INTRODUCTION

INFLAMMATION

Inflammation is a fundamental pathophysiological, localized, protective response to trauma or microbial invasion that destroys, dilutes, or walls-off the injurious agent and the injured tissue. Agents and means of provoking the response include mechanical trauma (especially crushing), radiation (thermal, UV, radioactive emanations), direct chemical damage (caustic and corrosive chemicals), secondary chemical or biochemical damage (metabolic inhibitors, anoxia), invading organisms (viruses, bacteria, parasites) and last but by no means least antibody-antigen reactions (1-3). The primary purpose of the inflammatory response is to eliminate the pathogenic insult and remove injured tissue components. This process accomplishes either regeneration of the normal tissue architecture and return of physiological function or the formation of scar tissue to replace what cannot be repaired (4-6). Inflammatory reactions are divided into two types: acute and chronic inflammation (1;6;7).

Acute inflammation is the initial response to tissue injury; it is a short lived process developing in response to a single episode of injury. Duration of the process is usually measured in days. It is mediated by the released of autocoids. Some of the autocoids involved are histamine, serotonin, bradykinin, prostaglandins (PGs) and leukotrienes (LTs) (8). Histamine and serotonin are believed to mediate the initial phase of inflammation (1 – 1.5 h), and kinins the second phase (1.5 – 2 h), PGs probably exert their proinflammatory effects in the late phase of inflammation (2.5-6 h) (6). Acute inflammation is characterized by the classic signs of pain, heat, redness and swelling with an accompanying loss of function. Microscopically, it involves a complex series of events

including dilation of arterioles, capillaries and venules with increased permeability and blood flow; exudation of fluids, including plasma proteins; and leukocytic migration (polymorphonuclear leukocytes or PMNs) into the inflammatory focus (2). If the initiating stimuli for an inflammatory reaction are not eliminated by the reaction or controlled adequately, a continuing state of inflammation persists (1).

Chronic inflammation is considered to be inflammation of prolonged duration (weeks or months). It is a long-lived reaction after the initial exposure to the damaging agent (7;9). It arises under the following settings 1) persistent infection by certain microorganisms and certain fungi; 2) prolonged exposure to potentially toxic agents; 3) autoimmunity: under certain conditions, immune reactions are set up against the individual's own tissues, leading to autoimmune diseases (7). Characteristically, there is an abundance of exudate, granulomatous tissue, monocytosis with many multinuclear giant cells formed by their fusion, lymphocytosis and accumulation of plasma cells. The connective tissue invasion results in the formation of much fibrous tissue (fibrosis) (1). The cellular components are macrophages, plasma cells, lymphocytes, and, in certain conditions, eosinophils (5;7). The macrophage is a central figure in chronic inflammation because of the great number of substances the activated macrophage can produce (e.g., eicosanoids, cytokines, chemokines, growth factors, nitric oxide) (7). Chronic inflammation involves the release of a number of mediators that are not prominent in the acute response. Some of these are interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-3 (IL-3), tumor necrotic factor alpha (TNF-α) and interferons. One of the most important conditions involving these mediators is rheumatoid arthritis, in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to severe disability and in which systemic changes occur that can result in shortening of life (8).

MEDIATORS OF INFLAMMATION

The criteria used to determine whether an endogenous substance can be positively considered as an inflammatory mediator, were first considered by Dale (10) and restated by Vane (11). These criteria are as follows:

1. The mediator should be detectable, at the site of inflammation, at the right time, in amounts adequate to account for the effect under consideration.
2. The mediator, when administered in concentrations of the order of those found in the lesion, should produce the observed effects, and no other.
3. Specific blocking agents or antagonists of the effects of the proposed mediator should prevent or attenuate the effect.
4. Prevention of release of the mediator should abolish or prevent the effect.
5. Agent or procedures preventing the breakdown or removal of the mediator should prolong or potentiate the effect.

Mediators which suit the above criteria and are specific as inflammatory mediators are as follow:

I. Vasoactive Amines

Histamine

Histamine is an amine formed by decarboxylation of the natural amino acid, histidine. It is stored in inactive form and widely distributed in tissues, particularly in the mast cells that are normally present in the connective tissue adjacent to vessels, as well as in circulating basophils and platelets (7;12). Preformed histamine is normally present in mast cell granules and is releaseds by mast cell degranulation in response to velocity of stimuli: 1) physical injury such as trauma, cold, or heat; 2) immune reactions involving binding of antibodies to mast cells; 3) fragments of complement called anaphylatoxins (C3a and C5a); 4) histamine-releasing proteins derived from leukocytes; 5) neuropeptides

(e.g., substance P); and 6) cytokines (IL-1, IL-8) 7). Histamine is clearly important in acute inflammation associated with mast cell degranulation in non-rodent species including man (13). Pharmacological analysis of the receptor involvement in these component parts of the inflammatory response has shown that the vasodilatation involves both H_1-and H_2-receptors (7;12) . Histamine can also stimulate free nerve endings (mainly H_1-receptors), cause pain and itching (12). An alternative role for histamine might be as a co-mediator of inflammation. In acute inflammation, histamine could both act as the vasodilator and increase vascular permeability but in chronic inflammation it would only fulfill the vasodilator role, perhaps serving to potentiate the increase in microvascular permeability caused by second mediators such as prostaglandins. Histamine is relatively unimportant in the late stage response. Thus, inhibition of histamine responses delays but does not prevent the inflammatory response (13).

