin (Jia *et al.*, 1999b). Other constituents of the plant include nine cucurbitacin glycosides, apocynin, drosin (Stuppner and Wagner, 1989a,b) as well as acetophenones, triterpenoids, and benzoic acid derivatives. Picroside I and kutkoside (Picroliv) has been shown to possess potent hepatoprotective (Dhawan, 1995) and significant antiarthritic activities (Singh *et al.*, 1993). The ethanol extract of *P. kurroa* as well as its standardized fraction Picroliv have been shown to protect liver against carbon tetrachloride, ethanol, paracetamol, thioacetamide etc. induced damage in rats (Pandey and Chaturvedi, 1969; Kanitkar, 1976; Dwivedi *et al.*, 1990, 1991; Rastogi *et al.*, 1995) as well as reduce blood sugar in alloxan-induced diabetics in rats (Joy and Kuttan, 1999).

*P. kurroa* extract also scavenges ROS effectively (Joy and Kuttan, 1995) as well as induce superoxide dismutase in vivo (Rastogi *et al.*, 1995). Recent studies indicate that *P. kurroa* and Picroliv, could enhance the endogenous antioxidant enzymes in vivo and could induce glutathione-S-transferase, a key enzyme in detoxification (Labadie *et al.*, 1989; Rastogi *et al.*, 1995). Apocynin, a low molecular weight compound isolated from *P. kurroa*, was found to be a potent inhibitor of the superoxide anion generating NADP
oxidase in human neutrophil membrane (Simsons et al., 1990). Recently, its inhibitory capacity against N-nitrosodiethylamine-induced hepato carcinogenesis has been reported in rats (Jose et al., 1999). The anti-carcinogenic and antitumour activity of *P. kurroa* extract has been attributed to scavenging of oxygen free radicals, stimulation of phase II enzymes and inhibition of topoisomerase I and II.

2.11.2. *Myristica malabarica*: The fruit rind of the plant *M. malabarica* (fam. Myristicaceae) (popularly known as rampatri, Bombay mace, or false nutmeg) is used as an exotic spice in various Indian cuisines. This is credited with hepatoprotective, anticarcinogenic and antithrombotic properties, and is found as a constituent in many Ayurvedic preparations such as pasupasani and Muthu-Marunthu. However, most of the medicinal attributes of the spice have not been substantiated adequately. Recently, the superoxide-scavenging and prolyl endopeptidase inhibitory activities of the methanol extract of rampatri have been reported (Khanom et al., 2000). The phenolic compounds present in the resin of rampatri seeds were also found to prevent oxidation of various edible oils more efficiently than butylated hydroxytoluene (Duggal and Karthe, 1956).

It is very likely that the traditional uses especially in the treatment of abdominal pain, hepatotoxicity and chronic ulcer may be related to the anti-inflammatory and antioxidant properties of *M. malabarica*. The *M. malabarica* extracts, constituting several herbal formulations, have also been claimed to possess antiulcer (Banerjee et al., 2007) and antitumor effect (Kirtikar et al., 1975a,b; Palani et al., 1999). From the above traditional usages and later scientific findings suggested that rampatri is a potential candidate as an antioxidant and anticancer agent. Although many benefits of *M. malabarica* have been claimed, only few authentic scientific studies are available as an antiulcerogen. In that light, the spice (rampatri) merits further investigation for use as an antioxidative and antiulcerogenic agent. Hence, we evaluated the antiulcerogenic property of one of its constituent phenolics, malabaricone C, which showed very strong in vitro antioxidant activity.