CHAPTER 1

Introduction

Plants in Taccaceae family have been used not only as food, but also as folk medicine for controlling blood pressure; improving sexual function; treating urticaria, fever, inflammation, peptic ulcer and tumor effects (1-3). Supporting data for anti-inflammatory, analgesic, and anti-ulcerogenic activities of the leaves of *T. integrifolia* have not yet been reported.

1.1 Historical Background

Inflammation is a mechanism as a consequence of defensive response intended to eliminate the initial cause of cell injury as well as the necrotic cell and tissue resulting from the original insult. It is a prevention mechanism of the body against multitude of different pathogens including viruses, bacteria, fungi, protozoan and metazoan parasites as well as tumors and a number of various harmful agents which are capable to derange its homeostasis. In addition, it is commonly associated with several symptoms or diseases, such as pain, fever, rheumatoid arthritis (RA), psoriatic arthritis, osteoarthritis (OA), gout, etc (4).

Drugs for the treatment of patients with inflammatory diseases can be divided into steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are used to treat pain and inflammation in a variety of conditions and their effects produced by inhibition of cyclooxygenase (COX). A major drawback of NSAID use is the high incidence of gastrointestinal side effects, which lead to the development of the selective COX-2 inhibitors. Consequently, the side effects of anti-inflammatory drugs are one of the major problems in developing medicine today (5, 6). In the recent years, a widespread search has been launched to identify new anti-inflammatory drugs from synthetic and natural resources. It has been reported that many plants with anti-inflammatory activity have been found to lack an ulcerogenic effect,

for examples, *Curcuma longa* Linn. (7, 8), *Turnera ulmifolia* (9, 10), and *Zingiber officinale* Roscoe (11-13).

Herbal medicines are popular and extensively used in the world. In many places, they offer readily available and more affordable alternatives to pharmaceutical drugs. The World Health Organization (WHO) estimates that a large proportion of the world's population relies heavily on traditional practitioners and medicinal plants. One-third of the world's population and up to half of the populations in the poorest parts of Asia and Africa do not have access to essential drugs. For these populations, traditional medicine presents a particularly promising opportunity to bridge the gap between those who need health care and those who provide health services. The WHO has passed a number of resolutions in response to a resurgence of interest in the study and the use of traditional medicines in health care, and in recognition of the importance of medicinal plants to the health systems of many developing countries (14, 15).

Plants of Taccaceae family are used as folk medicines for the relief or treatment of pain, fever, inflammation, incised wounds, burn, gastric ulcers, enteritis, hepatitis, blood dysentery and diarrhea. *T. plantaginea* has been used for the treatment of pain, fever, inflammation, and incised wounds (1). Rhizomes of *T. integrifolia* are used for controlling blood pressure, improving sexual function; whole plants and leaves are used for urticaria, anti-tumor, and as food. Since there is no supporting data for anti-inflammatory, analgesic, and anti-ulcerogenic activities of the leaves of *T. integrifolia*, therefore it is reasonable to evaluate anti-inflammatory, analgesic, and anti-ulcerogenic activities of the leaves of *T. integrifolia*. In addition, acute toxicity of *T. integrifolia* should be performed as well.

1.2 Literature review

1.2.1 Pain

Classification of pain

Pain can be categorized according to several variables, including its duration (acute, convalescent or chronic pain), its pathophysiologic mechanisms (physiologic, nociceptive or neuropathic pain), and its clinical context (eg, postsurgical, malignancy related, neuropathic, degenerative). Acute pain follows traumatic tissue injuries, is generally limited in duration, and is associated with temporal reductions in intensity.

Chronic pain may be defined as discomfort persisting 3–6 months beyond the expected period of healing. In some chronic pain conditions, symptomatology, underlying disease states, and other factors may be of greater clinical importance than definitions based on duration of discomfort.

With regard to a more recent classification, pain states may be characterized as physiologic, inflammatory (nociceptive), or neuropathic pain. Physiologic pain is defined as a rapid sensation of nontraumatic discomfort for very short period. Physiologic pain alerts the individual to the presence of a potentially injurious environmental stimulus, such as a hot object, and initiates withdrawal reflexes that prevent or minimize tissue injury.

Nociceptive pain is defined as noxious perception resulting from cellular damage following surgical, traumatic, or disease-related injuries. Nociceptive pain has also been termed inflammatory pain because peripheral inflammation and inflammatory mediators play major roles in its initiation and development. In general, the intensity of nociceptive pain is proportional to the magnitude of tissue damage and the release of inflammatory mediators.

Neuropathic pain is defined by the International Association for the Study of Pain as "pain initiated or caused by a pathologic lesion or dysfunction" in peripheral nerves and the central nervous system (CNS). Some authorities have suggested that any chronic pain state associated with structural remodeling or "plasticity" changes should be characterized as neuropathic pain. Neuropathic pain is usually continuous and described as burning, electric, shock-like, tearing, shooting or lancinating pain. Disease states associated with classic neuropathic sysmptoms include infection (eg, herpes zoster), metabolic derangements (eg, diabetic neuropathy) and Wallerian degeneration secondary to trauma or nerve compression (16).

Pathophysiology

Nociception, or sensation of pain, is composed of four basic processes: transduction, transmission, modulation, and perception (Figure 1.1). Transduction is the process by which noxious stimuli are translated into electrical signals at peripheral receptors sites (free nerve endings located throughout the skin, muscle, joints, fascia,

and viscera). Normal sensory stimuli do not activate the pain signal, but if the stimulus is powerful enough to surpass the threshold for innocuous activation, the receptors become nociceptors (pain receptors). These sensory receptors are targets for mechanical (crushing or pressure), chemical (endogenous or exogenous), or thermal (hot or cold) stimuli. Some nociceptors are polymodal, transducing more than one type of stimuli. One of these types of nociceptors is called the transient receptor potential (TRP). This family has a large number of members that are activated by the whole spectrum of thermal stimuli (very hot to very cold), as well as some mechanical and various chemical stimuli. Other receptors are "silent," but are recruited if the stimulus is more intense or prolonged.

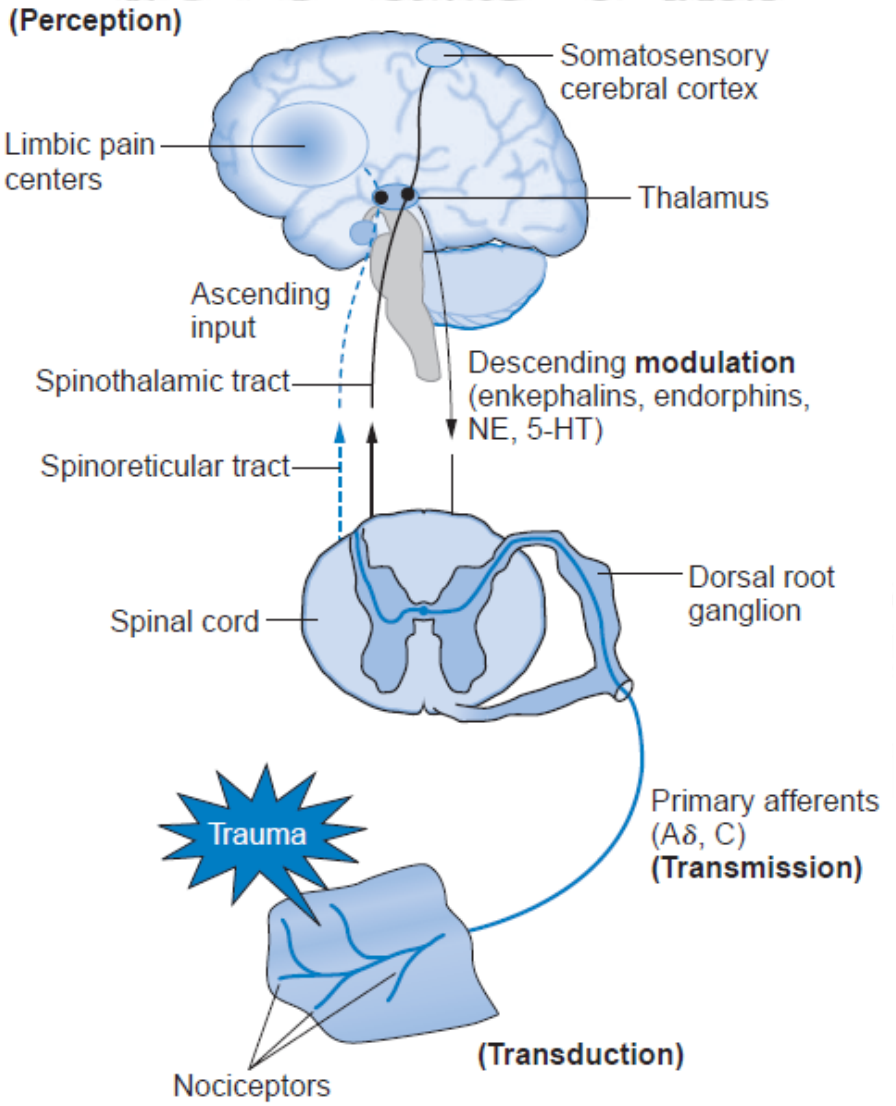

Figure 1.1 Pain pathways (17).

