CHAPTER IV

Discussion

Wound healing is critical process for the organism. Numerous molecules appear to overlap each other's function. During wound healing, several molecules that are usually present only during embryonal development are found in the granulation tissue. Epithelial cells start to express extracellular matrix receptors that are not normally present in the resting epithelium. Fibroblastic cells with a special phenotype are found in the healing (29) (30). In this study, we found that phenotype of oral fibroblastic cells have different shape as compare to oral epithelial cells, and its produce HA high level. In previous studies found that periodonal fibroblasts produce HA (31) and six major proteoglycans, versican, a high-molecular-mass chondroitin sulphate proteoglycan (CSPG); decorin, a dermatan sulphate proteoglycan (DSPG); a membrane-associated heparan sulphate proteoglycan (HSPG); two medium- or matrix-associated HSPGs; and a 91 kDa membrane-associated CSPG (32). HA is a key component of chronic wound, also induces the production of a series of proinflammatory cytokines by fibroblasts, epithelium, cementoblasts and osteoblasts (33)(34).

The rapid production of HA by fibroblasts in the early stages of wound healing may be a crucial role as HA stimulates the migration and mitosis of mesenchymal and epithelial cells (4)(35). Increased levels of HA, as observed during fetal wound healing or as achieved by the topical application of HA during wound dressing, are associated with brisker healing and reduced scarring (36). Gingival epithelium contains hyaluronate, but there is little histochemical information about its localization (37), and the expression of extracellular matrix proteins and the metabolic activity of fibroblasts can be modulated by oral epithelial cells (38) (39)(40). Glucosamine availability appears to be rate-limiting for HA synthesis (41). Thus the administration of adequate amounts of glucosamine by mouth during the first few days after surgery or trauma can be expected to enhance HA production in the wound, promoting swifter healing and possibly diminishing complications related to scarring (42). HA has a multi-functional roles in the formation of some pathologic condition of connective tissue, such as inflammatory process and edema during wound healing. High concentrations of HA, particularly in fetal skin, have long been noted to be associated with rapid

healing with little scarring. It is postulated that HA is the extra cellular matrix (fluid between skin cells) that is the natural transportation system for the events of wound healing (inflammatory cell migration, fibroblast cell migration, cytokine migration and epithelial cell migration) to smoothly occur. HA always seems to surround proliferating and migrating cells in regenerating, remodeling, or healing tissues (43). HA found in ECM of oral connective tissue is mainly produced by oral fibroblasts. The wound tissue in the early inflammatory phase of repair is rich in HA within inflamed sites. HA production from oral fibroblasts were decreased by the ethanol extract of *Zingiber cassumunar* Roxb., this results indicate that the ethanol extract can inhibit the production of a series of proinflammatory cytokines by fibroblasts and epithelium by HA induction. This suggests that this extract may reduce tissue hydration (HA) and swelling during wound healing in oral inflammatory disorders, consistent with its effects found *in vivo*.

Zingiber cassumunar Roxb. (Plai) was traditionally used for relieving edema and inflammation at the wound healing site in alternative medicine. (E)-1-(3,4-Dimethoxyphenyl) butadiene (DMPBD, compound D) (43)(44) is an active ingredient of the essential oil derived from the rhizome of Plai by Thailand Institute of Scientific and Technological Research (TISTR) (23). Its potential anti-inflammatory and anti-edematous effects led to investigate its ability to the release of ECM biomolecules, such as HA, sulfated-GAG and MMP-2, 9 by using an *in vitro* culture model (45)(46). The result of three extract fractions of Plai, hexane, ethanol and water, we found that hexane extract seemed to potent effect to oral fibroblasts and epithelium than ethanol and water extracts. 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 (47)(48).

In this study, the results showed the decreases of the HA release in culture media from oral fibroblasts by Plai extract. The Plai hexane extract showed less HA in culture medium than the ethanol extract in the same concentration. From oral epithelia, the hexane extract showed the more level of HA release than the ethanol extract. The hexane extract seemed to possess a potent anti-inflammatory activity which the major component is compound D, exhibited a strong inhibitory activity on the edema formation in carrageenan-induced rat paw edema. The decreasing of HA in oral

fibroblast culture media, exhibited the inhibition during inflammatory phase, and the increasing of HA in oral epithelium media showed the rapidly re-epithelialization and granulation tissue formation in wound healing process.

TPA or 12-O-tetradecanoyl-phorbol-13-acetate, as a well-known phorbol ester, induced cutaneous oxidative stress and toxicity in murine skin and the increasing of inflammation in skin. Retinoids or vitamin A have long been associated with wound healing. Vitamin A deficiency retards repair, and retinoids restore steroid-retarded repair toward normal. Because vitamin A tends to suppress fibroblasts in cell culture and stimulate steroid-treated macrophages to initiate reparative behavior in tissue, the researcher favor the hypothesis that retinoids are particularly important in macrophagic inflammation, which plays a central role in the control of wound healing (49). Probably all patients who take anti-inflammatory steroids should control their retinoid intake, but how they should control it is as yet unknown. Profound metabolic changes take place in keratinizing epithelia in the presence of retinoic acid. *In vivo* as well as *in vitro* the proliferative activity of epidermal cells is greatly enhanced. Together with the increased rate of new cell production cellular differentiation (keratinization and cornification) is also altered. The effects are species-unspecific, probably tissue-specific and dose-dependent. The precise action of retinoic acid, however, still remains unknown.

IL-1β, a pro-inflammatory cytokine produced by several cell types, including endothelial cell, synoviocyte, and chondrocyte. Stimulation of IL-1β has been shown the wide spread matrix degradation, including loss of tissue proteoglycan (PGs) and collagens. Increasing of the gelatinase activity in the culture media treated with IL-1β also indicated the effect of IL-1β on the elevation of MMPs activities (50). 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.

The mechanism of IL-1β and RA induced ECM degradation involves the release of matrix metalloproteinase and other degradative products. In agreement with this study both IL-1β and RA induce the release of MMP, both MMP-2 and MMP-9 into culture media. MMPs are the key enzyme in various diseases that collectively degrade all the components of the ECM. The role of MMP in the pathological destruction of tissue is promoted by various pro-inflammatory cytokines

that perturb the balance between synthesis and degradation of ECM components to favor 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. The gelatinases (MMP-2 and MMP-9) is inhibited by the *Zingiber cassumunar* extract in oral epithelial cells. This result demonstrate that the *Zingiber cassumunar* extract may involve in periodontitis by suppression of degradation of MMPs activity

These results demonstrate the ability of *Zingiber cassumunar* Roxb. ethanol extract to inhibit HA production from oral fibroblasts, and inhibit the gelatinase activity from stimulated oral epithelial cells, corresponding with its potent anti-inflammatory activity. The findings from this *in vitro* study will be essential for future development of a new drug in the management of inflammatory oral diseases.

Further studies

The results show the high potent of anti-inflammation in Plai treatment so, the further study should be the purification and investigate the active compound in hexane and ethanol extract whether they contain the wound healing activity, including the investigation in animal model and clinical trial should be done.