INTRODUCTION

INFLAMMATION

Inflammation is a fundamental pathophysiological response designed to eliminate any noxious stimulus introduced into the host. Such noxious stimuli include radiant, mechanical, chemical, physical, infectious, and immune provocations. 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 (Lee and Katayama, 1992; Fantone and Ward, 1999; Benjamini *et al.*, 2000).

Under certain conditions, the ability to clear injured tissue and foreign agents is impaired, or the regulatory mechanisms of the inflammatory response are altered. In these circumstances, inflammation is harmful to the host and leads to excessive tissue destruction and injury. In other instances, an immune response to altered tissue components also triggers a persistent inflammatory reaction (Fantone and Ward, 1999; Benjamini *et al.*, 2000). Excessive inflammation caused by abnormal recognition of host tissue as foreign body or prolongation of the inflammatory process may lead to inflammatory diseases such as rheumatoid arthritis, whereas deficiencies of inflammation compromise the host. Diseases characterized by inflammation are an important cause of morbidity and mortality in humans (Gallin and Snyderman, 1999).

Generally, inflammatory reactions are divided into two types: acute and chronic inflammation (Bowman and Rand, 1980; Lee and Katayama, 1992; Collins, 1999). The hallmarks of acute inflammation include (1) accumulation of fluid and plasma components in the affected tissue, (2) intravascular stimulation of platelets, and (3) the presence of polymorphonuclear leukocytes (PMNs). By contrast, the characteristic cell components of chronic inflammation are lymphocytes, plasma cells, and macrophages (Fantone and Ward, 1999).

Acute inflammation is the initial (almost immediate) 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 hours or days. It is mediated by the release of autacoids. Some of the autacoids involved are histamine, serotonin, bradykinin, prostaglandins (PGs) and leukotrienes (LTs) (Furst and Munster, 2001). Whereas histamine and serotonin are believed to mediate the initial phase of inflammation (1 - 1½ h), and kinins the second phase (1½ - 2 h), PGs probably exert their proinflammatory effects in the late phase of inflammation (2½ - 6 h) (Lee and Katayama, 1992). 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 (1) dilation of arterioles, capillaries and venules with increased permeability and blood flow; (2) accumulation of protein-rich extravascular fluid, which forms the exudate; and (3) leukocytic migration into the inflammatory focus (Collins, 1999; Gallin and Snyderman, 1999). If the initiating stimuli for an inflammatory reaction are not eliminated by the reaction or controlled adequately, a continuing state of inflammation persists (Bowman and Rand, 1980).

Chronic inflammation may be a sequel to acute inflammation or an immune response to a foreign antigen. The process may become chronic if the inflammatory response is unable to eliminate the injurious agent or restore injured tissue to its normal state. The long-lived reaction persists for weeks or months after the initial exposure to the damaging agent (Collins, 1999; Fantone and Ward, 1999). Characteristically, there is an abundance of exudate, granulomatous tissue, monocytosis with many multi-nuclear giant cells formed by their fusion, lymphocytosis and accumulation of plasma cells. The connective tissue invasion results in the formation of much fibrous tissues (fibrosis) (Bowman and Rand, 1980). The cellular components are macrophages, plasma cells, lymphocytes, and, in certain conditions, eosinophils (Fantone and Ward, 1999). One of the products of the arachidonic acid cascade, LTB_4, is extremely chemotactic and may be importantly involved in the chronic as well as the acute

inflammatory response (Lee and Katayama, 1992). In addition, 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), IL-2, IL-3, tumor necrosis 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 (Furst and Munster, 2001).

Granulomatous inflammation is a distinctive pattern of chronic inflammation characterized by aggregations of activated macrophages that have acquired an enlarged, squamous cell-like (called epitheloid) appearance. It is typical of the tissue response elicited by fungal infections, tuberculosis, leprosis, schistosomiasis, and the presence of foreign bodies or indigestible particles (e.g. suture, talc or splinters of wood). The principal cells involved in granulomatous inflammation are macrophages and lymphocytes (Collins, 1999; Fantone and Ward, 1999).

