INTRODUCTION

INFLAMMATION

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

Commonly, inflammation occurs as a defensive response to invasion of the host. Inflammation can be thought to proceed as follows:

1. **Initiation** of the mechanisms responsible for the localization and clearance of foreign substances and injured tissues is stimulated by the recognition that injury to tissues has occurred.
2. **Amplification** of the inflammatory response, in which both soluble mediators and cellular inflammatory systems are activated, follows recognition of injury.
3. After generation of inflammatory agents and elimination of the foreign agent, **termination** of the inflammatory response is accomplished by specific inhibitors of the mediators (Fantone and Ward, 1999; Gallin and Snyderman, 1999).

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 (Fantone and Ward, 1999). Excessive inflammation caused by abnormal recognition of host tissue as foreign or prolongation of the inflammatory process may lead to inflammatory diseases. Diseases characterized by inflammation are an important cause of morbidity and mortality in humans. The centrality of the inflammatory response in these varied

disease processes makes its regulation a major element in the prevention, control or cure of human disease (Gallin and Snyderman, 1999).

Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms; acute, chronic and granulomatous inflammation (Fantone and Ward, 1999). Acute inflammation is the initial response to tissue injury; it is mediated by the release of autacoids. Some of the autacoids involved are histamine, serotonin, bradykinin, prostaglandins and leukotrienes (Katzung and Furst, 1998). Acute inflammation is characterized by the classic signs of pain, heat, redness, swelling and 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, especially polymorphonuclear leukocytes (PMNs) into the inflammatory focus (Fantone and Ward, 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 persist (Bowman and Rand, 1980).

Chronic inflammation is a long-lived reaction, the reaction persisting for weeks or months after the initial exposure to the damaging agent (Hurley, 1983). 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 cellular components of the inflammatory response are (1) macrophages, (2) plasma cells, (3) lymphocytes and (4) eosinophils. Chronic inflammation is mediated by both immunological and nonimmunological mechanisms and is frequently observed in conjunction with reparative responses, namely, granulation tissue and fibrosis (Fantone and Ward, 1999). Chronic inflammation involves the release of a number of mediators that are not prominent in the acute response. Some of these are interleukins 1, 2 and 3 (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 (Katzung and Furst, 1998).

Granulomatous inflammation is typical of the tissue response elicited by indigestible substances such as fungal infections, tuberculosis, leprosy, schistosomiasis, and the presence of foreign material (e.g., suture or talc). The principal cells involved in granulomatous inflammation are macrophages and lymphocytes. It is characteristically associated with epithelioid cells and multinucleated giant cell. Some diseases of unknown etiology, especially sarcoidosis, are distinguished by florid granulomatous inflammation, although the inciting agent is not apparent (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 and Laidlaw (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 others.
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. Agents 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:

1. Vasoactive amines

Histamine

Histamine is widely distributed in tissues, the richest source being the mast cells that are normally present in the connective tissue adjacent to blood vessels. It is also found in blood basophils and platelets. Preformed histamine is present in mast cell granules and is released by mast cell degranulation 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) 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). Its important role in acute inflammation is associated with mast cell degranulation in man but is relatively unimportant in the later stages of the inflammatory response. Thus, inhibition of histamine response delays but does not prevent the inflammatory response. The acute inflammatory response to histamine comprises vasodilatation, an increase in microvascular permeability and edema formation, however there are other effects, such as stimulation of sensory nerve endings, especially those mediating pain and itching (Owen, 1987; Cotran *et al.*, 1994). In additional, histamine exerts powerful effects on smooth and cardiac muscle, on certain endothelial and nerve cells, and on the secretory cells of the stomach (Katzung and Julius, 2001).

Serotonin (5-Hydroxytryptamine)

Serotonin (5-Hydroxytryptamine) or 5-HT is a second preformed vasoactive mediator with actions similar to those of histamine. It is present in platelets and enterochromaffin cells, and in mast cells in rodents but not humans (Cotran *et al.*, 1994). Serotonin causes increased gastrointestinal motility and contraction of

isolated strips of intestine. Large vessels, both arteries and veins, are usually constricted by 5-HT, though the sensitivity varies greatly. This is a direct action on vascular smooth muscle cells, mediated through 5-HT_{2A} receptors. 5-HT can also cause vasodilatation by several mechanism, all operating through 5-HT_1 receptors. In the microcirculation, 5-HT cause dilatation of arterioles, together with constriction of venules, with the result that capillary pressure rises and fluid escapes from the capillaries (Rang *et al.*, 1995).