Serotonin (5-Hydroxytryptamine)

Serotonin (5-Hydroxytryptamine: 5HT) is a second preformed vasoactive mediator with actions similar to histamine. It is present in platelets and enterochromaffin cells, and in mast cells in rodents but not humans (7). In acute inflammation associated with mast cell degranulation in certain rodents, serotonin may be of equal or greater effect than histamine (14;15). In the microcirculation, serotonin causes dilation of arterioles, together with constriction of venules, with the result that capillary pressure rises and fluid escapes from the capillaries. In large vessels, both arteries and veins are usually constricted by serotonin, though the sensitivity varies greatly. This is a direct action on vascular smooth muscle cells, mediated through 5-HT_{2A} receptors. Serotonin can also cause vasodilatation by several mechanisms, all operating through 5-HT_1 receptors (16).

II. Plasma protease

Kinin system

The kinin system generates vasoactive peptides from kininogen precursors following the activation of tissue or plasma kallikreins by pathophysiological stimuli such as inflammation or tissue damage (7;12) . Three kinins have been identified in mammals: bradykinin, lysylbradykinin (kallidin) and methionyllysylbradykinin. Note that each kinin contains bradykinin in its structure (17). A variety of factors including tissue damage, allergic reactions, viral infections and other inflammatory events activate a series of proteolytic reactions that generate bradykinin and kallidin in the tissue (12). The kinins, particularly bradykinin, are important mediators involved in the initiation, development and maintenance of inflammatory and nociceptive processes (18). They generate local contributions to the acute and possibly the chronic phase of the inflammatory reaction by producing vasodilation, increase of vascular permeability, plasma extravasation, pain and synthesis of prostaglandins (12;19). Kinins may also modulate migration of white blood and tissue cells that take part in the inflammatory process. Kinins are among the most potent activators of prostaglandins, histamine and/or 5-hydroxytryptamine release. Moreover, some of their major actions for instance the endothelium mediated vasodilation, the production of pain, the smooth muscle contraction or relaxation in various organs are associated with release of prostaglandin. Indeed, it has also been shown that kinins promote the release of prostacyclins from vessels and from cell cultures (rat adipocyte, human endothelium cell) possibly by interacting with membrane phospholipases. Recent findings indicate also that kinins and other peptides activate PGE_2 production by deriving their arachidonic acid from phospholipid. (19). The major effects of kinin are mediated by the activation of at least two distinct receptors named B_1 and B_2. The B_2 subtypes are constitutively and widely expressed throughout the central and peripheral nervous system,

mediating most of physiological effects of kinins, while B_1 receptors are induced following tissue inflammation and damage. Activation of B_1 receptors produces a range of proinflammatory effects including edema, pain and promotion of blood-borne leukocyte trafficking (12;18). Considerable effort has been directed toward developing kinin receptor antagonists, since such drugs have considerable therapeutic potential as anti-inflammatory and antinociceptive agents. Actions of kinins mediated by prostaglandin generation can be blocked nonspecificially by inhibitors of prostaglandin synthesis such as aspirin (17).

Complement system

The complement system of blood plasma and extravascular tissue fluid plays an important role in many immune defense reactions and absence of a functional complement system reduces many inflammatory reactions. It consists of a group of 20 plasma proteins as inactive forms and sequentially activated by three convergent pathways including 1) a classical pathway, directly activated by complexes of antibody and antigen; 2) an alternative pathway, activated by bacteria and bacterial product; 3) a mannose-binding lectin pathway, activated by lectin binding to sugar on the bacterial cell surface (5;15;20). Whether the three complement pathways are activated or not, the end results are the same.

Complement activation promotes acute inflammation, recruitment of leukocytes and killing of pathogens by phagocytosis, and lysis or release of toxic products. The large fragment of C3 cleavage, C3b, binds covalently to the activator and promotes the important defence reactions of immune adherence and phagocytosis. Addition of C3b to C3 convertases results in the formation of a C5 convertase which cleaves C5 into C5a and C5b. This is the last recognized enzymatic step in the complement cascade because the

larger fragment, C5b, and the later complements, C6, C7, C8 and C9, form the lytic complex by a series of hydrophobic interactions (7).

Among the complement components, C3 and C5 are the most important inflammatory mediators. C3 and C5 can be activated by several proteolytic enzymes present within the inflammatory exudate. These include plasmin and lysosomal enzymes released from neutrophils. Thus, the chemotactic effect of complement and the complement-activating effects of neutrophils can set up a self-perpetuating cycle of neutrophil emigration (7). C3a and C5a are anaphylactoxins because they release histamine and stimulate smooth muscle contraction. The actions of C3a include histamine release and increased vascular permeability in human skin. Moreover, C5a may also contribute to inflammatory reactions by stimulating the release of other mediators. Apart from the release of histamine, C5a may also release protein mediators such as degenerative enzymes, cationic proteins and IL-1. Indeed, C5a can release lipid mediators such as LTs and PAF. PG synthesis has been reported to be stimulated by C5a and vasodilator PGs are well known to potentiate inflammatory reactions. Thus inflammatory response begun by complement activation may be prolonged and potentiated by the actions of mediators released by C5a (7).