After stimulation of nociceptors, several processes occur. Proinflammatory mediators, including histamine, substance P, prostaglandins (PGs), bradykinins, and serotonin (5-HT), are released at the site of injury (Figure 1.2). Immune mediators are also released, including tumor necrosis factor (TNF), nerve growth factors, interleukins (ILs), and interferons (IFN). These mediators sensitize the nociceptors, lowering the pain threshold in and around the injury site (peripheral sensitization). The sensitized nociceptors may fire more frequently and erratically and are stimulated by much weaker stimuli (hyperalgesia). More frequent firing is correlated with an increase in pain intensity.

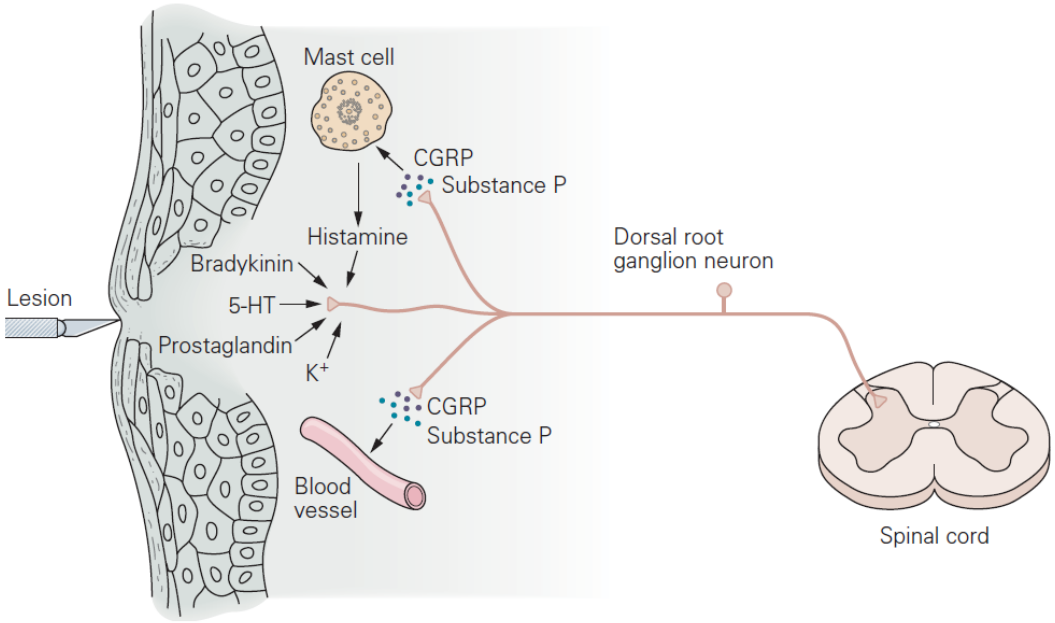

Figure 1.2 The chemical mediators released at the site of injury. In response to tissue injury, chemical mediators can sensitize and activate nociceptors. These factors contribute to hyperalgesia and allodynia. Tissue injury releases 5-HT, bradykinin and PGs that sensitize or activate nociceptors, which in turn releases substance P and calcitonin gene-related peptide (CGRP). Substance P acts on mast cells to cause degranulation and release histamine, which activates nociceptors. Substance P causes plasma extravasation and CGRP dilates blood vessels; the resulting edema causes additional release of bradykinin. In addition, 5-HT is released from platelets and activates nociceptors (18).

Transmission is the propagation of the electrical signal along primary afferent nerves, through the dorsal horn of the spinal cord to CNS. Painful impulses are generated at the nociceptor, with voltage-gated sodium channels initiating the action potentials. Voltage-gated calcium channels are responsible for allowing calcium influx to the presynaptic terminal, causing neurotransmitter release. The message is then transmitted to the spinal cord via two primary afferent nerve types: myelinated A fibers and unmyelinated C fibers. The Aδ fibers are responsible for rapidly conducting impulses associated with thermal and mechanical stimuli. Transmission of signals along Aδ fibers results in sharp or stabbing sensations that alert the patient to an injury (also called "first pain"). This produces reflex signals, such as musculoskeletal withdrawal, to prevent further injury.

The smaller, unmyelinated C fibers respond to mechanical, thermal and chemical stimuli but conduct impulses at a much slower rate compared with Aδ fibers. Transmission of electrical impulses via C fibers results in pain that is dull, aching, burning, and diffuse (called "second pain"). Prolonged stimulation of C fibers causes an additive effect on the perceived intensity of second pain, called wind-up (17).

1.2.2 Inflammation (19)

Inflammation is a reaction, both systemic and local, of tissues and microcirculation to a pathogenic insult. It is characterized by elaboration of inflammatory mediators and movement of fluid and leukocytes from the blood into the extravascular tissues. Many chemical mediators are integral to initiation, amplification and termination of inflammatory processes (Figure 1.3). This response localizes and eliminates altered cells, foreign particles, microorganisms and antigens and paves the way for the return to normal structure and function.

The clinical signs of inflammation, termed *phlogosis* by the Greek physician Galen, and *inflammation* in Latin, were described in classical times. In the first century AD, the Roman encyclopedist Aulus Celsus described the four cardinal signs of inflammation, namely, **rubor** (redness), **calor** (heat), **tumor** (swelling) and **dolor** (pain). These features correspond to inflammatory events of vasodilation, edema and tissue damage.

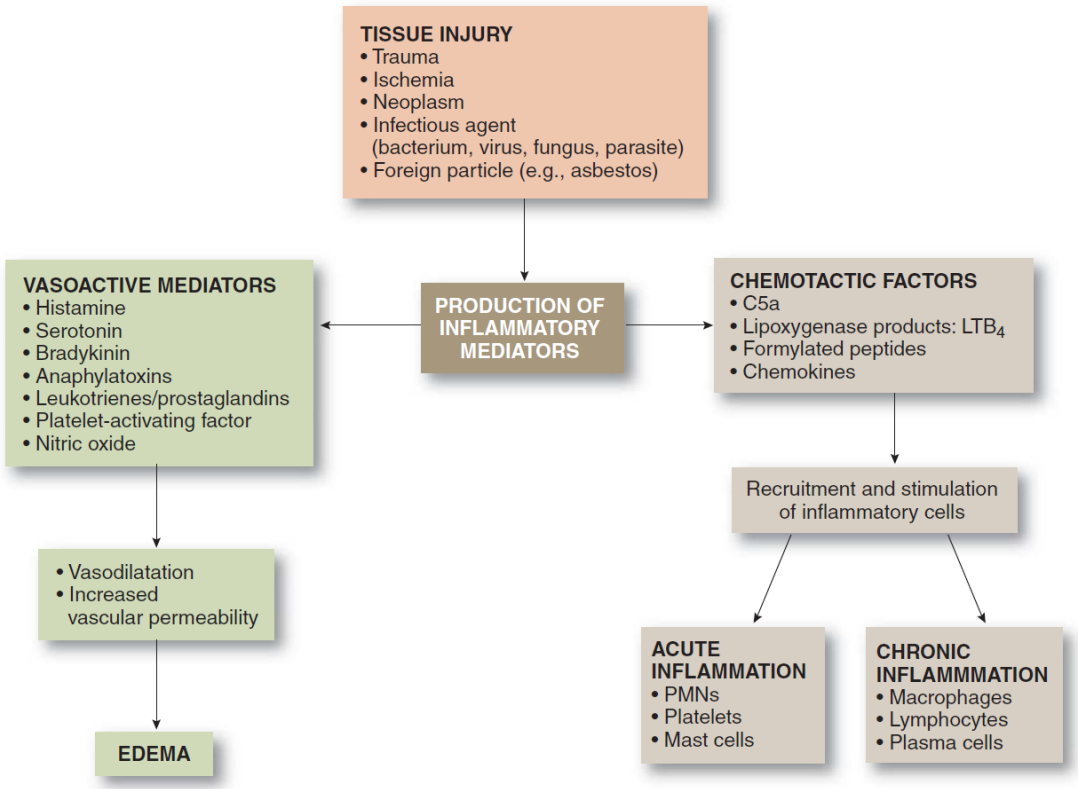

Figure 1.3 Mediators of the inflammatory response. Tissue injury stimulates the production of inflammatory mediators in plasma and released in the circulation. Additional factors are generated by tissue cells and inflammatory cells. These vasoactive and chemotactic mediators promote edema and recruit inflammatory cells to the site of injury. *PMNs* = polymorphonuclear neutrophils (19).