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 (1911) and restated by Vane (1972). 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 specified as inflammatory mediators are as follows:

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 mast cells that are normally present in the connective tissue adjacent to vessels, as well as in circulating basophils and platelets (Babe and Serafin, 1999; Collins, 1999). Preformed histamine is normally present in mast cell granules that are released in response to a variety of stimuli: (1) physical injury such as trauma, cold, or heat; (2) immune reactions involving binding of antibodies to mast cells; (3) C3a and C5a fragments of complement, the so-called anaphylatoxins; (4) histamine-releasing proteins derived from leukocytes; (5) neuropeptides (e.g. substance P); and (6) cytokines (IL-1, IL-8) (Collins, 1999). Histamine is clearly important in acute inflammation associated with mast cell degranulation in non-rodent species including man (Owen, 1987). The acute inflammatory response to histamine comprises vasodilatation, an increase in microvascular permeability and edema formation. Pharmacological analysis of the receptor involvement in these component parts of the inflammatory response has shown that the vasodilatation involves H_1- and H_2-receptors (Babe and Serafin, 1999; Collins, 1999). Histamine can also stimulate free nerve endings (mainly H_1- receptors), cause pain and itching (Babe and Serafin, 1999). Histamine is relatively unimportant in the later stages of the response. Thus, inhibition of histamine response delays but does not prevent the inflammatory response (Owen, 1987).

Serotonin (5-Hydroxytryptamine)

Serotonin (5-Hydroxytryptamine: 5-HT) is a second preformed vasoactive mediator with actions similar to those of histamine, in that it causes constriction of smooth muscle and increase vascular permeability. It is present in platelets and enterochromaffin cells, and in mast cells in rodents but not humans (Collins, 1999). In acute inflammation associated with mast cell degranulation in certain rodents, serotonin

may be of equal or greater importance than histamine (Abbas *et al.*, 1997; Chandrasoma and Taylor, 1998). In the microcirculation, serotonin causes dilatation 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 (Rang *et al.*, 1995).

II. Plasma proteases

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 (Babe and Serafin, 1999; Collins, 1999).

Kinins have been identified in mammals: bradykinin, lysylbradykinin (kallidin) and methionyllysylbradykinin (Reid, 2001). The kinins, particularly bradykinin, are important mediators involved in the initiation, development and maintenance of inflammatory and nociceptive processes (Calixto *et al.*, 2000). They generated locally contribute to the acute and possibly the chronic phase of the inflammatory reaction by producing vasodilation, increase of vascular permeability, plasma extravasation as well as cell migration (Babe and Serafin, 1999; Calixto *et al.*, 2000). Several biological effects of bradykinin are mediated by endogenous agent such as PGs and histamine and/or serotonin (Regoli, 1987). Moreover, kinins seem to be directly implicated in neurogenic inflammatory events through activation of C-fibers and consequent production of neuropeptides such as substance P (Geppetti, 1993). The major effects of kinins 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. On the other hand, the 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 (Babe and Serafin, 1999; Calixto *et al.*, 2000). Kinins are rapidly inactivated following their activation by degradative kininases present in plasma and tissues (Babe and Serafin, 1999; Collins, 1999). 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 PG generation can be blocked nonspecifically by inhibitors of PG synthesis such as aspirin (Reid, 2001).

Complement system

The complement system consists of a group of 20 plasma proteins. In addition to being a source of vasoactive mediators, components of the complement system are an integral part of the immune system and play an important role in both immunity and inflammation (Cooper, 1999). Complement components are present in plasma as inactive forms and sequentially activated by three convergent pathways including (1) classical pathway, directly activated by complexes of antibody and antigen; (2) alternative pathway, activated by bacteria and bacterial products; (3) mannose-binding lectin pathway, activated by lectin binding to sugars on the bacterial cell surface (Chandrasoma and Taylor, 1998; Cooper, 1999; Fantone and Ward, 1999). Whether the three complement pathways are activated, the end results are the same.