2. Plasma proteases

The kinin system

The kinin system generates vasoactive peptides from plasma proteins called kininogens by specific proteases called kallikreins (Collins, 1999). 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 tissues. Bradykinin is a nonapeptide. Kallidin has an additional lysine residue at the amino-terminal position and is sometimes referred to as lysylbradykinin. The two peptides are cleaved from $\alpha 2$ globulins that are synthesized by the liver and circulated in the plasma. These peptides are autacoids that act locally to produce pain, vasodilatation, increased vascular permeability, and the synthesis of prostaglandins. Thus, they comprise a subset of the large number of mediators that contribute to the inflammatory response (Babe and Serafin, 1996). The action of bradykinin is short-lived because it is quickly inactivated by an enzyme called kininase (Collins, 1999). Kinins may also modulate migration of white blood and tissue cells that take part to the inflammatory process. Several of the biological effects of bradykinins are mediated by endogenous agent such as prostaglandins and histamine and/or 5-hydroxytryptamine (Regoli, 1987). There are at least two distinct receptors for kinins, which have been designated B_1 and B_2 (Babe and Serafin, 1996). B_2 receptors mediate a large number of rapidly occurring biological effects, particularly the symptoms and signs of inflammation, while B_1 receptors appear to

be involved in some retarded, long lasting effects of kinins such as collagen synthesis and cell multiplication (Regoli, 1987). 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 nonspecifically by inhibitors of prostaglandin synthesis (Reid, 1998).

The complement system

The complement system consists of a group of 20 plasma proteins which plays an important role in many immune defense reactions. Complement components present as inactive form in plasma are numbered C1 through C9. The proteins involved in the activation are sequentially activated by three convergent pathways, termed classical, alternative, and lectin binding. The classical pathway is initiated by antigen-antibody immune complexes. The cascade that leads from activation to the formation of the membrane attack complex (MAC). The activation of the classical pathway requires recognition of the inflammatory agent by the first component of complement, C1, which consists of three separate proteins, C1q, C1r and C1s. The activation of the lectin-binding pathway is similar to the classical pathway and results from the binding of a serum protein, mannose-binding protein (MBP), which is synthesized in the liver and the structure of the molecule is similar to C1q. The alternative pathway is typically activated by derivative products of infectious organisms and by foreign materials through a cascade-like interaction of specific plasma proteins called the properdin system (properdin [P], factor B and D). The functional roles of the complement system are as follows:

(1) A source of vasoactive mediators: C3a, C4a and C5a are anaphylatoxins. They increase vascular permeability and have potent effects on smooth muscle contraction by releasing histamine from mast cells. C5a also activates the lipoxygenase pathway of arachidonic acid (AA) metabolism in

neutrophils and monocytes, causing further release of inflammatory mediators.

(2) The production of leukocyte chemoattractants: C5a is a potent chemotactic factor for neutrophils, monocytes, eosinophils and basophils.

(3) Enhanced leukocyte phagocytosis (opsonization): C3b and its degradation products (e.g. iC3b) when fixed to the bacterial cell wall, act as an opsonin and favor phagocytosis by neutrophils and macrophages, which bear cell surface receptors for C3b.

(4) Cell lysis: The insertion of the C5b-C9 or MAC into the plasma membrane creates a cylindrical hole, an effect that destroys the barrier function of the plasma membrane and leads to cell lysis.

The role of the complement system is normally beneficial but when the immune system operates inappropriately, as in some hypersensitivity reactions, complement may contribute to the ensuing tissue damage (Jose, 1987; Cotran *et al.*, 1994; Fantone and Ward, 1999).

3. Arachidonic acid (AA) metabolites

AA is a 20-carbon polyunsaturated fatty acid (5,8,11,14-eicosatetraenoic 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. AA metabolites, also called eicosanoids are synthesized by two major classes of enzymes: cyclooxygenases (COX) and lipoxygenases (Collins, 1999). The scheme of the major metabolic transformations of arachidonic acid is shown in Figure 1.

The cyclooxygenase pathway, mediated by two different enzymes (COX-1 and COX-2), leads to generation of prostaglandins (PGs) (Collins, 1999). COX-1, also called constitutive COX, is present in the platelets, endothelium, kidney and stomach mucosa whereas COX-2, also called inducible COX, may be induced by an inflammatory stimulus in macrophages or other cells