III. Arachidonic Acid Metabolites

Arachidonic Acid (AA) is a 20 carbon polyunsaturated fatty acid derived primarily from dietary linoleic acid and present in the body only in esterified form as a component of cell membrane phospholipids. It is released from membrane phospholipids through the activation of cellular phospholipases (e.g. phospholipase A_2) that have been activated by mechanical, chemical, physical stimuli, or by inflammatory mediators (e.g. C5a). AA

metabolites, also called eicosanoids, are synthesized by two principal enzyme pathway (7;21;22). The scheme of the major metabolic transformations of AA is shown in Figure 1.

The cyclooxygenase pathway leads to the generation of prostanoids, include prostaglandins (PGs) and thromboxanes (TXs) (5;7). During inflammation, PGE_2 and PGI_2 are the most important. These products are both potent vasodilator and hyperalgesic agents and since they have been detected at sites of inflammation, it is believed that they contribute to the erythema, edema and pain, which are characteristics of the inflammatory response (7;23). Although PGs do not appear to have direct effects on vascular permeability, both PGE_2 or PGI_2 markedly enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed region in combination with other mediators such as bradykinin and histamine. Moreover, they potentiate the pain-producing activity of bradykinin and other autacoids. Similarly, the combination of PGE_2 or PGI_2 with chemotactic factors results in plasma leakage from the microcirculation by a mechanism dependent on the circulating PMNs (21;22). PGE_2 is also a powerful pyrogenic substance and its production is thought to account for the fever induced by IL-1, an endogenous pyrogen (23). TXA_2 is a major product of AA metabolism in platelets which promotes platelet aggregation and vasoconstriction (21).

It is now clear that there are two isozymes of cyclooxygenase (COX) called COX-1 and COX-2. COX-1, also called constitutive COX, is expressed in most tissues and cells such as platelets, endothelium, kidney and stomach mucosa. In contrast, COX-2, also called inducible COX, has low or undetectable levels in peripheral tissues under basal condition. It may be induced by an inflammatory stimulus in macrophages or other cells, and tends to facilitate the inflammatory response (8;23). Ideal anti-inflammatory drugs should have an inhibitory action on PG synthesis mediated by COX-2 but not by COX-1.

Thus, an inhibitor of COX-2 may be an anti-inflammatory drug without the gastrointestinal and renal side effects (8;24).

The lipoxygenase pathway is mediated by a family of lipoxygenase enzymes. 5-Lipoxygenase, the predominant AA-metabolizing enzyme in neutrophils, is the most important of these enzymes. It leads to the synthesis of the LTs (21) . The LTs can be divided, on the basis of their chemical structures and pharmacological actions, into two groups including 1) the dihydroxy leukotrine: LTB_4, and 2) the cysteinyl leukotrienes: LTC_4, LTD_4 and LTE_4 (25). LTC_4, LTD_4 and LTE_4 are collectively known as slow-reacting substance of anaphylaxis (SRS-AS) (5). LTB_4 is the strongest candidate as an inflammatory mediator. It has powerful effects on PMN function; it is a potent chemokinetic, chemotactic and degranulating agent for PMNs. The actions of LTB_4 on PMNs are stereospecific and are not shared by other LTs. In human skin, LTB_4, LTC_4 and LTD_4 cause transient wheal and flare responses either by a direct action or through the release of other endogenous mediators (e.g. PGs) (22). In addition, LTC_4 and LTD_4 appear to act on the endothelial lining of postcapillary venules to cause exudation of plasma. They also are bronchoconstrictors in man (5;21).

IV. Platelet-Activating Factor (PAF)

Platelet-Activating Factor (PAF) is a bioactive phospholipid-derived mediator formed by different cells including eosinophils, macrophages, neutrophils, vascular endothelium and platelets (26). Its biosynthesis involves the acetylation of a precursor released from membrane phospholipids by activated phospholipase A_2 (27).

Two pathways have been described for PAF synthesis: *remodelling* and *de novo*. The remodelling was described first and probably is the more important in inflammation.

PAF is not produced by this pathway in resting cells; they must be activated to initiate the synthesis. The synthesis is initiated by phospholipase A_2, and arachidonate release and PAF synthesis are closely coupled. The arachidonic acid is converted to eicosanoids, which also have diverse, potent actions. The lyso-PAF is converted to PAF by specific acetyltransferase, which is activated by phosphorylation on cell stimulation (26) . PAF activates most inflammatory cells and induces a variety of *in vivo* effects related to inflammation, particularly to immediate hypersensitivity and accordingly to bronchial asthma (27). Like the eicosanoids, PAF is not stored in cells but is synthesized in response to stimulation. It is elaborated by leukocytes and mast cells and exerts proinflammatory effects. In addition to platelet stimulation, PAF causes vasoconstriction and bronchoconstriction, and at extremely low concentrations it induces vasodilation and increases venular permeability with potency 100 to 10,000 times greater than that of histamine. Higher doses produce hyperalgesia (7). Moreover, PAF also causes increased leukocyte adhesion to endothelium (by enhancing leukocyte integrin binding), chemotaxis, degranulation, and the oxidative burst. Thus, PAF can elicit most of the cardinal features of inflammation. PAF also boosts the synthesis of other mediators, particularly eicosanoids, by leukocytes and other cells (7;21). Furthermore, PAF may be of particular importance in late phase reactions, in which it can activate inflammatory leukocytes. In this situation, the major source of PAF may be basophils or the surface of vascular endothelial cells (stimulated by histamine or LTs) rather than mast cell (14). It is still impossible to determine clearly the role of PAF as a potential mediator in inflammation. The possibility that it plays an important role is nevertheless as likely, if not more so, than in case of the eicosanoids (27)