Inflammation can be divided into two phases, acute and chronic inflammation. Initiation of an inflammatory response results in activation of soluble mediators and recruitment of inflammatory cells to the area. Molecules are released from the offending agent, damaged cells and the extracellular matrix that alter the permeability of adjacent blood vessels to plasma, soluble molecules and circulating inflammatory cells. This stereotypic, immediate response leads to rapid flooding of injured tissues with fluid, coagulation factors, cytokines, chemokines, platelets and inflammatory cells, neutrophils in particular. This overall process is called **acute inflammation.**

Certain types of injury trigger a sustained immune and inflammatory response with the inability to clear injured tissue and foreign agents. Such a persistent response is termed **chronic inflammation.** Chronic inflammatory infiltrates are composed largely of lymphocytes, plasma cells and macrophages.

Acute inflammation

Circulating platelets, basophils, PMNs, endothelial cells monocyte/macrophages, tissue mast cells and the injured tissue itself are all potential cellular sources of vasoactive mediators. In general, these mediators are 1) derived from metabolism of phospholipids and arachidonic acid [e.g., PGs, thromboxanes, leukotrienes (LTs), lipoxins, plateletactivating factor (PAF)], 2) preformed and stored in cytoplasmic granules (e.g., histamine, 5-HT, lysosomal hydrolases) or 3) derived from altered production of normal regulators of vascular function [e.g., nitric oxide (NO) and neurokinins] (Figure 1.4, 1.5).

Phospholipids and fatty acid derivatives released from plasma membranes are metabolized into mediators and homeostatic regulators by inflammatory cells and injured tissues. One pathway involves liberation of arachidonic acid from the glycerol backbone of cell membrane phospholipids (in particular, phosphatidylcholine) by stimulus-induced activation of phospholipase A_2 (PLA_2). Once generated, arachidonic acid is further metabolized through two pathways: 1**) cyclooxygenation**, with subsequent production of PGs and thromboxanes; and 2) **lipoxygenation**, to form LTs and lipoxins. As part of a complex regulatory network, prostanoids, LTs and lipoxins, which are derivatives of arachidonic acid, both promote and inhibit inflammation (Figure 1.6). Net impact depends on several factors, including levels and profiles of prostanoid production, both of which are changed during an inflammatory response.

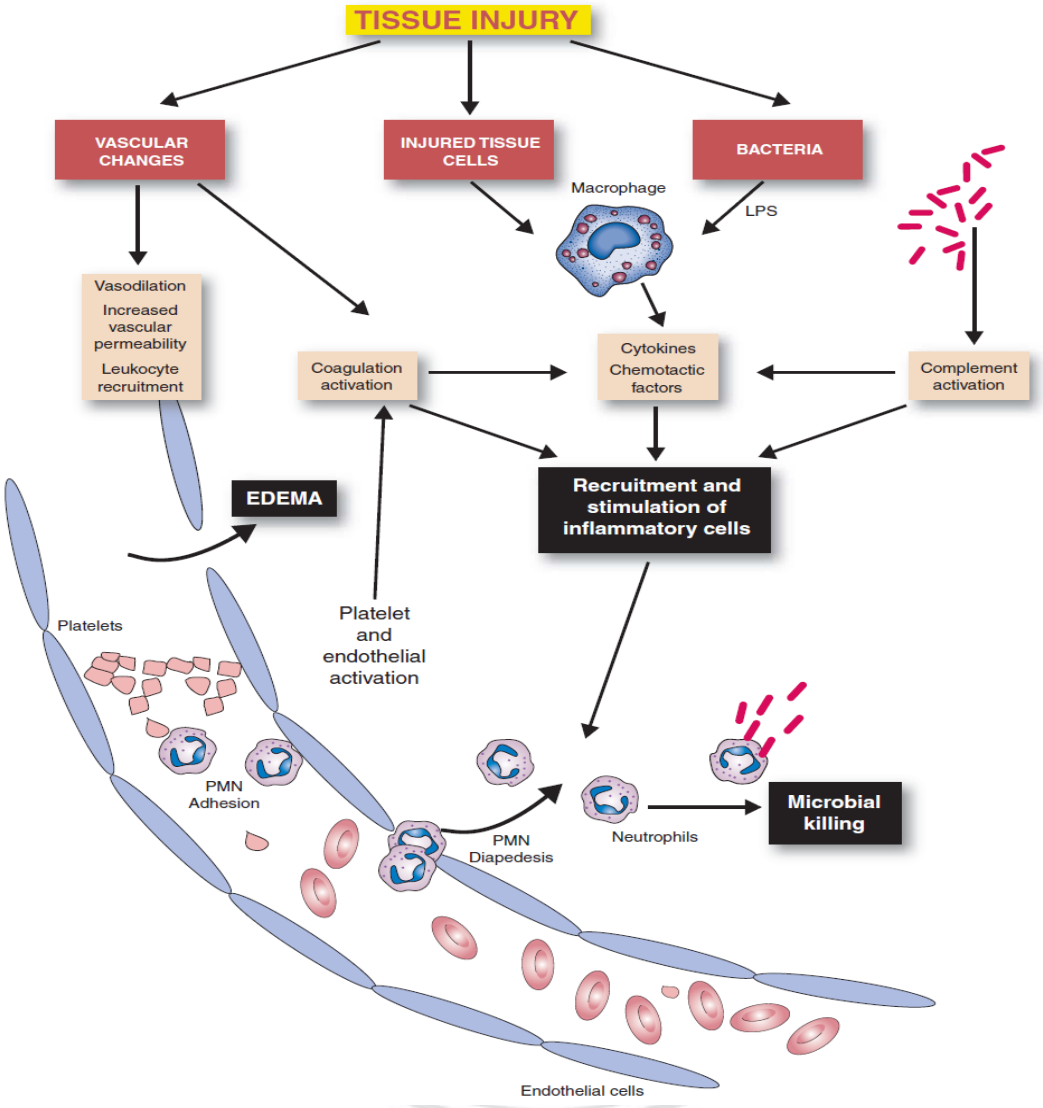

Figure 1.4 The inflammatory response to injury. Chemical mediators and cells are released from plasma following tissue injury. Vasodilation and vascular injury lead to leakage of fluid into tissues (edema). Platelets are activated to initiate clot formation and hemostasis and to increase vascular permeability via histamine release. Vascular endothelial cells contribute to clot formation, retract to allow increased vascular permeability and anchor circulating neutrophils via their adhesion molecules. Microbes (*red rods*) initiate activation of the complement cascade, which, along with soluble mediators from macrophages, recruit neutrophils to the site of tissue injury. Neutrophils eliminate microbes and remove damaged tissue so that repair can begin. *PMN* = polymorphonuclear neutrophil (19).

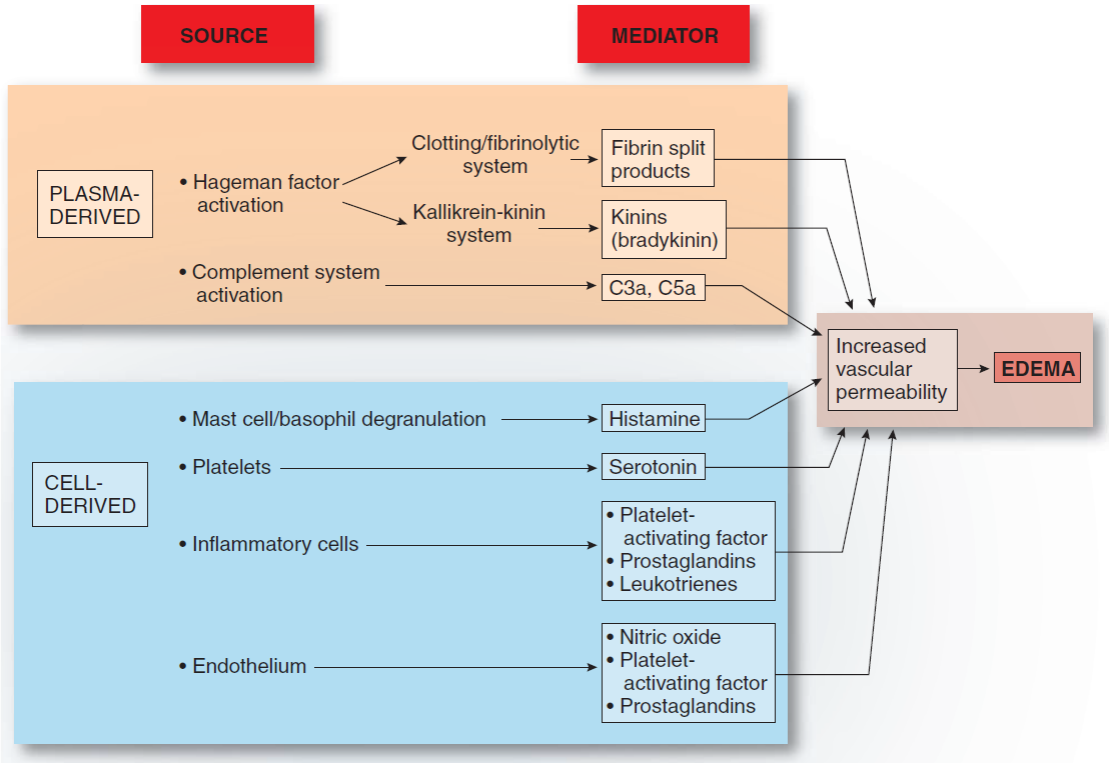

Figure 1.5 Inflammatory mediators of increased vascular permeability (19).