Activation by either pathway initiates a cascade of proteolytic events that produce chemotactic factors to attract phagocytic and inflammatory cells to the site, increase vascular permeability to allow access to the site of infection, bind to the agent to promote their phagocytosis (opsonization) and elimination, and also directly kill the infecting agent (Cooper, 1999). The biologic functions of the complement system fall into two general categories; cell lysis by the membrane attack complex (MAC), and the biologic effects of the proteolytic fragments of complement. Complement-derived factors affect a variety of phenomena in acute inflammation. They increase vascular

permeability and cause vasodilation mainly by releasing histamine from mast cells (Collins, 1999; Fantone and Ward, 1999).

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. Moreover, C5a also activates the lipoxygenase pathway of arachidonic acid metabolism in neutrophils and monocytes, causing further release of inflammatory mediators. Thus, inflammatory responses begun by complement activation may be prolonged and potentiated by the actions of mediators released by C5a (Collins, 1999).

III. Arachidonic Acid Metabolites

Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid. It is released from membrane phospholipids through the activation of cellular phospholipases (e.g. phospholipase A_2) by mechanical, chemical, and physical stimuli or by other mediators (e.g. C5a). AA metabolites, also called eicosanoids, are synthesized by two principal enzyme pathways, the cyclooxygenase, and the lipoxygenase pathway (Salmon and Higgs, 1987; Campbell and Haluska, 1999; Collins, 1999). The scheme of the major metabolic transformations of AA is shown in Figure 1.