V. Cytokines

Cytokines are potent polypeptide/proteins (glycoproteins) with both local and (frequently) systemic effects that produce specific receptor-mediated effects on target cells or on the producer cell. It is important to note that most cytokines are not constitutively produced, but require cell activation for their synthesis. This definition therefore includes the ILs, growth factors, colony-stimulating factors, tumor necrosis factor (TNF) etc. (28). Long known to be involved in cellular immune responses, cytokines have additional effects that play important roles in both acute and chronic inflammation (7). Cytokines are responsible for important homeostatic functions such as the maintenance of normal levels of circulating blood leukocytes (lymphocytes, myelomonocytic cells and erythrocytes) and the acute-phase response to infections, it is now clear that they can also be responsible for tissue pathology in a variety of diseases ranging from acute infectious lesions to chronic infectious diseases and to chronic idiopathic diseases, and chronic destructive arthritis (28).

IL-1, IL-2 and TNF are the inflammatory cytokines which play key roles in the acute-phase response to infection and tissue injury. As well as their important local effects, the cytokines produced by macrophages and neutrophils have long range effects that contribute to the host defense. One of these is the elevation of body temperature which is caused by TNF-α, IL-1, IL-6 and other cytokines. These are termed "endogenous pyrogens" (29). IL-1 and TNF produce many proinflammatory responses which include mobilization and activation of PMNs; induction of cyclooxygenase and lipoxygenase enzymes; increase in adhesion molecule expression; activation of B-cells, T-cells, and natural killer cells; and stimulation of production of other cytokines (30). Clearly, many of the events associated with acute inflammatory reaction can be mediated by IL-1 and TNF (7). Other actions of these agents likely contribute to fibrous and tissue

degeneration in the chronic proliferation phase of inflammation; stimulation of fibroblast proliferation, induction of collagenase and activation of osteoblasts and osteoclasts (30) . Other cytokines, including IL-8 and interferon-γ, exert additional effects such as increased chemotaxis for leukocytes and increased phagocytosis. All these effects result in the accumulation of fluid (edema) and leukocytic cells in the injured areas. These, in turn, amplify the response further since additional biologically active compounds are transported in the fluid and also are released from the accumulated cells, attracting and activating still more cells (4).

ANTI-INFLAMMATORY DRUGS

The treatment of patients with inflammatory disease involves two primary goals; first, the relief of pain which is often the presenting symptom and the major continuing complaint of the patient; and second, the slowing or - in theory - arrest of tissue-damaging processes (8). The drugs employed in the treatment of inflammatory diseases can be divided into nonsteroidal anti-inflammatory drugs (NSAIDs), anti-inflammatory corticosteroids and disease modifying anti-rheumatic drugs (DMARDs) (8).

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g. indomethacin, ibuprofen, naproxen) are, as a group, the most frequently consumed drugs worldwide (31). These drugs also exert antipyretic and analgesic effects, but it is their anti-inflammatory properties that make them most useful in the management of disorders in the pain which is produced according released to the intensity of inflammatory process (8). The mechanism