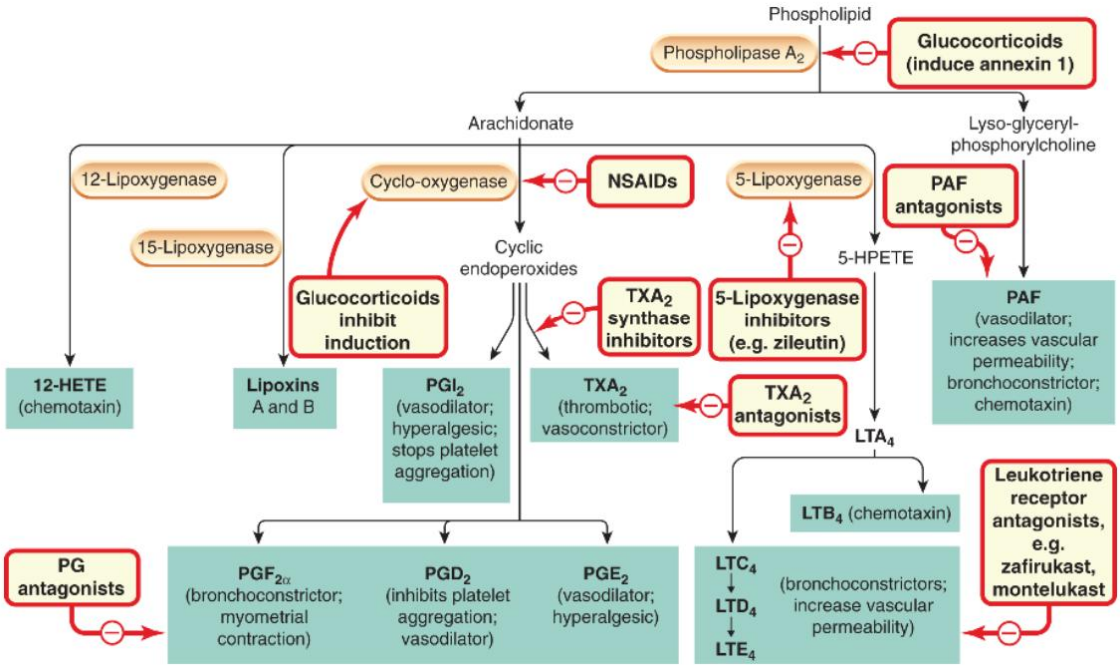

Figure 1.6 Summary diagram of the inflammatory mediators derived from phospholipids with an outline of their actions, and the sites of action of anti-inflammatory drugs. The arachidonate metabolites are 'eicosanoids'. The glucocorticoids inhibit transcription of the gene for cyclooxygenase-2, which is induced in inflammatory cells by inflammatory mediators. The effects of PGE_2 depend on which of the three receptors for this prostanoid are activated. (PGI_2, prostacyclin; TX, thromboxane; LT, leukotriene; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid) (20).

Chronic inflammation

When acute inflammation does not resolve or come to be disordered, chronic inflammation occurs. Inflammatory cells persist, stroma responds by becoming hyperplastic and tissue destruction and scarring lead to organ dysfunction. This process may be localized, but more commonly it progresses to disabling diseases such as chronic lung disease, rheumatoid arthritis, asthma, ulcerative colitis, granulomatous diseases, autoimmune diseases and chronic dermatitis. Inflammation ends of a dynamic continuum with overlapping morphologic features: 1) inflammation with continued recruitment of chronic inflammatory cells is followed by 2) tissue injury due to prolongation of the inflammatory response and 3) an often disordered attempt to restore tissue integrity.

Granuloma formation is a protective response to chronic infection (e.g., fungal infections, tuberculosis, leprosy, schistosomiasis) or the presence of foreign material (e.g., suture or talc), isolating a persistent offending agent, preventing its dissemination and restricting inflammation, thereby protecting the host tissues. Neutrophils ordinarily remove agents that incite acute inflammatory responses. However, there are circumstances in which reactive neutrophils cannot digest those substances. Such a situation is potentially dangerous, because it can lead to a vicious circle of 1) phagocytosis, 2) failure of digestion, 3) death of the neutrophil, 4) release of the undigested provoking agent and 5) rephagocytosis by a newly recruited neutrophil (Figure 1.7).

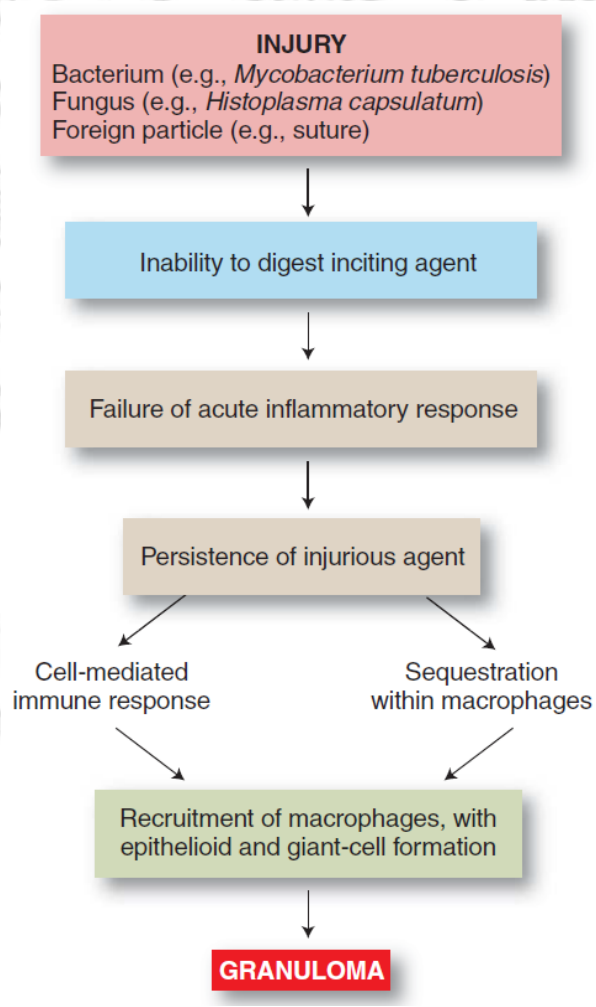

Figure 1.7 Mechanism of granuloma formation (19).

Anti-inflammation drugs

The treatment of patients with inflammatory diseases involves two primary goals; first, the relief of pain which is often the presenting symptom and the major continuing complaint of the patients; and second, the slowing or arrest of the tissue-damaging process. At present, anti-inflammatory drugs can be divided into NSAIDs and glucocorticoids.

1) NSAIDs (21)

Anti-inflammatory activity of NSAIDs is mediated chiefly through inhibition of PG biosynthesis (Figure 1.6). Various NSAIDs have additional possible mechanisms of action, including inhibition of chemotaxis, down-regulation of IL-1 production, decreased production of free radicals and superoxide, and interference with calcium-mediated intracellular events. Aspirin irreversibly acetylates and blocks platelet cyclooxygenase, while the non-COX-selective NSAIDs are reversible inhibitors. Selectivity for COX-1 versus COX-2 is variable and incomplete for the older NSAIDs, but selective COX-2 inhibitors have been synthesized. The selective COX-2 inhibitors do not affect platelet function at their usual doses. In testing using human whole blood, aspirin, ibuprofen, indomethacin, piroxicam, and sulindac are somewhat more effective in inhibiting COX-1. The efficacy of COX-2-selective drugs equals that of the older NSAIDs, while GI safety may be improved. On the other hand, selective COX-2 inhibitors may increase the incidence of edema and hypertension. As of August 2011, celecoxib and the less selective meloxicam are the only COX-2 inhibitors marketed in the USA. Rofecoxib and valdecoxib, two previously marketed, selective COX-2 inhibitors, were withdrawn from the market because of their association with increased cardiovascular thrombotic events. It has been recommended that all NSAIDs product labels be revised to mention cardiovascular risks.

The NSAIDs decrease the sensitivity of vessels to bradykinin and histamine, affect lymphokine production from T lymphocytes, and reverse the vasodilation of inflammation. To varying degrees, all newer NSAIDs are analgesic, anti-inflammatory, and antipyretic, and all (except the COX-2-selective agents and the nonacetylated salicylates) inhibit platelet aggregation. NSAIDs are all gastric irritants and can be associated with GI ulcers and bleeding as well, although as a group the newer agents

tend to cause less GI irritation than aspirin. Nephrotoxicity has been observed for all of the drugs for which extensive experience has been reported. Nephrotoxicity is due, in part, to interference with the autoregulation of renal blood flow, which is modulated by PGs. Hepatotoxicity can also occur with any NSAID.