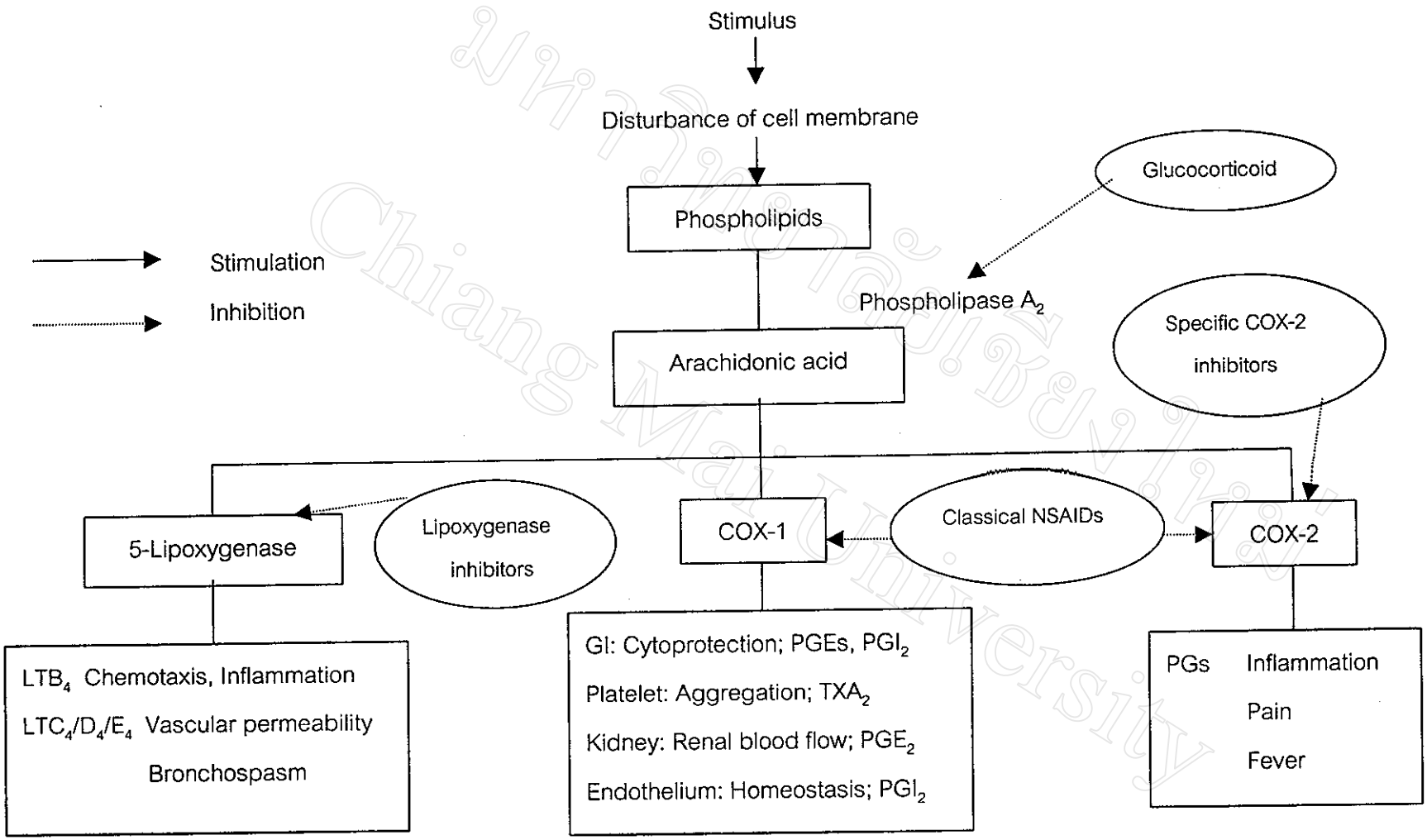

Figure 1. Scheme of the major metabolic transformations of arachidonic acid

(Antonio and Souza Brito, 1998). COX-1 and COX-2 differ in their sensitivity to inhibition by certain anti-inflammatory drugs. Selective inhibition of COX-2 may be of therapeutic advantage, since this isozyme is probably involved in prostaglandin production at the site of inflammation but not at other sites such as the gastrointestinal tract and kidney. Thus, an inhibitor of COX-2 may be anti-inflammatory drug without the side effects of reducing renal function or producing gastric ulcerations (Campbell and Halushka, 1996).

Prostaglandins are divided into series based on structural features as coded by a letter (PGD, PGE, PGF, PGG and PGH) and a subscript numeral (e.g., 1, 2), that indicates the number of double bonds in the compound. The most important ones in inflammation are PGE_2, PGD_2, $PGF_{2\alpha}$, prostacyclin (PGI_2), and thromboxane A_2 (TXA_2) (Collins, 1999). Prostaglandins have been detected in almost all experimental models of inflammation studied and in the synovial fluid of patients with arthritis. In most cases, the major prostaglandins found are PGE_2 and PGI_2 (Griffiths, 1999). Both PGE_2 and PGI_2 markedly enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed region in combination with 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 polymorphonuclear leukocytes (PMNs) (Salmon and Higgs, 1987; Campbell and Halushka, 1996). TXA_2 is a major product of arachidonic acid metabolism in platelets, which promotes platelet aggregation and vasoconstriction (Campbell and Halushka, 1996).

