CHAPTER V

DISCUSSION

The major objective of this study was to investigate the therapeutic effects of aloe vera on burned wound area, especially on dermal microvascular changes.

The results of this study showed that aloe vera could facilitate the wound healing process during the experimental period of seven and fourteen days. The results is agreed with those of previous studies which reported that aloe vera could facilitate wound healing.

The average healing time of the aloe vera gel treated area was 11.89 days and the vaseline gauzed treated area was 18.18 days (Visuthikosol et al., 1995). In the partial thickness guinea-pig burn model (Zawacki, 1974) complete healing was achieved in 21 days for the aloe products and antithromboxane agents (Heggers et al., 1993). Active component e.g. lectin-like compound, polysaccharides, amino acid, ascorbic acid, lignin in aloe vera (Coats, 1979; Engel et al., 1987; Winter et al., 1981) stimulated increased collagen of fibroblasts in number a dose-response fashion (Danof, 1987; Davis et al., 1988).

Studies of arteriole vascular changes, postcapillary venular permeability, and leukocyte adhesion in burn wound-rat on seventh day and fourteenth day after burning.

On day 7

1. Arteriole vascular changes

On the seventh day after burn, the present experiment exhibited marked enhance of second-and third-order arteriolar diameters. The present finding is in accordance with those of previous studies which reported that thermal stimulus caused the inflammatory conditions by release of numerous vasoactive mediators. At 24 hours, the burned tissue was found high levels of PGE_2 and TXA_2 and low levels of $PGF_{2\alpha}$ and PGI_2 (Robson et al., 1980). In electrical injury model, high level of TXA_2 continued over 72 hours, while PGE_2 and PGI_2 had lower levels than TXA_2 over 72 hours. (Robson et al., 1984).

The response to ultraviolet light was mediated by the inducible form of nitric oxide synthase (NOS). This enzyme generated greater quantities of NO than constitutive nitric oxide synthase (cNOS), which might explain the effectiveness of this stimulus in causing vasodilation (Warren et al., 1993). Besides, the agonists of acetylcholine, ATP, and bradykinin were potent stimuli to both NO and prostaglandin release. The vasodilation was abolished by the inhibition of either cyclooxygenase or NOS, suggesting a link between NO and prostaglandin. The significant inhibition of vasodilator PGE_2 was equally effective as L-NAME, nitric oxide synthase inhibitor, in attenuating arachidonic acid-mediated vasodilation. The result suggested that in the microcirculation of rabbit skin, the arachidonic acid-PGD_2-NO-pathway was

involved in acetylcholine and bradykinin mediated vasodilation (Warren et al., 1994). The result of present study showed that the second and third order arteriolar vasodilation markedly existed after burning on the seventh day. It is possible that a variety of vasoactive mediators, such as PGE_2, PGI_2, PGD_2 $PGF_{2\alpha}$, bradykinin, ATP, serotonin and histamine may have the synergistic effects. Vasodilation may be also related to NO pathway.

The result of present study exhibited the formation of new blood vessels. Fibroblast growth factors (FGFs) had been known to be the potent stimulators of vascular endothetial cell proliferation and angiogenesis. Acidic FGF (aFGF) and basic FGF(bFGF) significantly increased arteriolar diameter in a dose-dependent and time dependent manners. This effect was abolished during inhibition NOS but was not affected by cyclooxygenase inhibitor. The vasodilation induced by FGFs was not observed in endothelium-denuded vessels. These results suggest that aFGF and bFGF induced vasodilation through an endothelium-dependent and NO-mediated signaling pathway, leading to relaxation of arteriolar smooth muscle (Wu et al., 1996).

In addition, the result in the present study also presented angiogenesis which was the increased permeability. Angiogenesis in situ included coordinated interaction of various microvasular cell types, i.e. , endothelial cells, pericytes, and perivascular fibroblasts (Nehls and Drenckhaln, 1995). Vascular endothelial growth factor (VEGF) was a polypeptide mitogen that stimulated the growth of endothelial cell in vitro and promoted the growth of blood vessels in vivo, and was also found fms-like tyrosine kinase (Flt) acting its receptor (Peter et al., 1993). VEGF increased leakage of venules and capillaries as a result of opening endothelial intercellular junction, as well as, other drastic morphological modifications, including the induction of

fenestrae in venular and capillary endothelium which normally were not fenestrated.

2. Postcapillary venular permeability and leukocyte adhesion

On the seventh day after burn, the present experiment exhibited marked enhancement of postcapillary venular permeability, leukocyte adhesion and transmigration. The results of this study agreed with those of previous studies (Robbins, 1994; Garcia et al., 1986; Bizios, et al., 1988). The increase in vascular permeability induced by inflammatory mediators including histamine, serotonin and bradykinin is the cause of venular endothelial contraction and widening of the interendothelial cell junction.