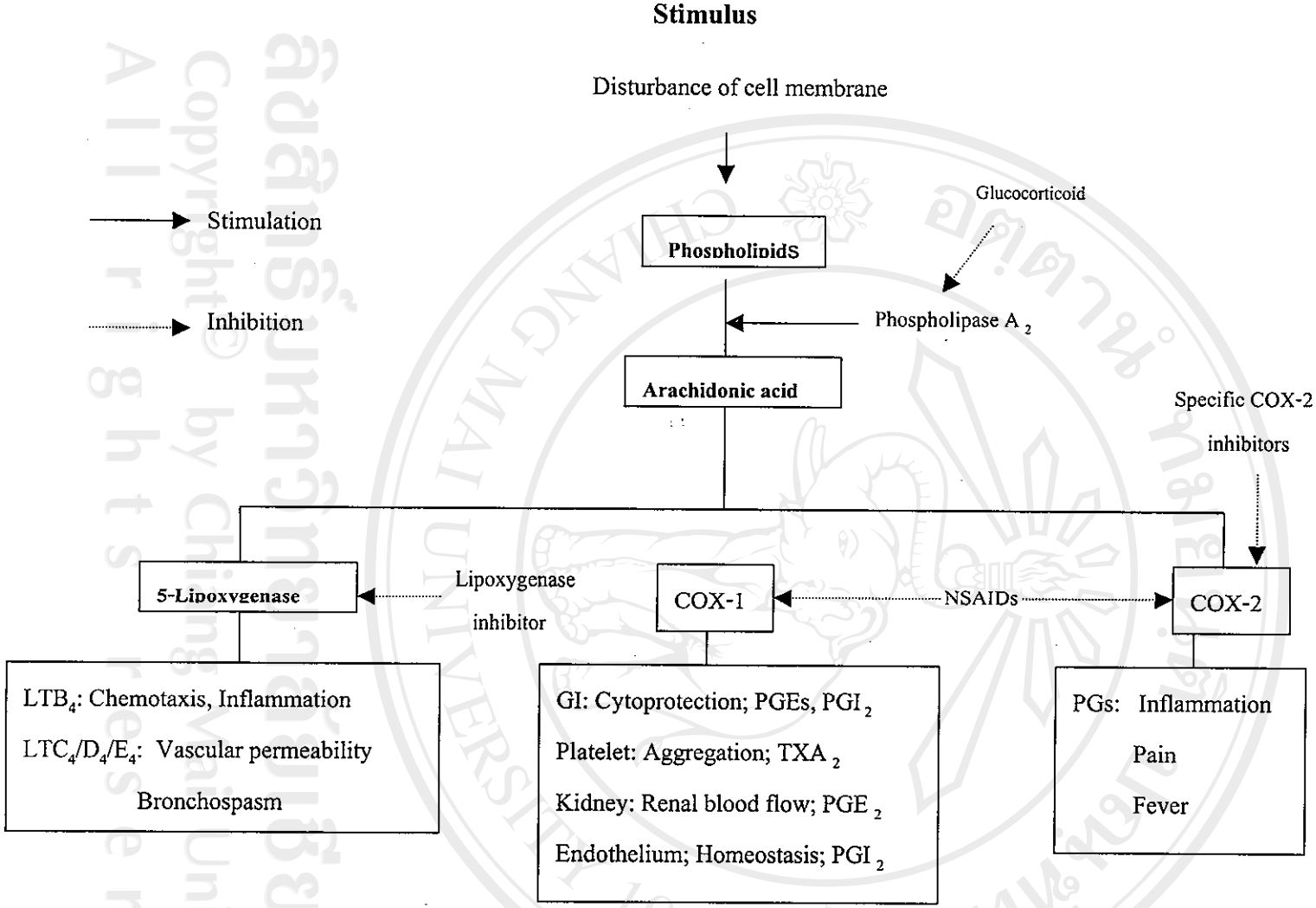

Figure 1. Scheme of the major metabolic transformation of arachidonic acid

COX = cyclooxygenase, LT = leukotriene, NSAIDs = non-steroidal anti-inflammatory drug, GI = gastrointestinal tract

of action is widely believed to involve inhibition of COX mediated biosynthesis of PGs, TXs and certain related autacoids (30). During therapy with these drugs, inflammation is reduced by decrease of release of mediators from granulocytes, basophils and mast cells. The NSAIDs decrease the sensitivity of vessels to bradykinin and histamine, affect lymphokine production from T lymphocytes and reverse vasodilation (8). In addition to sharing many therapeutic activities, NSAIDs share several unwanted side effects. The most common is a propensity to induce gastric or intestinal ulceration.

Two isozymes of COX (COX-1 and COX-2) catalyze the conversion of arachidonic acid to PGH_2, the most important step in the formation of both PGs and thromboxane A_2 (TXA_2). COX-1 is a wide-ranging essential enzyme which produces PGs involved in cytoprotective and regulatory functions in gastrointestinal mucosa, platelet and renal cells whereas COX-2 produces PGs that mediate pain and inflammation (32). The inhibition of COX-2 is thought to mediate, at least in part, the antipyretic, analgesic, and anti-inflammatory action of NSAIDs, but the simultaneous inhibition of COX-1 results in unwanted side effects, particularly those leading to gastric ulcers. However, most currently available NSAIDs inhibit both COX-1 and COX-2 (30;33). Overall, there is probably little difference in the analgesic and inflammatory efficacy between the different NSAIDs (33;34).

As a refinement of the mechanism proposed by Vane in 1972 it has therefore been suggested that inhibition of COX-2 underlies the therapeutic efficacy of NSAIDs whilst inhibition of COX-1 underlies their side effects. Since this discovery, enormous resources have been invested in developing highly selective COX-2 inhibitors, which selectively block the activity of COX-2 without affecting COX-1 (8;24). One can now distinguish the following four major groups of NSAIDs on the basis of their inhibitory activity on COX-1 and COX-2 (24;31).

1. Selective COX-1 inhibitors: such as low dose aspirin, where the concentrations in the system circulation are too low to cause inhibition of COX elsewhere.
2. Nonselective COX inhibitors: These drugs inhibit both COX-1 and COX-2. This category includes high dose aspirin, indomethacin, piroxicam, diclofenac, ibuprofen and many others.
3. Selective COX-2 inhibitors or COX-2 preferential inhibitors: These drugs, such as meloxicam and nimesulide, inhibit COX-2 rather than COX-1.
4. Highly selective COX-2 inhibitors or COX-2 specific inhibitors: These drugs, such as celecoxib and rofecoxib, inhibit COX-2 but have no effect on COX-1.

However, selectivity for COX-1 versus COX-2 is still variable and incomplete. Furthermore, during therapy with NSAIDs, inflammation is also reduced by decrease of release of mediators from granulocytes, basophils and mast cells. NSAIDs also decrease the sensitivity of vessels to bradykinin and histamine, affect lymphokine production from T lymphocytes and reverse vasodilation (8).

Anti-inflammatory corticosteroids

Anti-inflammatory corticosteroids such as dexamethasone and prednisolone, have powerful anti-inflammatory and immunosuppressive effects (8;35). They block all the known pathways of eicosanoid synthesis, perhaps by stimulating the synthesis of several inhibitory proteins collectively called annexins or lipocortins. They inhibit phospholipase A_2 activity, probably by interfering with phospholipid binding and thus preventing the release of AA (8). Thus the anti-inflammatory action of these agents may also be related to the inhibition of prostaglandin and leukotriene synthesis (36). Corticosteroids have powerful anti-inflammatory effect. Unfortunately, the toxicity associated with chronic corticosteriod therapy inhibits their use except in the control of

acute flare-ups of joint diseases (8). Long term use of glucocorticoids is associated with major adverse events in a dose-dependent manner. Although some studies have shown the relative safety of long term low dose glucocorticoids, other studies highlight the cumulative toxicity that leads to osteoporosis, infections and peptic ulcer (37).

