CHAPTER IV

DISCUSSION

Prevention of articular cartilage destruction has long been a goal in the treatment of arthritis. Normal tissue turnover can be viewed as a balance between degradation and synthesis of the macromolecules that constitute the extracellular matrix (ECM). This process is tightly regulated because highly degradative proteinases are controlled at several levels, including synthesis, secretion, activation, and inhibition. Tissue destruction occurs when proteinase-mediated degradation exceeds synthesis, which is markedly influenced by cytokines and growth factors that stimulate matrix synthesis. Metalloproteases, a family of zinc-dependent enzymes, and more specifically the matrix metalloproteases (MMP), play a premier role in joint articular tissue degeneration (22;24).

The chondrocytes synthesize a number of proteinases, which are capable of degrading the component molecules of this specialized extracellular matrix. So the cartilage explants were used in this study as a model to investigate the catabolism of ECM biomolecules such as sulfated-glycosaminoglycan, uronic acid and hyaluronan, which were activated by retinoic acid (RetA) or interleukin-1β (IL-1β).

RetA, a naturally occurring vitamin A metabolite, promotes cartilage degradation *in vitro*. It mediates its effects primarily through binding to RetA receptor, nuclear receptors that are members of the steroid/thyroid hormone receptor superfamily. A previous study showed that RetA promoted the release of both proteoglycan and collagen from porcine articular cartilage explants (19). In the present study, the degradation of ECM biomolecules from cartilage explants was stimulated by RetA. After treatment, it was found the increases of ECM biomolecules degradation such as sulfated-GAG and HA, while uronic acid content in cartilage tissues was decreased in response to RetA. These results were in excellent agreement with the previously reported data. In addition, the increasing in gelatinolytic activity in culture medium from cartilage explants was found when induced with RetA. These indicated the elevation of matrix metalloproteinase inresponse to RetA.

IL-1β, a pro-inflammatory cytokine produced by several cell types, including endothelial cell, synoviocyte, and chondrocyte, interacts with chondrocytes through a receptor on

the cell surface. Stimulation of cartilage explants with IL-1β has been shown the wide spread matrix degradation, including loss of tissue proteoglycan (PGs) and collagens (55;56). In this study, the results showed the increases in degradation of ECM biomolecules which were released to culture media from cartilage explants, and uronic acid content in cartilage tissue was decreased in response to IL-1β stimulation. Increasing of the gelatinase activity in the culture media of cartilage explant treated with IL-1β also indicated the effect of IL-1β on the elevation of MMPs activities. These results were in agreement with the previous studies reporting that IL-1β played a major role in the pathology of cartilage degeneration by stimulation of MMP activity and consecutive matrix component degradation (23).

The mechanism of IL-1β and RetA induced ECM degradation involves the release of matrix metalloproteinase and other degradative products which contributes to the chronic sequelae leading to bone and cartilage destruction (57). In agreement with this study both IL-1β and RetA induce the degradation of ECM biomolecules releasing the products into culture media. Moreover the release of matrix metalloproteinase, both MMP-2 and MMP-9 was also induced, resulting in the increase degradation of ECM biomolecules such as GAG and HA into the culture media.

MMPs are the key enzyme in joint diseases that collectively degrade all the components of the ECM. They have received considerable attention with respect to arthritic tissue destruction because their expression correlates strongly with collagen. The role of MMP in the pathological destruction of cartilage is promoted by various pro-inflammatory cytokines that perturb the balance between synthesis and degradation of ECM components to favour matrix breakdown. Proteoglycan loss is a rapid event following pro-inflammatory stimulation but it can be readily replaced once the stimulus is removed. Collagen is more resistant to degradation but is much more difficult to replace (25). The mechanism to induce the degradation of ECM biomolecules by IL-1 is involved the activation of JNK, which in turn activates the transcription factor AP-1 (Figure 8), a key regulator of MMP production. The dual operation of tyrosine kinase-mediated NF-kappa B stimulation and c-Jun/AP-1 activation is essential to the induction of MMP-9 by IL-1β (58).

The therapeutic goals for arthritis are the decrease the symptoms of the disease and control the progression of the disease process. Pharmaceutical approaches are well known and

consist largely of the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. While these pharmaceuticals have definite beneficial symptomatic effects, their uses are also associated with relative high rate of well known side effects such as gastrointestinal ulceration, renal necrosis, and platelet dysfunction (59;60). Moreover, some of these drugs can down-regulate chondrocyte metabolism and actually decrease GAG synthesis (59). From these reasons, it needs to develop agents which are capable to ameliorate the arthritic symptoms by modifying the underlying pathological condition remains. Therefore, this is an important research objective. The aim of this study was to investigate chondroprotective effect of the Plai extracts using the cartilage explant model. Diacereine, the anti-osteoarthritis drug was also used in this study as the positive control