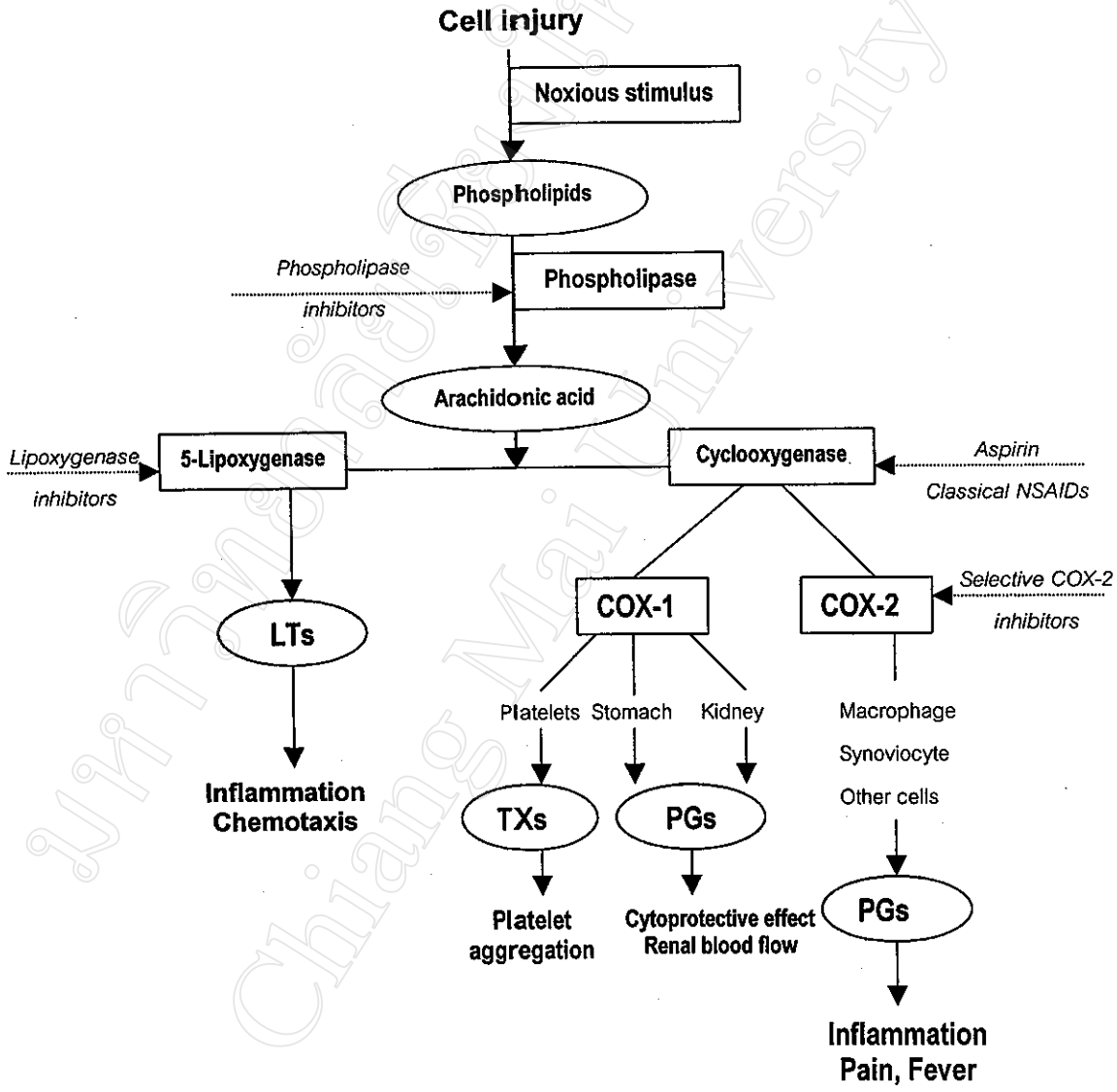

Figure 1. Scheme for mediators derived from arachidonic acid (AA) and sites of drug action (dashed arrows).

The cyclooxygenase pathway leads to the generation of prostanoids, include prostaglandins (PGs) and thromboxanes (TXs) (Collins, 1999; Fantone and Ward, 1999). Of the various cyclooxygenase products formed 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 (Collins, 1999; Griffith, 1999). Although PGs do not appear to have direct effects on vascular permeability, both PGE_2 and 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 circulating PMNs (Salmon and Higgs, 1987; Campbell and Halushka, 1999). 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 (Griffith, 1999). TXA_2 is a major product of AA metabolism in platelets, which promotes platelet aggregation and vasoconstriction (Campbell and Halushka, 1999).

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 (Griffith, 1999; Furst and Munster, 2001). 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 anti-inflammatory drug without the gastrointestinal and renal side effects (Hawkey, 1999; Furst and Munster, 2001).

The lipoxygenase pathway is mediated by a family of lipoxygenase enzymes. 5-lipoxygenase, predominant AA-metabolizing enzyme in neutrophils, is the most important of these enzymes, since it leads to the synthesis of the leukotrienes (LTs) (Campbell and Halushka, 1999; Collins, 1999). The LTs can be divided, on the basis of their chemical structures and pharmacological actions into two groups include (1) the dihydroxy leukotriene: LTB_4, and (2) the cysteinyl leukotrienes: LTC_4, LTD_4 and LTE_4 (Penrose *et al.*, 1999). LTC_4, LTD_4 and LTE_4 are collectively known as slow-reacting substances of anaphylaxis (SRS-As) (Fantone and Ward, 1999).

LTB_4 is the strongest candidate as an inflammatory mediator. It is a potent chemokinetic, chemotactic and degranulating agent for PMNs. It promotes neutrophil-induced inflammation by causing cell degranulation and release of lysosomal enzymes (Salmon and Higgs, 1987; Penrose *et al.*, 1999). The actions of LTB_4 on PMNs are stereospecific, 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) (Salmon and Higgs, 1987). Furthermore, 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 (Campbell and Halushka, 1999; Fantone and Ward, 1999).