2) Glucocorticoids (22)

Glucocorticoids dramatically reduce the manifestations of inflammation. This is due to their profound effects on the concentration, distribution, and function of peripheral leukocytes and to their suppressive effects on the inflammatory cytokines and chemokines and on other mediators of inflammation. Inflammation, regardless of its cause, is characterized by the extravasation and infiltration of leukocytes into the affected tissue. These events are mediated by a complex series of interactions of white cell adhesion molecules with those on endothelial cells and are inhibited by glucocorticoids. After a single dose of a short-acting glucocorticoid, the concentration of neutrophils in the circulation is increased while the concentration of lymphocytes (T and B cells), monocytes, eosinophils, and basophils are decreased. The changes are maximal at 6 h and are dissipated in 24 hours. The increase in neutrophils is due both to the increased influx into the blood from the bone marrow and to the decreased migration from the blood vessels, leading to a reduction in the number of cells at the site of inflammation. The reduction in circulating lymphocytes, monocytes, eosinophils, and basophils is primarily the result of their movement from the vascular bed to lymphoid tissue. In addition to their effects on leukocyte function, glucocorticoids influence the inflammatory response by reducing the PG, LT, and PAF synthesis that results from activation of phospholipase A_2 . Finally, glucocorticoids reduce expression of COX-2, the inducible form of this enzyme, in inflammatory cells, thus reducing the amount of enzyme available to produce PGs. Glucocorticoids cause vasoconstriction when applied directly to the skin, possibly by suppressing mast cell degranulation. They also decrease capillary permeability by reducing the amount of histamine released by basophils and mast cells. The anti-inflammatory and immunosuppressive effects of glucocorticoids are largely due to the actions described above. In humans, complement activation is unaltered, but its effects are inhibited. Antibody production can be reduced by large doses of steroids, although it is unaffected by moderate doses (eg, 20 mg/d of prednisone). The anti-inflammatory and immunosuppressive effects of these agents are

widely useful therapeutically but are also responsible for some of their most serious adverse effects.

Glucocorticoids have important effects on the nervous system. Adrenal insufficiency causes marked slowing of the alpha rhythm of the electroencephalogram and is associated with depression. Increased amounts of glucocorticoids often produce behavioral disturbances in humans: initially insomnia and euphoria and subsequently depression. Large doses of glucocorticoids may increase intracranial pressure (pseudotumor cerebri). Glucocorticoids given chronically suppress the pituitary release of ACTH, growth hormone, thyroid-stimulating hormone, and luteinizing hormone. Large doses of glucocorticoids have been associated with the development of peptic ulcer, possibly by suppressing the local immune response against *Helicobacter pylori.* They also promote fat redistribution in the body, with increase of visceral, facial, nuchal, and supraclavicular fat, and they appear to antagonize the effect of vitamin D on calcium absorption. The glucocorticoids also have important effects on the hematopoietic system. In addition to their effects on leukocytes, they increase the number of platelets and red blood cells.

1.2.3 Peptic ulcer

Peptic ulcer disease (PUD) is a common disorder that affect millions people in the world. The average annual age-adjusted hospitalization rate was 63.3/100,000 population (1998-2005) in the United States and The hospitalization rate was highest for adults >65 years of age (299.8/100,000 population) and decreased with decreasing age group. The age-adjusted hospitalization rates were significantly higher for male patients than for female patients and were significantly lower for whites than for each of the other racial/ethnic groups (23). In Thailand the incidence rate of peptic ulcer is approximately 1 in 73.

1.2.3.1 Physiology of the stomach

The stomach is an organ of the gastrointestinal tract between esophagus and duodenum. Functions of the stomach are mix food with gastric juice, reservoir for food before releasing into the small intestine, and secretion of gastrin and gastric juice (e.g. HCl, pepsin, lipase). Histology of the stomach consists of three main layers (mucosa, submucosa and muscularis externa). In the mucosa, epithelial cells extend downward into the lamina propria to form gastric glands. These glands secrete many important

substances and contain four types of which produce different secretion. Mucous neck cells secrete mucus, parietal cells produce HCl and intrinsic factor, chief cells secrete pepsinogens and gastric lipase, and G cells secrete gastrin. (Figure 1.8)

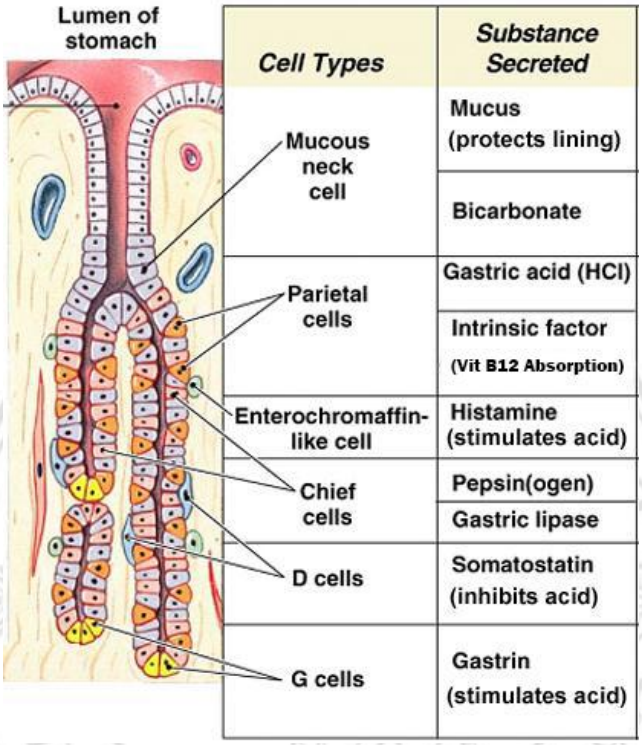

Figure 1.8 The exocrine cells of a gastric gland. (From http://teachmeanatomy.info; Physiology of the Stomach, David W., 2014)

Acid secretion is regulated by neural, endocrine and paracrine mechanisms. Central mechanisms of acid secretion involve with stimulations of medullary nuclei by the thought, sight, or smell of food and then consequent activation of the dosal motor nucleus (DMN) of the vagus nerve. The neurons that project from the medullary raphae nuclei activate the DMN through the release of thyrotropin-releasing hormone (TRH), which stimulates the preganglionic vagal fiber to release acetylcholine (ACh) at the ganglia in the gastric wall. Peripheral mechanisms of acid secretion involve with secretion of hydrogen ions (H^+) from the parietal cells which stimulated by histamine, gastrin and ACh (Figure 1.9). ACh binds to muscarinic-3 receptor (M_3) on parietal cell, resulting in phospholipase C-activated rise, amplification of H^+/K^+-ATPase on the apical surface, and secretion of H^+. In addition, the enterochromaffin-like cell (ECL), the main source of histamine, secretes histamine in response to stimulation by both the vagus nerve and gastrin. Gastrin from G cell in the antrum is stimulated and released by

luminal food or by the vagus acting through the release of gastrin-releasing peptide (GRP) and inhibited by somatostatin, which release from delta cells. Antral acidification stimulates the release of somatostatin from delta cells to turn off gastrin release. This constitutes the negative feedback regulation of gastrin release (24).