Diacereine is a drug belonging to the chemical of antraquinone class and has been used in OA treatment. It is a potent inhibitor of proinflammatory cytokine synthesis and activity, particularly for IL-1β (61). The previous study showed the activity of Diacereine to inhibit IL-1β induced collagenase synthesis by chondrocytes and to partially reverse the IL-1β induced inhibition of proteoglycan synthesis(62). Moreover, Diacereine has shown the protective effects on cartilage matrix degradation in OA animal models (62;63). This study also showed the results similar to previous studies, which indicated that Diacereine could be able to significantly inhibit RetA and IL-1β stimulated release of ECM biomolecules from cartilage explants. Moreover, the cartilage explants were treated with IL-1β or RetA in the presence of Diacereine, showed significant decrease of gelatinase activity in the culture media. Diacereine has a unique mode of action that distinguishes it from other drugs used in OA, such as NSAIDs or corticosteroids. The effects of therapeutic levels of Diacereine have shown *in vitro* (64) and *in vivo* (65) to be primarily due to potent inhibition of the synthesis and activity of the excessive levels of IL-1 and other catabolic cytokines present in osteoarthritis. Tamura, T., et al (66) have demonstrated that Diacereine down-regulates the gene-expression and production of pro-MMPs and up- regulates the TIMP-1 production. Diacereine also has anabolic properties. It has been shown to stimulate the production of transforming growth factor-β (TGF-β) (67). TGF-β is the most potent stimulator of chondrocyte proliferation, consequently increasing collagen and proteoglycan synthesis. These results suggested that the therapeutic effects of Diacereine on OA may be due to

the chondroprotective activity. The effect of Diacereine on the inflammatory cascade showed in Figure 23.

Plai (*Zingiber cassumunar* Roxb.) has long been regarded by Thai massage therapists as one of those oils necessary to combat joint and muscle problems. (E)-1-(3,4-Dimethoxyphenly) butadiene (DMPBD, compound D) is an active ingredient of the essential oil derived from the rhizome of Plai reporting by Thailand Institute of Scientific and Technological Research (TISTR). Preliminary study *in vivo* revealed the effect of DMPBD on both cyclooxygenase (COX) and lipoxygenase (LOX) pathways (4). The hexane extract seemed to possess potent anti-inflammatory activity. The major component isolated from the hexane extract is Compound D, showed a strong inhibitory activity on the edema formation in carrageenan-induced rat paw edema (45). In the TPA induced rat ear edema, if DMPBD possess the same pharmacological effect as diclofenac, it should possess inhibitory activity on the COX pathway (45;46). The results obtained from the *in vivo* models of inflammation suggested that DMPBD exerted a clear anti-inflammatory effect. Terpinen-4-ol and -pinene significantly inhibited edema formation, whereas sabinene and -terpinene were inactive up to 6 mg/paw (45;46).

In this study three fractions of Plai extract namely hexane , ethanol and water extracts were studied for the chondroprotective effects by measuring ECM molecules released to culture media via IL-1β or RetA stimulated cartilage explant model. The results showed high chondroprotective effects in both hexane and ethanol extracts of Plai, which demonstrated decreasing of GAG and HA release but increased uronic acid content in cartilage tissue. Moreover, the decrease in MMPs activity was found when co treated IL-1β with ethanol and hexane extracted Plai. The water extracted Plai, however, did not show any chondroprotective activities. According to the HPLC elution profiles of the extracts (see appendix), the water extracted Plai contained only one peak at Rt 4.90 while the ethanol and hexane extracted residues contained two peaks (Rt 13.7 and 24.8) and three more peaks (Rt 13.7, 24.8 and 26.7) of non-polar compounds, respectively. These results suggested that the active compound of Plai which contained the chondroprotective activities may be a group of non-polar compounds which were reported to have anti-inflammatory activity such as Terpinen-4-ol, sabinene, pinene and DMPBD (45;46).

This study confirms that the active compound of Plai is a unique topically active chondroprotective agent. It is a potential for local therapeutic application in inflammatory disease. A drug development program could be systematically initiated to determine the possibility of making Plai to a new pharmacological agent for the treatment of arthritic disorders.

Further studies

The further studies will be the purification and investigation the active compound in hexane and ethanol extracts particularly the fraction 2-4 from HPLC chromatogram whether they contain the chondroprotective activity.

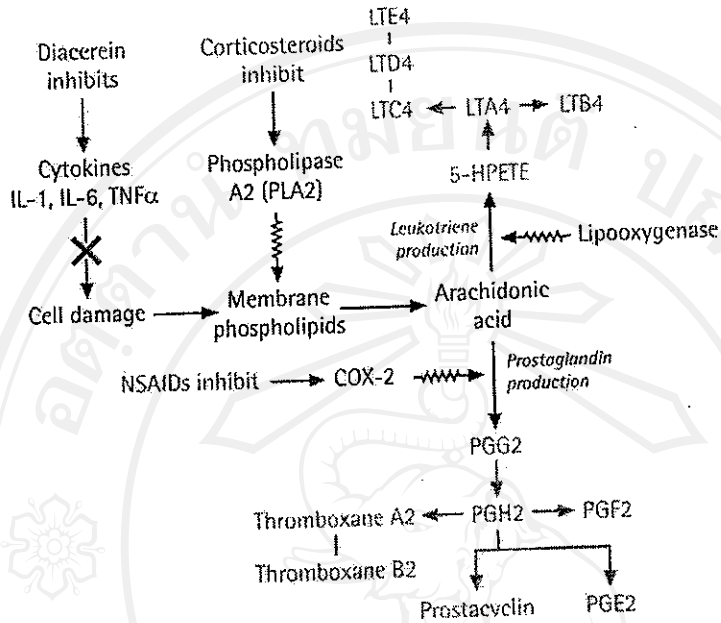

Figure 23. Effect of Diacereine on the inflammatory cascade.

CONCLUSION

In conclusion, the hexane and ethanol extracts of Plai possesses a potent chondroprotective activity using the cartilage explant model stimulated with Retinoic acid (RetA) or Interleukin 1β (IL-1β). Therefore, it is possible to use this compound as a new pharmacological agent for the management of degenerative joint diseases.