Disease modifying anti-rheumatic drugs (DMARDs)

Members of the group DMARDs or slow-acting anti-rheumatic drugs (SAARDs) include methotrexate, azathioprine, penicillamine, hydroxychloroquin, chloroquin, organic gold compounds and sulfasalazine. The effects of DMARDs may take 6 weeks to 6 months to become evident. Very little is known about their mechanism of action, but they may slow the bone damage associated with rheumatoid arthritis and are thought to affect more basis inflammatory mechanisms than do the NSAIDs (8). In contrast, newer drugs have been designed with strict reference to proven pathophysiology in rheumatoid arthritis (RA) and the intended action of these agents is highly likely to be the explanation for the observed efficacy (38). For example, the major mode of action of leflunomide is believed to be inhibition of dihydroorotate dehydrogenase, a key enzyme in *de novo* synthesis of pyrimidines. Blocking of the pyrimidine pathway prevents the proliferation of activated T cells, which are thought to play an important role in the pathogenesis of RA (39). The discovery that numerous cytokines are present in joints affected by the disease process suggests that one or more of these may be useful targets of disease modifying drug therapy. Unfortunately, they are also more toxic than the NSAIDs (8).

Among the three groups of anti-inflammatory drugs, NSAIDs are the most used agents, since they can effectively relief the symptoms of inflammation and have less severe side effects when compare to the other two groups of drugs (8).

EXPERIMENTAL MODELS

1. Inflammatory models

Ethyl phenylpropiolate (EPP)-induced ear edema in rats: Edema is a useful parameter to look at when testing for agents which may be active in treating acute inflammation (40). Ear edema induced in rats by EPP was suggested to serve as a more useful model for the rapid *in vivo* screening of agents with anti-inflammatory activity, since only a small amount of a test substance is needed. By using inducer EPP, the mechanism involved can be suggested. Kinins, serotonin and PGs are released in EPP-induced ear edema (41;42).

Carrageenin-induced paw edema in rats: The hind paw edema induced in rat by subplantar injection of irritants including formalin, kaolin, dextran, carrageenin, arachidonic acid, etc., has long been known and used for testing substances for anti-inflammatory property. The most commonly used irritant is carrageenin (43). Carrageenin is a sulphate polysaccharide which has been fractionated with potassium chloride into two seperate components, kappa and lamda carrageenin (44). The lamda carrageenin is more active in eliciting either acute or chronic inflammatory responses. The edema is produced by a sequential release of pharmacological mediators; histamine, serotonin, kinins and PGs (45;46). It is important when using this model to assess the effect of the potential anti-inflammatory agent at the appropriate time during the swelling of hind paw. Ideally the foot should be measured at more than one time point but certainly at 3-4 h. This allows for the participation of all the chemical mediators. The test is excellent for detecting inhibitors of COX (40). Moreover, the advantage of carrageenin-induced edema in comparison with the edema elicited by other phlogistic agents is its responsiveness to doses of all clinically used anti-

inflammatory drugs at well below the toxic level, with the degree of edema inhibition being in a dose-related manner (43).

Arachidonic acid (AA)-induced paw edema in rats: AA-induced paw edema in rats is an *in vivo* model to distinguish between COX and lipoxygenase inhibitors (47). The choice of AA is based on the knowledge that LTs are involved in the formation of edema when AA is used as inducer (42).

AA-induced hind paw edema in rats by subplantar injection produces a severe edematous response. One of the unique aspects of the AA-induced rat paw edema model, is that lipoxygenase metabolites, especially LTs, have an important role in producing vascular permeability and edema formation whereas COX products have low or no activity. In addition, AA-provoked paw edema is highly sensitive to inhibition by dual inhibitors of AA metabolism (i.e. phenidone) and steroids but is insensitive to selective COX inhibitors (47).

Cotton pellet-induced granuloma formation in rats: Meire *et al.*, (48) first introduced the method using cotton pellet to induce granuloma formation. The response to a subcutaneously implanted cotton pellet in rat has been divided into three phases, namely (1) a transudative phase, defined as the increase in wet weight of the pellet which occurs during the first three hours after implantation (2) an exudative phase, defined as a leakage of fluid from the bloodsteam around the granuloma and occurring between 3 and 72 hours after implanting the pellet, and (3) a proliferative phase, measured as the increase in dry weight of the granuloma which occurs between three and six days after implantation (49). The net dry weight of granuloma tissue indicates the intensity of the subchronic inflammation. This method is generally

employed to measure the interfering capacity of agents on the proliferative phase of inflammatory process.

Measurement of alkaline phosphatase activity in the serum (50)**:** Alkaline phosphatase is a lysosomal enzyme. It is widely distributed in many tissues, including the osteoblasts (the bone-building cells), the cells lining the sinusoids and bile canaliculi in the liver. It was reported that the activity of alkaline phosphatase in serum is markedly increased during inflammation. Alkaline phosphatase activity in pouch wall was elevated during cotton pellet-induced granuloma formation on the seventh day and decreased on the fourteenth day when healing occurred. Measurement of alkaline phosphatase activity in serum of this group of rats indicates the activity of agents on the production and release of alkaline phosphatase (51;52).