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. Chemically, its biosynthesis involves the acetylation of a precursor released from membrane phospholipids by activated phospholipase A_2. 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 (Vargaftig and Braquet, 1987; Collins, 1999). Like the eicosanoids, PAF is not stored in cell 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 (Collins, 1999). 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 boots the synthesis of other mediators, particularly eicosanoids, by leukocytes and other cells (Campbell and Halushka, 1999; Collins, 1999). 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 (Abbas *et al.*, 1997). 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 (Vargaftig and Braquet, 1987).

V. Cytokines

Cytokines are polypeptide products of many cell types (but principally activated lymphocytes and macrophages) that modulate the function of other cell types. Long known to be involved in cellular immune responses, these products have additional effects that play important roles in both acute and chronic inflammation (Collins, 1999).

IL-1 and TNF are the most relevant of cytokines, which exercise an influence on the inflammatory process (Collins, 1999; Benjamini *et al.*, 2000). IL-1 and TNF produce many of same 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 (Insel, 1999). Clearly, many of the events associated with acute inflammatory reaction can be mediated by IL-1 and TNF (Collins, 1999). Other actions of these agents likely contribute to the fibrosis and tissue degeneration of chronic proliferation phase of inflammation; stimulation of fibroblast proliferation, induction of collagenase and activation of osteoblasts and osteoclasts (Insel, 1999). As well as their important local effects, the cytokines produced by

macrophages and neutrophils have long range effects that contribute 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" (Janeway *et al.*, 1999). 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 (Benjamini *et al.*, 2000).

ANTI-INFLAMMATORY DRUGS

At present, 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) (Furst and Munster, 2001).

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are, as a group, the most frequently consumed drugs worldwide (Frolich, 1997). The mechanism of action is widely believed to involve inhibition of COX mediated biosynthesis of PGs, TXs and certain related autacoids (Insel, 1999). It is now known that COX exists in at least two isoforms, a constitutive COX-1, and an inducible COX-2. 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 (Insel, 1999; Davies *et al.*, 2000). Overall, there probably little difference in the analgesic and inflammatory efficacy between the different NSAIDs (Heller *et al.*, 1985; Davies *et al.*, 2000).

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 investigated in developing high selective COX-2 inhibitors, which selectively block the activity of COX-2 without affecting COX-1 (Hawkey, 1999; Furst and Munster, 2001). One can now distinguish the following four major groups of NSAIDs on the basis of their inhibitory activity on COX-1 and COX-2 (Frolich, 1997; Hawkey, 1999).

(1) Selective COX-1 inhibitors: such as low dose aspirin, which the concentrations in the systemic 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 now variable and incomplete. Furthermore, during therapy with NSAIDs, inflammation is reduced by decreasing 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 (Furst and Munster, 2001).

Anti-inflammatory corticosteroids

Anti-inflammatory corticosteroids such as dexamethasone and prednisolone, have powerful anti-inflammatory and immunosuppressive effects (Schimmer and Parker, 1999; Furst and Munster, 2001). 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 (Furst and Munster, 2001). In addition to their effects on lymphocyte number, corticosteroids

profoundly alter the immune response of lymphocytes. Although the use of corticosteroids as anti-inflammatory agents does not address the underlying cause of the disease, the suppression of inflammation is of enormous clinical utility and has made these drugs among the most frequently prescribed agents. Similarly, corticosteroids are of immense value in treating diseases that result from undesirable immune reactions (Schimmer and Parker, 1999). Unfortunately, the toxicity associated with chronic corticosteroid therapy inhibits their use (Furst and Munster, 2001).

Disease modifying anti-rheumatic drugs (DMARDs)

Members of the group slow-acting antirheumatic drugs (SAARDs) or DMARDs include methotrexate, azathioprine, penicillamine, hydroxychloroquin, chloroquin, organic gold compounds and sulfasalazine as well as new DMARDs i.e. leflunomide, etanercept and inbliximab. 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 basic inflammatory mechanism than do the NSAIDs (Furst and Munster, 2001). In contrast, newer drugs were designed with strict reference to proven pathophysiology in rheumatoid arthritis (RA) and the intended action of these agents is highly likely the explanation for the observed efficacy (Case, 2001). 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 pyrimidine pathway prevent the proliferation of activated T cells, which are thought to play an important role in the pathogenesis of RA (Emery, 2000). 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 (Furst and Munster, 2001).