The lipoxygenase pathway mediated by three different lipoxygenases (Collins, 1999). 5-lipoxygenase is the most important of these enzymes. It leads to the synthesis of the leukotrienes (LTs) (Campbell and Halushka, 1996). The LTs can be divided, on the basis of their chemical structures and pharmacological actions into LTB_4, LTC_4, LTD_4, LTE_4 and LTF_4 (Piper and Samhoun, 1987). LTB_4 is

the potent chemotactic agent and activator of neutrophil functional responses, such as aggregation and adhesion of leukocytes to venular endothelium and release of lysosomal enzymes. The cysteinyl-containing leukotrienes C_4, D_4 and E_4 (LTC_4, LTD_4 and LTE_4) known as the "slow-reacting substance of anaphylaxis" (SRS-A), cause intense vasoconstriction, bronchospasm, and increased vascular permeability (Campbell and Halushka, 1996; Collins, 1999).

4. Platelet-activating factor (PAF)

PAF is another bioactive phospholipid-derived mediator. Its biosynthesis involves acetylation of a precursor released from membrane phospholipids by activated phospholipase A_2 (Vargaftig and Braquet, 1987; Collins, 1999). Like the eicosanoids, PAF is not stored in cell but reality synthesized in response to stimulation (Campbell and Halushka, 1996). A variety of cell types, including platelets, basophils (and mast cells), neutrophils, monocytes/ macrophages, and endothelial cells, can elaborate PAF, in both secreted and cell-bound forms. In addition to platelets stimulation, PAF causes vasoconstriction and bronchoconstriction, and at extremely low concentrations it induces vasodilation and increases venular permeability with a potency 100 to 10,000 times greater than that of histamine (Collins, 1999). 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 leukotrienes) rather than mast cell (Abbas *et al.*, 1997). PAF also causes chemotaxis, degranulation, and the oxidative burst. Thus, PAF can elicit most of the cardinal features of inflammation (Collins, 1999). Signs and symptoms of inflammation include increased vascular permeability, hyperalgesia, edema and infiltration of neutrophils (Campbell and Halushka, 1996; Collins, 1999).

5. Cytokines

Cytokines are proteins produced by many cell types (principally activated lymphocytes and macrophages, but also endothelium, epithelium, and connective

tissue cells) 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. Cytokines can be grouped into five classes, depending on their major function or on the nature of target cell (Collins, 1999).

(1) Cytokines that regulate lymphocyte function: These cytokines regulate lymphocyte activation, growth, and differentiation. Within this category are IL-2 and IL-4, which favor lymphocyte growth, as well as IL-10 and tumor growth factor beta (TGF-β), which are negative regulators of immune responses.

(2) Cytokines involved with natural immunity: This group of cytokines includes two major inflammatory cytokines, TNF-α and IL-1β; type I interferons (IFN-α and IFN-β); and IL-6.

(3) Cytokines that activate inflammatory cells: These cytokines activate macrophages during cell-mediated immune responses and include IFN-γ, TNF-α, TNF-β (lymphotoxin), IL-5, IL-10, and IL-12.

(4) Chemokines: This group of cytokines is characterized by chemotactic activity for various leukocytes. In addition to their roles in regulating leukocytes recruitment and activation, chemokines act on stromal cells such as fibroblasts and smooth muscle cells as well as hematopoietic progenitor cells.

(5) Cytokines that stimulate hematopoiesis: These mediate immature leukocyte growth and differentiation. Examples include IL-3, IL-7, granulocyte-macrophage colony-stimulating factor (GM-CSF).

The major cytokines that mediate inflammation are IL-1 and TNF (Collins, 1999). 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, 1996). 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, 1996). On occasions, IL-1 or TNF may be the sole mediators; it seems more likely that the cytokines act in concert with other classes of inflammatory mediators in defense of the host. Where the cytokines differ from so many other classes of inflammatory mediators, however, is in their potential to mediate the tissue destruction of chronic diseases such as rheumatoid arthritis (Billingham, 1987).

ANTI-INFLAMMATORY DRUGS

The treatment of patients with inflammation 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 the tissue-damaging process. Anti-inflammatory drugs can be divided into nonsteroidal anti-inflammatory drugs (NSAIDs), anti-inflammatory corticosteroids and disease modifying anti-rheumatic drugs (DMARDs) (Furst and Munster, 2001).

1. Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are widely used for a large number of painful and inflammatory conditions (Hawkey, 1998). There is a marked variation in the response of individuals. One patient does achieve therapeutic benefit from one NSAIDs but it may not have a therapeutic effect on another patient (Payan and Katzung, 1995). NSAIDs provide only symptomatic relief from the pain and inflammation associated with the disease and do not arrest the progression of pathological injury to tissue during severe episodes (Insel, 1996). Most currently available NSAIDs inhibit both COX-1 (constitutive) and COX-2 (induced in settings of inflammation) activities. The inhibition of COX-2 is the antipyretic, analgesic, and anti-inflammatory action of NSAIDs. The simultaneous inhibition of COX-1 results in unwanted side effects, particularly leading to gastric ulcers, correlated with inhibition of the biosynthesis of gastric prostaglandins, especially PGI_2 and PGE_2, that serve as cytoprotective

agents in the gastric mucosa (Insel, 1996; Frolich, 1997). Drugs that have the highest COX-2 activity and a more favorable COX-2 : COX-1 activity ratio will have a potent anti-inflammatory activity with fewer side-effects than drugs with a less favorable COX-2 : COX-1 activity ratio (Vane and Botting, 1998). Hence, there has been an active search for "better" NSAIDs that have high anti-inflammatory efficacy with minimal side effects (Wu, 1998). The identification of selective inhibitors of COX-2 will therefore lead to advances in therapy (Vane and Botting, 1998). On the other hand, the search for a safe anti-inflammatory drug that is free from gastric intolerance continues unabated and a part of such research is the evaluation of medicinal plants known to be used for the treatment of inflammatory disorders (Singh *et al.*, 1989).

One can now distinguish the following three major groups of NSAIDs on the basis of their inhibitory activity on COX-1 and COX-2 (Frolich, 1997; Hawkey, 1999).

(1) **Nonselective COX**, these drugs similarly inhibit both COX-1 and COX-2. This category includes aspirin, indomethacin, piroxicam, diclofenac and ibuprofen.

(2) **COX-2 preferential**, these drugs inhibit COX-2 rather than COX-1, for examples meloxicam and nimesulide.

(3) **COX-2 specific**, these drugs inhibit COX-2 but have no effect on COX-1, such as celecoxib and rofecoxib.

Moreover, various NSAIDs have additional possible mechanisms of action such as indomethacin have been reported to reduce the synthesis of both prostaglandins and leukotrienes and may also inhibit phospholipase A and C, reduce PMN migration, and decrease T cell and B cell proliferation. In addition, aspirin also interferes with the chemical mediators of the kallikrein system. As a result, aspirin inhibits granulocyte adherence to damaged vasculature, stabilizes lysosomes, and inhibits the migration of polymorphonuclear leukocytes and macrophages into the site of inflammation (Furst and Munster, 2001).

2. Anti-inflammatory corticosteroids

Corticosteroids are the hormones secreted by the adrenal cortex. They are classified into glucocorticoids, which have pronounced effects on carbohydrate and protein metabolism and also anti-inflammatory actions, and mineralocorticoids, which are important in water and electrolyte balance (Flower and Dale, 1989). Glucocorticoids such as prednisolone are known to 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 arachidonic acid (Foegh *et al.*, 1998). In addition, glucocorticoids have recently been shown to selectively inhibit the expression of COX-2 (Furst and Munster, 2001). It is now clear that glucocorticoids inhibit the production by multiple cells of factors that are critical in generating the inflammatory response. As a result, there is decreased release of vasoactive and chemoattractive factors, diminished secretion of lipolytic and proteolytic enzymes, decreased extravasation of leukocytes to areas of injury, and ultimately, decreased fibrosis (Schimmer and Parker, 1996). The glucocorticoids have powerful anti-inflammatory effects. Unfortunately, the toxicity associated with chronic corticosteroid therapy inhibits their use except in the control of acute flare-ups of joint diseases (Furst and Munster, 2001).

3. Members of the group slow-acting antirheumatic drugs (SAARDs) or DMARDs

Rheumatoid arthritis is an immunologic disease that causes significant systemic effects shorten life in addition to joint disease. Very little is known about the mechanism of action of DMARDs on rheumatoid arthritis, but they may slow the bone damage and are thought to affect more basic inflammatory mechanism than do the NSAIDs. Unfortunately, they may also be more toxic than the NSAIDs. The effects of DMARDs therapies may take 6 weeks to 6 months to become evident. These therapies include methotrexate, azathioprine, penicillamine,

hydroxychloroquin and chloroquin, organic gold compounds, sulfasalazine, leflunomide, TNF-blocking agents, and immunoadsorption apheresis. Considerable controversy surrounds the long-term efficacy of these drugs. 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 (Furst and Munster, 2001).