2. Ulceration

Chronic administration of nonselective COX inhibitors (e.g. aspirin) or steroids (e.g. prednisolone) is known to induce gastric ulceration (8;24;33). The reason being attributed principally to the inhibition of biosynthesis of 'cytoprotective PGs' (e.g.PGE_2 and PGI_2) by inhibiting the constitutive COX-1 enzyme of AA metabolism. As described by Valle and Todisco (53), the presence of PGs in gastric juice and gastric mucosa may facilitate the diverse cytoprotective functions, including secretion of mucus and bicarbonate, maintenance of mucosal blood flow, and cell surface hydrophobicity. In addition, since it has been reported that a number of selective COX-2 inhibitors have low gastric toxicity (24), it seems that the mechanism of action of anti-inflammatory agents, which have low or no ulcerogenic effects, may be due to the selective COX-2 inhibition.

3. Algesic models

3.1 Acetic acid-induced writhing response in mice: Most NSAIDs usually possess analgesic activity. Inhibition of PG biosynthesis is considered to be a shared mechanism of the anti-inflammatory, analgesic and antipyretic actions of NSAIDs (8;54). The writhing response induced in rat or mice by intraperitoneal injection of a noxious agent is commonly used as a basis for testing analgesic activity. The response consists of a wave of constriction and elongation passing caudally along the abdominal wall, sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs. The latency and duration of writhing response depends on the characteristics of the challenge substances. The substance, which has a long latency, such as acetic acid or phenylbenzoquinone, may be supposed to act indirectly by liberating an endogenous substance that excites pain nerve endings (55). The inhibitory effect of a substance on writhing response in this test was found to be well correlated with clinical results in humans (56).

3.2 Formalin test: The formalin test in mice has an advantage over other frequently used tests as it involves a biphasic response with an early and a late phase representing neurogenic and inflammatory pain and agents can be screened for activity in these two models of pain. The first phase response is believed to represent a direct irritant effect of formation on sensory C fibers whilst the later phase response is most likely secondary to the development of an inflammatory response and the release of algesic mediators (57).

4. Antipyretic models

Yeast-induced hyperthermia in rats: Experimental models of fever have used microbial and antigenic agents that provoke inflammation and all have implicated

leukocytes, important participants in the inflammatory response, as being responsible for endogenous pyrogen release (58). Yeasts, either directly or by releasing pyrogenic material, activate cells within the body to synthesize and release an endogenous pyrogen. The cells capable of producing endogenous pyrogen include neutrophils, monocytes and lymphocytes (54). It is probable that these cells play an important role in fever associated with diseases where chronic inflammation is a prominent feature (58).

The pyrexia induced in rat by subcutaneous injection of brewer's yeast has been used to determine antipyretic activity of many compounds (59). The pyrexia reaches its peak at 18 h after induction and the assessment is also made at this period. It has been postulated that many chemical neuromediators are involved in hypothalamic regulation of body temperature (60). Milton (54) found that PGE_2 is an endogenous modulator responsible for fever and that antipyretic drugs such as aspirin and paracetamol produce their action by inhibiting PG biosynthesis and release.

Historical background of *Garcinia Hanburyi* Hook f.

Nowadays, medicinal plants may represent a useful source of new effective therapeutic agents (61). Various researchers have reported that most plants with anti-inflammatory property lack an ulcerogenic effect e.g. *G. speciosa* (62) and some even possess anti-ulcerogenic activity, e.g. *Zingiber officinale* Roscoe (63-65), *Curcuma longa* Linn. (66;67) and *Pluchea indica* Less. (68).

G. hanburyi Hook f. commonly know in Thailand as "Rong", "Rong Thong" or "Gamboge" (69-72) belongs to the family Guttiferae (Figure 2) *G. hanburyi* is widely grown in Thailand, China and Cambodia (73;74). The tree is up to 10.5-15 m high, with a trunk diameter of 30 cm and many spreading branches. The bark is thick, orange-brown color. The leaves are 10-17.5 cm long, laurel-like. Flowers are small,