EXPERIMENTAL MODELS

1. Inflammatory models

Ethyl phenylpropiolate (EPP)-induced ear edema in rats: Edema is a cardinal sign of acute inflammation. Thus, edema is a useful parameter to look at when testing for agents which may be active in treating acute inflammation (Sedgwick and Willoughby, 1989). 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 small amount of a test substance is needed. By using edema inducer (EPP), the mechanism involved can be suggested. Kinins, serotonin and PGs are released in EPP induced ear edema (Brattsand *et al.*, 1982).

Carrageenin-induced paw edema in rats: Carrageenin-induced hind paw edema in rats was first introduced by Winter *et al.* (1962). This model has become common as a test for anti-inflammatory activity.

The hind paw edema has been analyzed by a variety of different workers and it has been found that the reaction depends upon the activation of the complement system. The depletion of the amount of complement will cause a striking suppression of the reaction. The edema is produced by a sequential release of pharmacological mediators; histamine, serotonin, kinins and PGs (Vinegar *et al.*, 1969; Di Rosa *et al.*, 1971). 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 the 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 (Sedgwick and Willoughby, 1989).

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 (DiMartino *et al.*,1987). The choice of AA is based on the knowledge that LTs are involved in the formation of edema when AA is used as inducer (Yong *et al.*, 1984).

Since AA-induced ear inflammation in mice has been reported to be sensitive in detecting the anti-inflammatory acting of lipoxygenase inhibitor (Yong *et al.*, 1984; Carlson *et al.*, 1985), this model was initiated to characterize the model of AA-induced rat paw edema and evaluates its sensitivity to lipoxygenase inhibitors and other anti-inflammatory agents (DiMartino *et al.*, 1987).

AA-induced hind paw edema in rats by subplantar injection produces a severe edematous response. One of the unique aspects of AA-induced rat paw edema model, lipoxygenase metabolites, especially LTs, have an important role in producing vascular permeability and edema formation whereas COX products have low or no activity. Moreover, 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 (DiMartino *et al.*, 1987).

Cotton pellet-induced granuloma formation in rats: Meire *et al.* (1950) 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 occurred during the first three hours after implantation (2) an exudative phase, defined as a leakage of fluid from the bloodstream 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 (Swingle and Shideman, 1972). 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 (Bessy *et al.*, 1946): Alkaline phosphatase is a lysosomal enzyme. It is widely distributed in many tissues, including the osteoblasts (the bone-building cells), the cell lining the sinusoids and bile

canaliculi in the liver. It is reported that the activity of alkaline phosphatase in serum was 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 will indicate the activity of agents on the production and release of alkaline phosphatase (Nishikaze *et al.*, 1980).

2. Ulcerogenic model

Chronic administration of nonselective COX inhibitors (e.g. aspirin) or steroids (e.g. prednisolone) are known to induce gastric ulceration (Hawkey, 1999; Davies *et al.*, 2000; Furst and Munster, 2001). The reason being attributed principally to the inhibition of biosynthesis of 'cytoprotective PGs' (e.g. PGEs and PGI_2) by inhibiting the constitutive COX-1 enzyme of AA metabolism. As described by Valle and Todisco (1999), 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 there has been reported that a number of selective COX-2 inhibitors have low gastric toxicity (Hawkey, 1999), 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.

Test substance was administered once daily for 7 days and its effect on the gastric mucosa was compared with aspirin and steroid.

3. Analgesic models

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 anti-inflammatory, analgesic and antipyretic actions of NSAIDs (Milton, 1982; Furst and Munster, 2001). Therefore, it is interesting to investigate the analgesic activity of test drugs possessing anti-inflammatory activity.