EXPERIMENTAL MODELS

1. Inflammatory models

Various animal models of inflammation are used to evaluate the anti-inflammatory activity of test agents and to investigate the mechanism on the inflammatory process. Although many such models are employed in anti-inflammatory research, none of models provides adequate simulation of the pathological event underlying the clinical disorder. From this point of view, in order to predict the therapeutic effectiveness of test substances, use should be made concomitantly of several models, which together can mimic a broad spectrum of acute and chronic inflammatory events (Gryglewski, 1977).

Carrageenin-induced hind paw edema in rats: Edema is a useful parameter to look at when testing for agents which may be active in treating acute inflammation (Sedgwick and Willoughby, 1989). This model is useful in detecting orally active anti-inflammatory agents (Olajide, 1999). There is good correlation between the responsiveness to anti-inflammatory drugs shown by carrageenin-induced paw edema in rats and rheumatoid polyarthritis in humans. Therefore, this assay has been accepted to serve as a preliminary screening test for anti-rheumatic activity (Vinegar *et al.*, 1969). The main advantage of this model is the ease and reproducibility of the system (Sedgwick and Willoughby, 1989). Many phlogistic agents including formalin, kaolin, dextran, etc. have been used to induced paw edema. Edema produced by these irritants is not specifically influenced by anti-inflammatory compounds. Therefore, Winter *et al.* (1962) have concluded that the phlogistic agent of choice for testing anti-inflammatory drugs is

carrageenin. Carrageenin is a sulphate polysaccharide which has been fractionated with potassium chloride into two separate components, kappa and lambda carrageenin. The lambda carrageenin is more active in eliciting both acute and chronic inflammatory responses (Di Rosa, 1972). The other advantage of carrageenin-induced edema in comparison with the edema elicited by other phlogistic agents, is its responsiveness to doses of all clinical used anti-inflammatory drugs well below the toxic level with the degree of edema inhibition in a dose-related manner (Winter *et al.*, 1962).

Carrageenin-induced pleurisy in rats: Carrageenin-induced pleurisy in rats is an acute inflammatory model which is characterized by fluid and leukocyte accumulation associated with extravasation of plasma proteins (Capasso *et al.*, 1975; Lo *et al.*, 1981). It has been suggested that the pleural models can differentiate steroid and non-steroid activity (Sedgwick and Willoughby, 1989). The pleurisy model has been accepted as a reliable method to study inflammation allowing the determination of several parameters simultaneously. The parameters such as (1) measurement of exudate volume (2) measurement the white blood cell number in the exudate using a Coulter counter or a hematocytometer (3) determination of lysosomal enzyme activity (4) determination of PGE_2 (Vogel and Vogel, 1997a).

Cotton pellet-induced granuloma formation in rats: This method has been widely employed to evaluate the transudative, exudative and proliferative phases of chronic inflammation (Swingle and Shideman, 1972). It is a test, which reflects the effect of a drug on cell migration and proliferation (Sedgwick and Willoughby, 1989). The response to a subcutaneously implanted cotton pellet in rat has been divided into three phases. A transudative phase, a fluid that is low in protein and is noninflammatory in origin, defined as the increase in wet weight of the pellet which occurred during the first three hours after implantation. 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. A proliferative

phase, measured as the increases in dry weight of the granuloma which occurs between three and six days after implantation (Swingle and Shideman, 1972). Moreover, the body weight gain and dry thymus weight can be recorded to evaluate the mechanism of action of test substance in comparison with prednisolone and aspirin.

Measurement of the alkaline phosphatase activity in serum: In the cotton pellet-induced granuloma formation model, serum alkaline phosphatase activity can also be assessed. Alkaline phosphatase is a lysosomal enzyme. It has been reported by Nishikaze *et al.* (1980) that alkaline phosphatase activity in pouch wall was elevated during cotton pellet granuloma formation on the seventh day and decreased on the fourteenth day when healing occurred. The role of lysosomal enzymes as mediator of inflammation is well documented (Ismail *et al.*, 1997). In rheumatoid arthritis the erosion of cartilage matrix appears to be due to the release of lysosomal enzymes from the inflammatory cells of the invading pannus (Weissmann, 1965a). Measurement of alkaline phosphatase activity in serum of this group of rats will indicate the activity of agents in protecting lysosomal membrane system during chronic inflammation (Ismail *et al.*, 1997).

2. Ulcerogenic model

As most of anti-inflammatory drugs possess a common side effect, i.e. gastric ulcer. It is therefore reasonable to investigate the ulcerogenic effect of substances possessed anti-inflammatory activity. The model modified from the method of Murakami *et al.* (1982) and Saxena *et al.* (1987) is suggested to be used for studying of ulcerogenic effect of substances. Vogel and Vogel (1997b) found that there is a good correlation between gastro-intestinal side effects in man and the ulcerogenic effects in rats.