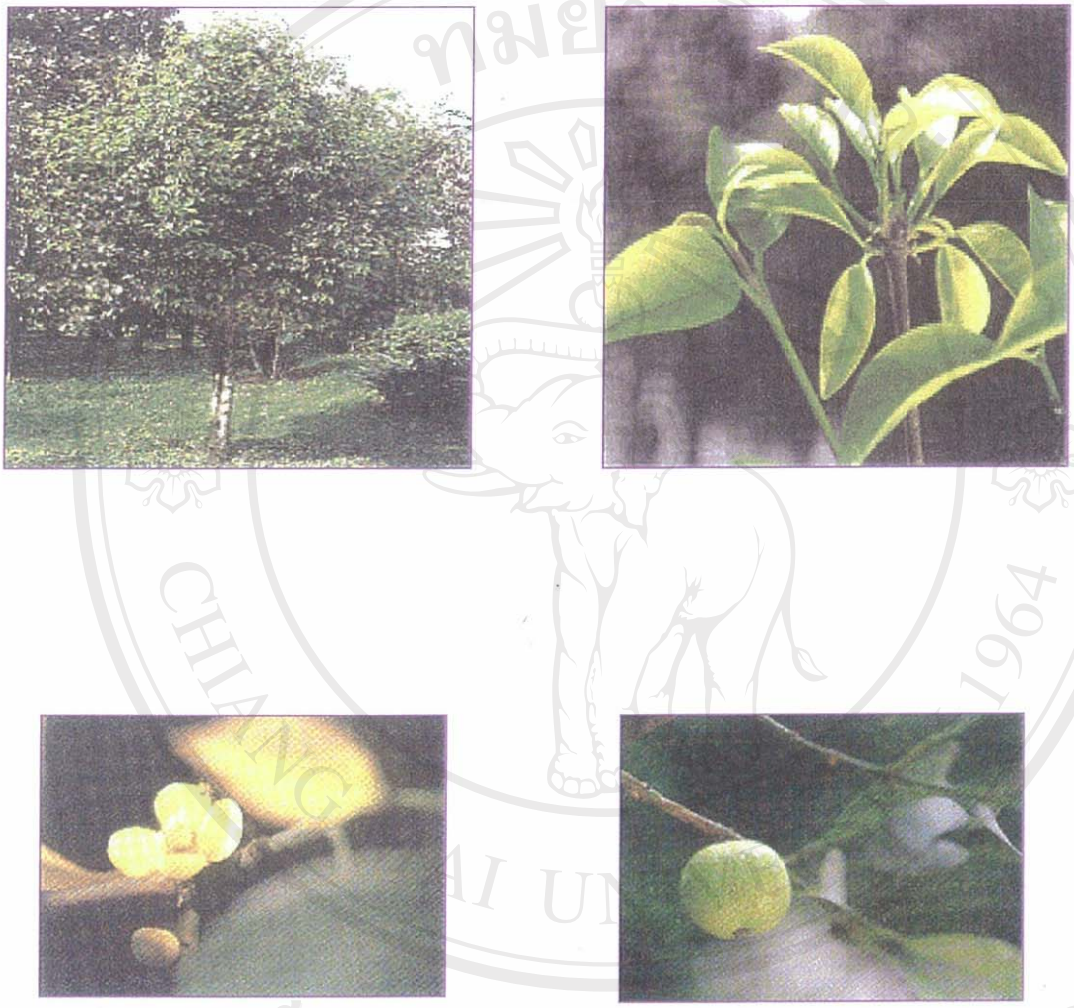

Figure 2. "Rong Thong"

(*Garcinia hanburyi* Hook f.)

dioecious, with yellow color, pedicels are 6 mm long. The fruit is crab apple size, 3 cm long. (69;71;75-77). Gamboge (gum-resin) secretes in latex-tubes (ducts) in the middle bark and to some extent in the pith, alburnum, leaves, flowers and fruits. It is at first a yellow milky juice obtained in drops from broken leaves, twigs or artificial incisions and being caught in leaves, coconut shells or bamboo joints. There are two varieties of gamboge: 1) Pipe, the best resulting from making, at the beginning of the rainy season, June-October. Gamboge is extracted by spiral incisions in the bark half around the tree trunk from the ground upward a number of feet, and collecting the slowly exuding juice in a hollow bamboo joint placed at the lower end of the incision, requiring 1-2 months to fill and harden, in which the contraction toward the sides often affords a central cylindrical cavity, upon cracking off the bamboo shell. 2) Cake, resulting form collecting the juice in leaves and various vessels, being subjected to exposure and adulterations, thereby becoming less uniform and brittle with dull brownish non-conchoidal fracture. It is usually in masses, 2-3 pounds (0.7-1 kg), sometime much larger, being pressed or run into boxes or tubs (73-77).

In Thai folklore medicine, it is used for infected wound, pain and edema. Moreover, it is a very powerful drastic hydragogue carthatic, very useful in dropsical conditions and for lowering blood pressure (69;72;76;78-80). It usually produces much griping, nausea and vomiting when taken in full dose, so that generally it is combined with other carthatics. It generally irritates the alimentary canal, especially the small intestines, when taken in excess, and 4 gm have occasioned death. It can be detoxified by wrapping with galangal leaf and lotus leaf, roast, and then powdering before use (77;78).

In the phytochemical study of gamboge, eleven novel cytotoxic xanthones, gambogin, morellin dimethyl acetal, isomorellin B, morellic acid, gambogenic acid, gambogenin, isogambogenin, desoxygambogenin, gamboganin dimethyl acetal, gambogellic acid and hanburin were isolated together with four known xanthones

gambogic acid, isomorellin, morellic acid and desoxymorellin, from the dry latex of *G. hanburyi* (81).

No pharmacological effects of Gamboge extract have been reported. However, some species of Garcinia are widely used for different types of inflammatory diseases. In Thailand, dried stembark of *G. cowa* is used as an antipyretic agent and fresh pericarp of *G. mangostana* is employed as a topical anti-inflammatory agent (82;83). The methanol extract from the bark of *G. speciosa* showed anti-inflammatory and analgesic action without ulcerogenic effects (62). In Nigeria dried fruit and root of *G. kola* are indicated to treat arthritis and inflammation of the respiratory tract, respectively (84;85). The hexane extract of dried seed from *G. kola* showed anti-inflammatory activity on carrageenin-indueced pedal edema model in rat and inhibited gastric lesions induced by indomethacin when orally administrated as a powder in 25% of diet (86;87).

PURPOSE OF THE STUDY

The purpose of the present study was to evaluate the anti-inflammatory, analgesic and antipyretic activities of the methanol extract from *G. hanburyi* in many inflammatory models in comparison with reference drugs i.e. aspirin, phenylbutazone, phenidone and prednisolone. The mechanism of action of the methanol extract from *G. hanburyi* on the inflammatory process and its effects on the gastric mucosa were also examined in comparison to some nonsteroidal and steroidal anti-inflammatory drugs.