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 (Collier *et al.*, 1968). The inhibitory effect of a substance on writhing response in this test was found to be well correlated with clinical results in humans (Taber *et al.*, 1969).

4. Antipyretic models

Yeast-induced hyperthermia: 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 responsible for endogenous pyrogen release (Atkins and Bodel, 1974). 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 (Milton, 1982). It is probable that these cells play an important role in fever associated with disease where chronic inflammation is a prominent feature (Atkins and Bodel, 1974).

The pyrexia induced in rat by subcutaneous injection of brewers yeast, has been used to determine antipyretic activity of many compounds (Teotino *et al.*, 1963). The pyrexia reaches its peak at 18 h after induction and the assessment is also made at this period. It has been postulate that many chemical neuromediators are involved in hypothalamic regulation of body temperature (Martin *et al.*, 1988). Milton (1982) 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 *Clerodendrum petasites* S. Moore.

Clerodendrum petasites S. Moore, commonly known in Thailand as "Thao yaai mom", belongs to the family Verbenaceae (Figure 2). *C. petasites* is widely grown in India, Malaysia and Thailand. It is erect, shrubs or herbs, 3 to 5 ft high, often with square stem. The leaf is 15 to 20 cm long and 1.5 to 2.5 cm wide, usually opposite and rarely alternate or whorled, mostly simple. Flowers are long tubes with white color, calyx is cup shaped, typically 5-lobed. The fruit is a drupe (or berry) with a large kernel.

In Thai folklore medicine, its leaf and root are traditionally used for the treatment of fever (Mokkhasmit *et al.*, 1976; Tiangburanatham, 1996), inflammation and skin diseases as well as asthma (Pongboonroud, 1976; Tiangburanatham, 1996). In India, a mixture of the fruits of *C. petasites* is used to produce sterility (Lal and Lata, 1980), whereas Chineses use it (part not specified) for the treatment of fever and malaria (Pie, 1985).

There are several phytochemical, biological and pharmacological studies of *C. petasites*. *C. petasites* has been found to yield several compounds including steroids (Prakash and Garg, 1981; Akihisa *et al.*, 1989) and flavonoids (Aziz Abdur Rahman *et al.*, 2000). In 1976, Mokkhasmit *et al.* observed acute toxicity of crude ethanol extract of *C. petasites* in mouse and found no toxicity after an subcutaneous administration 10 g/kg of extract. Anyhow, Bhakuni *et al.* (1969) found that it has toxic effect when it was administered intraperitoneally.

From the work of Aziz Abdur Rahman *et al.* (2000), two flavonoid compounds, which isolated from the stem and root of the plant, were screened against twelve pathogenic bacteria for their antibacterial activities. The test materials found to be significant *in vitro* antibacterial activities against almost all gram positive and gram negative bacteria. In addition, Chatluang (2000) had studied bronchodilator activity of the ethanol extract from the plant of *C. petasites* and found that the extract possessed the bronchodilator effect. Although the mechanism of action was unclear but it seemed that this relaxant effect was not caused by β-adrenergic receptor stimulation or muscarinic receptor inhibition. Moreover, the results obtained from the Hippocratic -

Figure 2. "Thao yaai mom"

(*Clerodendrum petasites* S. Moore., family Verbenaceae.)

screening test showed that intraperitoneal injection of high doses (2.5 - 5 g/kg) of the ethanol extract caused some changes in various behaviors such as a decrease of respiratory and motor activity, slight loss of righting reflex and screen grip.

PURPOSE OF THE STUDY

The purpose of the present study was to evaluate the anti-inflammatory, analgesic and antipyretic activities as well as the dose-response relationship of the methanol extract from *C. petasites* 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 *C. petasites* on the inflammatory process and the ulcerogenic activity were also examined in comparison to some nonsteroidal and steroidal anti-inflammatory drugs.