3. Anti-ulcerogenic models

Many plants with anti-inflammatory effect have been found to possess anti-ulcerogenic activity. It is therefore of interest to study this effect of substances with anti-inflammatory property. Various mechanisms may be associated with the formation of gastric mucosal damage in experimental models used to study anti-ulcer drugs in rats (Antonio and Souza Brito, 1998).

Indomethacin-induced gastric lesions: NSAIDs like indomethacin, is known to induce ulceration through inhibition of the cyclooxygenase pathway. Consequently, leading to inhibition of "cytoprotective prostaglandins" which is responsible for mucus production. It has suggested that agents, which exhibited an anti-ulcer activity in this model could be due to an enhancement of mucosal defensive factors such as gastric mucus (Pal and Nag Chaudhuri, 1991; Antonio and Souza Brito, 1998).

Pyrolus ligation: This model is used for evaluation of anti-secretory activity based on ligature of the pylorus has been published by Shay *et al.* (1945). The ligation caused an accumulation of intraluminal HCl as well as gastric secretion. Thus, the gastric volume, gastric secretory rate and total acidity can be determined. It has been postulated that histamine might be involved in the formation of pylorus-ligated ulcers and play a mediating role in the gastric secretion stimulated by gastrin, vagal excitation and cholinergic agents. It has suggested that agents, which exhibited an anti-ulcer activity in this model could be due to histamine inhibition (Antonio and Souza Brito, 1998).

LITERATURE REVIEW OF *Diospyros variegata* KRUZ.

Nowadays, medicinal plants may represent a useful source of new effective therapeutic agents (Sautebin, 2000). Various researchers have reported about many medicinal plants that have anti-inflammatory and anti-gastric ulcer activities, for example galangal (*Alpinia galanga* (Linn.) Sw.) (Mitsui *et al.*, 1976; Tseng *et al.*, 1992) and ginger (*Zingiber officinalis* Roscoe) (Kiuchi *et al.*, 1983; Yamahara *et al.*, 1988). The anti-gastric ulcer effects of these plants are postulated that they have

cytoprotective properties. Some species of *Diospyros* are widely used for different types of inflammatory diseases and gastric ulcer. The bark of *D. candolleana* is used for rheumatism and swellings, the bark of *D. paniculata* is used for rheumatism and ulcers, *D. tomentosa* is used for bronchitis and pleurisy; and the leaves of *D. leucomelas* showed anti-inflammatory activity in the carrageenan and serotonin paw edema tests and TPA and EPP ear edema tests (Mallavadhani *et al.*, 1998).

The present study on *Diospyros variegata* Kruz. was carried out because it is used in Thai folklore medicine for management of pain and inflammatory conditions (Pongboonroud, 1976). *D. variegata* belongs to the family Ebenaceae. Its Thai name is Phayaa-raak-dam. The tree is up to 20 m high. The leaves are simple, alternate, dark green, oval-shaped, 5-11 cm wide and 15-30 cm long. The flowers are yellow and solitary. The berry fruit is green and turn black after ripening. The leaves and fruit are illustrated in Figure 2. *D. variegata* is widely distributed in many regions of Thailand, especially in Sara Buri and Lop Buri province (Pongboonroud, 1976; Pupattanapong, 1987).

In Thai folklore medicine, stems of *D. variegata* are boiled in water and the water extract is heated until dark thick mass is formed, which is cooled down and sliced into pieced. A decoction of these slices is used as a tea for the management of pain and inflammatory conditions (Pongboonroud, 1976).

Pharmacological study of the extract from the root of *D. variegata* revealed that the extract did not possess antihistaminic and antispasmodic activity when tested using isolated ileum of guinea-pig and had no hypotensive action in anesthetized dogs (Mokkhasamit *et al.*, 1971a). In addition, phytochemical study of the root of *D. variegata* showed that the plant did not contain any alkaloid substance (Congdon *et al.*, 1981).

Mokkhasamit *et al.* (1971b) observed acute toxicity of the crude methanol extract of *D. variegata* in mice and found no toxicity after an oral and intraperitoneal administration of different doses i.e. 1,3 and 10 g/kg. The dose of