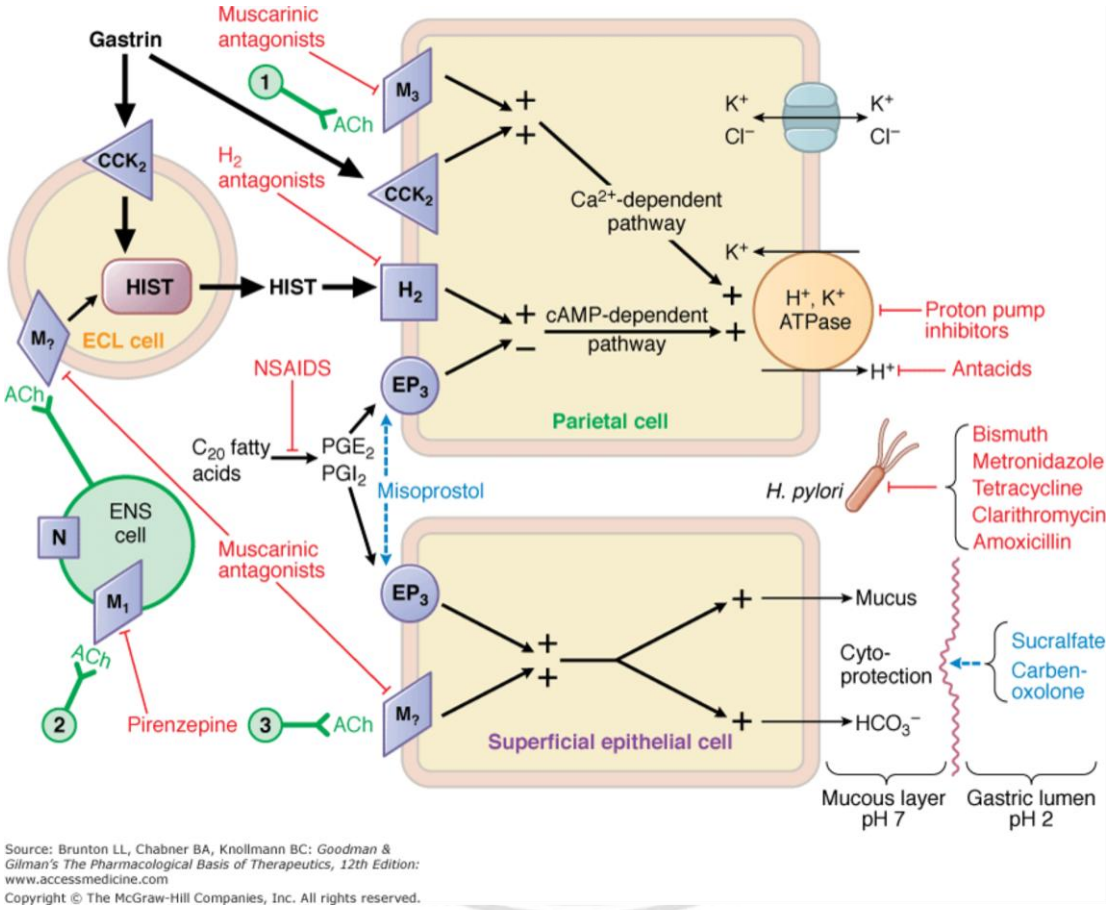

Figure 1.9 Physiological and pharmacological regulations of gastric secretion: the basis for therapy of acid-peptic disorders. The interactions among an enterochromaffin-like (ECL) cell that secretes histamine, a ganglion cell of the enteric nervous system (ENS), a parietal cell that secretes acid, and a superficial epithelial cell that secretes mucus and bicarbonate are shown. Physiological pathways, shown in solid black, may be stimulatory (+) or inhibitory (−). 1 and 3 indicate possible inputs from postganglionic cholinergic fibers; 2 shows neural input from the vagus nerve. Physiological agonists and their respective membrane receptors include acetylcholine (ACh), muscarinic (M), and nicotinic (N) receptors; gastrin, cholecystokinin receptor 2 (CCK_2); histamine (HIST), H_2 receptor; and prostaglandin E_2 (PGE_2), EP_3 receptor. NSAIDs are

nonsteroidal anti-inflammatory drugs, which can induce ulcers via inhibition of COX (25).

Gastric parietal cells are highly specialized for their unusual task of secreting concentrated acid. The cells are packed with mitochondria that supply energy to drive the apical H^+,K^+-ATPase, or proton pump, that moves H^+ ions out of the parietal cell against a concentration gradient of more than a million-fold. At rest, the proton pumps are sequestered within the parietal cell in a series of membrane compartments known as tubulovesicles. When the parietal cell begins to secrete, on the other hand, these vesicles fuse with invaginations of the apical membrane known as canaliculi, thereby substantially amplifying the apical membrane area and positioning the proton pumps to begin acid secretion (Figure 1.10). The apical membrane also contains potassium channels, which supply the K^+ ions to be exchanged for H^+, and Cl^- channels that supply the counterion for HCl secretion (Figure 1.11). The secretion of protons is also accompanied by the release of equivalent numbers of bicarbonate ions into the bloodstream, which as we will see, are later used to neutralize gastric acidity once its function is complete (Figure 1.11) (26).

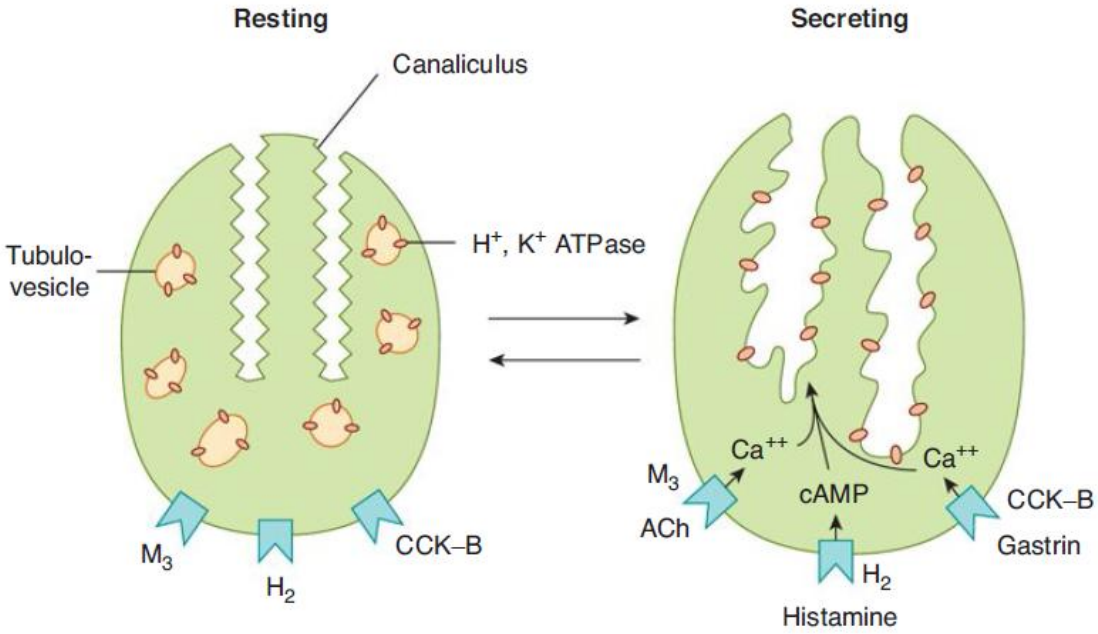

Figure 1.10 Parietal cell receptors and schematic representation of the morphological changes. Amplification of the apical surface area is accompanied by an increased density of H^+, K^+-ATPase molecules at this site. Note that acetylcholine (ACh) and gastrin signal via calcium, whereas histamine signals via cAMP (26).

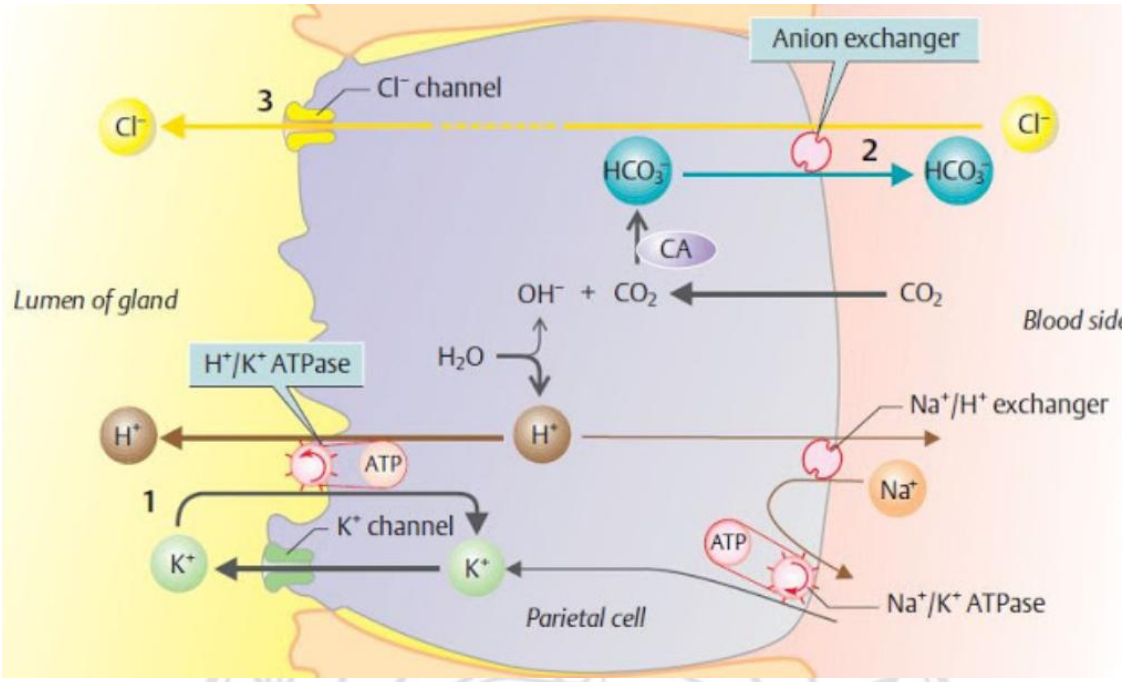

Figure 1.11 Mechanism of gastric acid secretion from the parietal cells. (From http://teachmeanatomy.info; Physiology of the Stomach, David W., 2014)

1.2.3.2 Pathogenesis of peptic ulcer

Peptic ulcer disease develops as a result of an imbalance between increased aggressive factors to overcome defensive factors. Ulcer is defined as a break in the mucosal surface, with depth to the submucosa (Figure 1.12). Gastric acid and pepsin play a major role in causing the mucosal breaks regardless of the causes of the inciting agents (e.g., *H. pylori*, aspirin or NSAIDs, emotional stress and alcohol) [27]. The aggressive-defensive factors and their roles in peptic ulcers are described as follows:

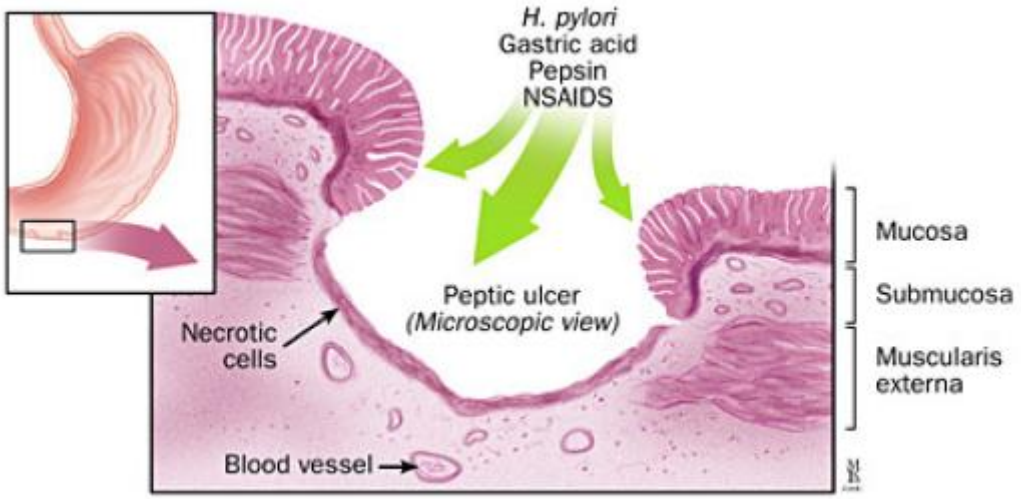

Figure 1.12 Pathogenesis of peptic ulcer. (From http://www.hopkinsmedicine.org/; 2013)

1. Aggressive factors

H. pylori, a major cause of peptic ulcer disease, is a spiral-shaped gram-negative bacillus that found in the deeper portions of the mucus gel coating the gastric mucosa or between the mucus layer and the gastric epithelium (Figure 1.13). It causes intense local inflammation. It releases bacterial proteases and phospholipases which break down the glycoprotein-lipid complexes in the gastric mucus. It also produces urease that breaks down endogenous urea to form ammonia. According to these causes the gastric pH increases locally. *H. pylori* also has adhesins used for its binding to the cells. Finally, they elaborate toxins that cause further damage like metaplasia.

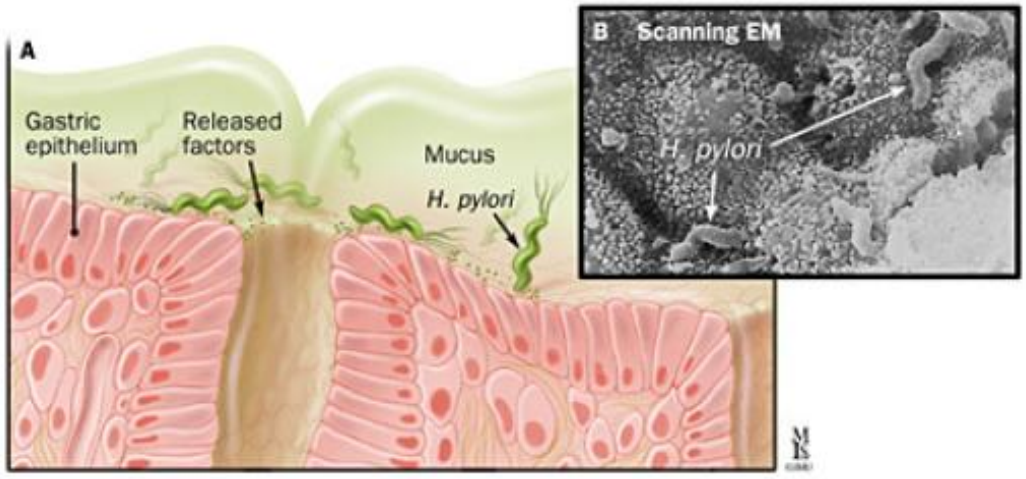

Figure 1.13 A: *H. pylori* resident on the gastric epithelium, B: electron micrograph. (From http://www.hopkinsmedicine.org/; 2013)

NSAIDs are weak acids that appear to have direct toxicity to gastric mucosal from ion trapping condition. NSAIDs remain in a nonionized lipophilic form when found within the acid environment of the stomach and migrate across lipid membranes of epithelial cells led to cell injury once trapped intracellularly in an ionized form [(28)]. NSAIDs inhibit the COX-1 (express in the stomach and responsible for maintaining gastric mucosal) and COX-2 (participates in inflammation) enzymes. It is believed that COX-1 inhibition reduces PGs synthesis. PGs play role in defense system to regulate the release of mucosal HCO_3^- and mucus, inhibit parietal cell secretion and are important in maintaining mucosal blood flow and epithelial cell restitution. Reduction of PGs leads to a reduction in mucosal blood flow, hypoxia, a reduction in mucosal defense (mucin, HCO_3^- and surface active phospholipid), a decreasing of epithelial cell proliferation and an increasing of HCl secretion. Other mechanism may contribute to damage by NSAIDs is an increased leukotriene production that occurs because arachidonic acid metabolism shifts to the alternative 5-lipooxygenase (LOX) pathway when the COX pathway is inhibited (29).

HCl and pepsin are the gastric secretory products capable of inducing mucosal injury. Acid secretion is regulated by neural, endocrine and paracrine mechanism. Neuronal (ACh), paracrine (histamine) and endocrine (gastrin) factors all regulate acid secretion. Gastric acid and pepsin have long been considered the principal inciting

agents in the pathogenesis of peptic ulcer. When levels of acid and pepsin overwhelm mucosal defense mechanisms led to peptic ulcer (30).

Emotional stress is proposed as a major cause of peptic ulcer disease. Stress may play a role in triggering overt injury is most commonly observed in the acid-producing portions of the stomach. Some studies suggest that stress stimulates the release of glucocorticoids which inhibit PGs synthesis thereby causing impairment of mucosal defense mechanism. Stress-induced gastric ulcer appears to be related to local ischemia by the decrease of local mucosal NO generation and the increase of endothelin-1 which is a potent vasoconstrictor (31, 32).

Alcoholic beverages stimulate gastric acid production. The role of alcohol appears to stimulate gastric secretions by exciting sensory nerves in the buccal and gastric mucosa and promoting the release of gastrin and histamine. Moreover, direct application of high concentrations of alcohol (more than 40% alcohol) to the gastric mucosa causes demonstrable mucosal injury (33). Nevertheless, alcohol exacerbates the clinical course and severity of ulcer symptoms and it appears to act synergistically with *H. pylori* to delay healing (34).

2. Defensive factors

The mucosal defense system can be divided as a three-level barrier including pre-epithelial (phospholipids, mucus and bicarbonate barrier), epithelial (cellular resistance, restitution, epithelial cell regeneration and cell proliferation), and subepithelial elements (blood flow and leukocyte) (35). Surface epithelial cells provide the next line of defense through several factors including mucus production, epithelial cell ionic transporters that maintain intracellular pH and HCO_3^- production, and intracellular tight junctions.

Mucus-HCO_3^--Phospholipid layer serves as a physicochemical barrier to multiple molecules including hydrogen ions (H^+). PGs stimulate mucus and HCO_3^- production. Mucus is secreted by epithelial cells of gastroduodenal surface. It is composed of 95% water and 5% a mixture of lipids and cross-linked mucin glycoproteins. The mucus gel that impends diffusion of ions and molecules such as pepsin. HCO_3^- forms a pH gradient ranging from 1 to 2 at the gastric luminal surface and reaching 6 to 7 along the epithelial cell surface.

NO is an important factor in modulating gastrointestinal mucosal defense mechanisms. Some of nitric oxide actions overlaps with that of PGs, as it modulates the activity of mucosal immunocytes and reduces leukocytic endothelial adhesion. In addition, it modulates mucosal blood flow and reduces epithelial permeability, resulting in enhanced mucosal resistance to ulceration. NO also prevents adherence of leukocytes to the vascular endothelium. This gaseous mediator also has a role in modulating gastric mucus and bicarbonate secretion. Suppression of NO synthesis renders the gastric mucosa more susceptible to injury, while administration of NO donors can protect the stomach from injury. Agents that release NO in small amounts over a prolonged period have been shown to greatly reduce inflammation and to accelerate ulcerative healing (36).

The continuous cell restitution from mucosal progenitor cells is modulated by several growth factors, including epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), and basic fibroblast growth factor (FGF). The vascular endothelial growth factor (VEGF) and FGF are important in regulating angiogenesis in the gastric mucosal (35).