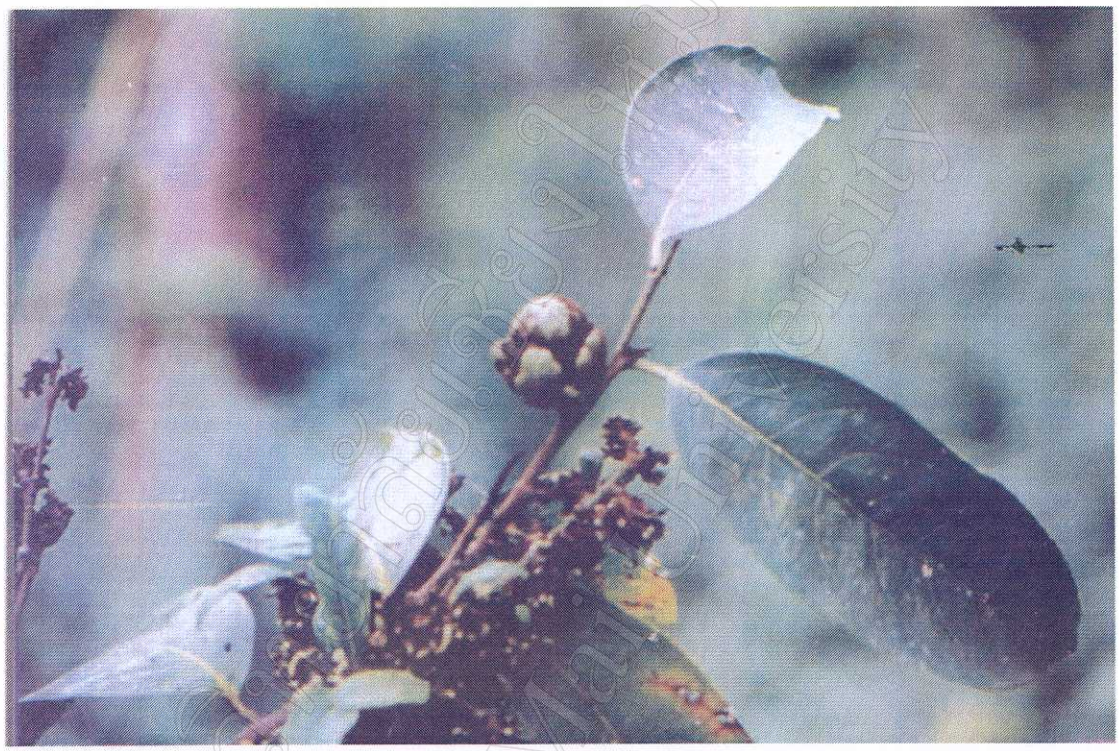

Figure 2. Leaves and fruit of *Diospyros variegata* Kruz.

10 g/kg in mice is about 200,000 times of the dose used in humans in Thai traditional practice (0.05 mg/kg).

The analgesic, antipyretic and anti-inflammatory activities of methanol and hexane extracts from the stem of *D. variegata* were assessed (Trongsakul, 1999). In analgesic test, the hexane extract of *D. variegata* possessed the same inhibitory intensity as aspirin on acetic acid-induced writhing response but elicited only a weak effect on the tail-flick response and on the early phase of formalin test when compared with morphine. The results obtained rather suggest the peripheral mechanism of analgesic activity of the hexane extract from *D. variegata*. The mechanism of analgesic action of the hexane extract is probably due to the inhibition of prostaglandin biosynthesis, since PGs, especially PGE_2 and $PGF_{2\alpha}$, are capable of sensitizing pain receptors. This postulation is supported by the marked inhibitory activity of the hexane extract on licking which occurred in the late phase of the formalin test. In addition, the hexane extract possessed on excellent antipyretic effect when tested using yeast-induced hyperthermia in rats. The hexane extract also elicited anti-inflammatory action when tested in ethyl phenylpropiolate (EPP) and arachidonic acid-induced rat ear edema. The above results suggest the inhibitory effect of the hexane extract on cyclooxygenase and lipoxygenase pathway of arachidonic acid metabolism. On the contrary, the methanol extract of *D. variegata* did not possess any analgesic, antipyretic or anti-inflammatory activities. Therefore, it is possible that the nonpolar substances present in the stem of *D. variegata* are responsible for these effects (Trongsakul, 1999).

PURPOSE OF THE STUDY

The anti-inflammatory activity of the hexane extract from the stem of *D. variegata* on skin inflammation has been reported (Trongsakul, 1999). Therefore, it is of interest to study the anti-inflammatory activity in detail using various animal models. The goal of this study was to

a) evaluate the anti-inflammatory activity of the hexane extract from the stem of *D. variegata* in both acute and subchronic inflammatory models.

b) examine the possible the mechanism(s) of action mediating anti-inflammatory activity of the hexane extract from the stem of *D. variegata* in comparison with some nonsteroidal and steroidal anti-inflammatory drugs.

c) evaluate the effect on gastric mucosa of the hexane extract from the stem of *D. variegata*.

d) evaluate the anti-ulcerogenic activity and examine the possible the mechanism(s) of action mediating anti-ulcerogenic activity of the hexane extract from the stem of *D. variegata* in comparison with reference drugs.