PGE_2 and PGI_2 stimulate mucus, HCO_3^-, and phospholipid production. Inhibition of these protective effects by NSAIDs increases the likelihood of injury to the epithelium and decreases its ability to respond and regenerate (37).

1.2.3.3 Therapy for peptic ulcer

The main of treatment for peptic ulcers (gastric and duodenal) are relief of pain, promotion of healing and prevention of recurrence. Drugs used in the treatment of peptic ulcers divided into two classes: agents that reduce intragastric acidity and agents that enhancing mucosal protection. The management of *H. pylori*-associated acid-peptic ulcers is eradication of *H. pylori* (38).

1. Agents reduce intragastric acidity (27, 38-40)

Antacids

Antacids are weak bases that neutralize gastric acid to form a salt, water and raising the gastric pH. They are often used for the relief of dyspepsia. Apart from neutralizing gastric acid, aluminum hydroxide containing antacids stimulate mucosal

PG synthesis, leading to increased mucus and bicarbonate secretion and improved mucosal blood flow. Antacids contained aluminum hydroxide may cause constipation whereas the most common side effect of magnesium hydroxide is diarrhea.

H_2 receptor antagonists

H_2 receptor antagonists inhibit gastric acid secretion by reversibly competing with histamine for binding to H_2 receptors on parietal cells, resulting in reduction of the volume of gastric acid and pepsin secretion. In addition, this class of drug is often used for the treatment of active ulcer in combination with antibiotics directed at eradicating *H. pylori*. However, these drugs are less potent than proton pump inhibitors and some side effects are reported. For example, prolonged receiving a high dose of cimetidine causes reversible gynecomastia and impotence. Cimetidine is able to inhibit cytochrome P450 and causes many drug interaction problems. In addition, the elevated levels of serum aminotransferases, creatinine, and serum prolactin are indicated with its long-term usage.

Proton pump inhibitors

Proton pump inhibitors inhibit both fasting and meal-stimulated gastric acid secretion. They are prodrugs that require activation in an acid environment. After absorption into the systemic circulation, the prodrug diffuses into the parietal cells and inhibits the H^+/K^+ ATPase (proton pump) that is the final step in acid production. Proton pump inhibitors are used to promote healing of gastroduodenal ulcers, and to treat gastroesophageal reflux disease (GERD) and gastric hypersecretory conditions. In addition, proton pump inhibitors are approved by Food and Drug Administration (FDA) for the treatment/prevention of recurrence of NSAIDs-associated gastric ulcers and reducing the recurrence associated with *H. pylori* infections.

2. Agents enhance mucosal protection (27, 38-41)

Sucralfate

Sucralfate is a complex sucrose salt which contains aluminum hydroxide and sulfate. This agent is insoluble in water, forms a viscous gel coat, and binds selectively at the stomach and duodenum sites of ulceration. In addition, sucralfate may act as barrier to prevent gastric acid attack, promote a trophic action by binding of EGF,

induce PGs synthesis, stimulate mucous and bicarbonate secretion, and enhance mucosal defense and repair. Adverse effect of this drug is rare.

Prostaglandin analogues

Misoprostol, a PGE_1 analog, has been approved by the FDA for the prevention of NSAIDs-induced ulcer disease. PGs analogues also prevent gastric ulcer by enhancing mucus bicarbonate secretion, inhibition of gastric acid secretion, stimulation of mucosal blood flow, and reduction of mucosal cell turnover. The most common adverse effects are diarrhea and abdominal cramp.

Colloidal bismuth compounds

The mechanisms of therapeutic activity of bismuth compounds are not clear. Bismuth compounds appear to work by several mechanisms including coating on top of stomach mucus, protecting the ulcer from gastric acid, stimulating mucus-bicarbonate and PGs secretion. In addition, they have been reported to have antimicrobial activity against *H. pylori* and approved by the FDA for using in combination with other agents for the treatment of *H. pylori* infection.

3. Eradication of *H. pylori* (42)

Many regimens for *H. pylori* eradication have been proposed and the combination therapy for 14 days provides the effective therapy. The combination therapy consists of two antibiotics plus either a proton pump inhibitor or H_2-receptor antagonist, or add on bismuth compound. However, some of these drugs have been reported to cause mild to severe side effects. Therefore, searching for herbal drug or medicinal plant which possesses gastroprotective activity and fewer side effects may be the potential reason.

1.2.4 The historical background of *Tacca integrifolia*

Taccaceae is a monogeneric family with 20 species mostly found in South Asia. In Thailand, so far only 5 species have been recorded (43).

Tacca integrifolia Ker Gawl. was named by John Bellenden Ker Gawler in 1812. It is a perennial herb with creeping rhizome. *T. integrifolia* is founded in China, India, Bangladesh, Burma, Laos, Malay Peninsula, Indonesia, and Thailand. Vernacular

names are Waan phangphon (ว่านพังพอน), Mann phlaen (ม่านแผลน), Nilaphusee (นิลพูสี), Ruesee lom (ฤษีลม) and Maa thonlak (ม้าถอนหลัก) (43).

T. integrifolia is a herb with oblong curved rootstock. Leaves entire, elliptic ovate, acuminate, 20-40 cm long, strongly nerved. Scape maroon-brown, about as long as the petiole, stout, recurved or bent to one side, few-flowered. Involucral bracts large, spreading, foliaceous, purplish-black; 2 inner larger than the 2 outer; bracteoles long, filiform, pendent. Flowers pale, perianth greenish purple and yellow or dirty lilac, outer lobes subulate, rather shorter than the obtuse inner. Berry almost 4 cm, oblong, fleshy (Figure 1.14).

Figure 1.14 *Tacca integrifolia*. (From www.botanicalgarden.ubc.ca, www.beverlyallen.com.au)

Plants of the genus *Tacca* are phenomenal resources of taccalonolide steroids, which possess a special pentacyclic steroidal skeleton, and some of which show antitumor activity (44-47). Up to now, the 25 taccalonolides A-Y have been isolated from *T. plantaginea*, *T. subflaellata*, and *T. paxiana* (48). *T. chantrieri* has been reported that the main active component comprising in its rhizome is a wide array of saponins (49). Saponins have been reported to possess antifungal properties. The

magnitude of the hemolytic activity of saponins has often been linked with the antifungal and anti-inflammatory activities of the saponins (50).

The rhizome of *T. plantaginea* has been used in China as folk medicine for pain, fever, inflammation, and incised wounds (1). The rhizome of *T. chantrieri* has been used also in China as folk medicine for the treatment of various diseases including high blood pressure, burn, gastric ulcers, enteritis, and hepatitis (2, 3). The leaves of *T. integrifolia* has been use in India for blood dysentery and diarrhea (51). In Thailand, rhizomes of *T. integrifolia* are used for controlling blood pressure and improving sexual function (52), whole plants are used not only as food but also for urticarial and anti-tumor. The activities of plants in genus *Tacca* are summarized in Table 1.1.

Preliminary study has shown that ethyl acetate extract of the leaves of *T. integrifolia* reduced the ear edema induced by ethyl phenyl propiolate (EPP) in rats. Because there are few studies of leaves for anti-inflammatory, analgesic, gastric ulcers, and safety profile, therefore *T. integrifolia* should be rigorously identified and investigated for their pharmacological activities, additionally toxicity study should also be carried out.

Table 1.1 Summary activities and uses of plants in genus *Tacca*.

Part used	**Treatment**	**Plant**	**References**
Rhizome	Control blood pressure	*T. integrifolia*	(53) (54)
		T. chantrieri	(2)
	Improving sexual function	*T. integrifolia*	(53)
	Anti-tumor	*T. integrifolia*	(44) (46)
		T. chantrieri	(45)
	Diarrhea	*T. integrifolia*	(44)
		T. chantrieri	(49)
		T. leontopetaloides	(55)
	Anti-inflammation	*T. chantrieri*	(49) (56)
		T. plantaginea	(1)
	Analgesic	*T. chantrieri*	(56)
		T. plantaginea	(1)
	Anti-pyretic	*T. chantrieri*	(56)
		T. plantaginea	(1)
	Anti-ulcerogenic	*T. chantrieri*	(57)
	Incised wounds	*T. plantaginea*	(1)
Leaves or whole plant	Diarrhea and blood dysentery	*T. integrifolia*	(51) (58)
	Urticaria	*T. chantrieri*	(59)
	Anti-tumor	*T. chantrieri*	(59)
	Control blood pressure	*T. integrifolia*	(54)

1.3 Hypothesis

The analgesic, anti-inflammatory and anti-ulcerogenic effects of Taccaceae plants were described as above. *T. integrifolia* is a plant in the Taccaceae family, and theoretically it may share those activities with plants in this family. Therefore, the hypothesis of this study was that *T. integrifolia* possesses analgesic, anti-inflammatory and anti-ulcerogenic effects.

1.4 Purposes of the study

The purposes of the present study were to investigate analgesic, anti-inflammatory, anti-ulcerogenic activities and acute oral toxicity of *T. integrifolia.*