Thrombin is generated at the site of injury by activation of the complement system, causing increased vascular permeability, chemotaxis and opsonization (Robbins, 1994), It appeared to be a reversible change in endothelial cell shape with formation of intercellular gaps (Garcia et al., 1986). In addition, thrombin acted on the endothelial cells and induced the generation of platelet activating factor (PAF), which in turn mediated the increased neutrophil adherence (Bizios, et al., 1988). PAF induces platelet aggregation at the sites of tissue injury, and enhances the release of serotonin, thus causing changes in vascular permeability. PAF also causes increased leukocyte adhesion to endothelium, chemotaxis, degranulation and the oxidative burst (Robbins, 1994). PGE_2 and PGI_2 contributed to vasodilation and exacerbate permeability elicited by primary stimuli such as histamine and bradykinin (Gerritsen, 1996). In contrast, PGE_2 could also reduce the release of plasma leakage evoked by histamine, and potentiation of plasma leakage and leukocyte accumulation evoked by indomethacin (Headvist, 1990).

In the lipoxygenase pathway of arachidonic acid, 5-lipoxygenase which is the predominent enzyme in neutrophil, is converted into a family of compounds collectively called leukotrienes (LTs) (Robbins, 1994). The leukotrienes (LTC_4, LTD_4, LTE_4) elicited a dose-dependent extravasation of plasma in the hamster cheek pouch with a potency exceeding that of histamine by approximately 1,000 - fold. The permeability increase was localized to postcapillary venules (Dahlen, et al., 1981) by leakage via gap formation (Headvist et al., 1987). On the other hand, local administration of LTB_4 to hamster cheek pouch caused leukocyte to adhere to the endothelium of venule of all sizes and subsequently to the perivascular interstitium (Headvist et al., 1994).

The complement system is found in greatest concentration in plasma and has functions in immune system. In the process of inflammation, a number of complement components are elaborated that cause increased vascular permeability, chemotaxis and opsonization (Robbins, 1994). Thus, on the seventh day after burn, these findings suggest that markedly increased postcapillary venular permeability may be related to vasoactive mediators including histamine, serotonin, thrombin, PGE_2, PGI_2, PAF, leukotrienes, compounds of the complement system and NO.

These findings exhibited the marked enhancement of postcapillary venular permeability and leukocyte adhesion after burning on the seventh day, These results agreed those of with previous studies which reported that the adhesion of leukocyte with endothelium was an important factor contributing to increased endothelial permeability (Siflinger-Birnboim and Malik, 1996). Studies using endothelial cell monolayer in culture have shown that neutrophil (PMN) activation increased endothelial permeability both in the presence and absence of PMN - endothelial monolayer contact. Hydrogen

peroxide (H_2O_2), an oxidant released by PMN activation, played an important role in PMN - induced increase in endothelial permeability. It was suggested that some mediators of inflammation (e.g. , histamine, thrombin) activated H_2O_2 production, caursing increase in endothelial permeability through activation of endothelial protein kinase C (PKC) and increase in endothelial cytosolic Ca^{2+} (Siflinger-Birnboim and Malik, 1993, 1996). Ramirez et al. (1996). showed that modulation of microvascular permeability through PKC activation required NOS involvement and suggested that a complex signal transduction pathway operated in response to PAF.

On day 14

On the fourteenth day after burn, the present study exhibited the reduction of the second - and third - order arteriolar diameters. Previous studies proposed that prostaglandins and thromboxanes might play role in the long- term inflammatory response in tissue injuries, and progressive dermal ischemia (Heggers and Robson, 1983, 1985 ; Robson et al., 1980)

Studies of arteriole vascular changes, postcapillary venular permeability and leukocyte adhesion in aloe-treated burn wound-rats at seventh day and fourteenth day after burning

On day 7

1. Arteriole vascular changes

On the seventh day the present study demonstrated that daily application of aloe vera markedly reduce the second-and third-order arteriolar vasodilation. A number of investigations have demonstrated that many active

substances of aloe vera had antiinflammatory activities ; however, a direct effect on arteriolar changes, postcapillary venular permeability and leukocyte adhesion has rarely been studied. Aloe vera contained bradykininase (Fujita and Shosuke, 1976) and carboxypeptidase (Fujita et al., 1979) which could hydrolyze bradykinin and angiotensin I into angiotensin II (Fujita et al., 1979; Rubel, 1983), leading to suppressing vasodilation and pain (Rubel, 1983). In vitro study of Yagi et al in 1982 found that glycoprotein present in aloe vera had antibradykinin activity. Hirata and Suga (1977) found magnesium lactate in aloe vera which inhibited the conversion of histidine to histamine in mast cells by inhibiting histidine decarboxylase (Lehniraer, 1987), resulting in the suppression of inflammation and vasodilation (Rubel, 1983). The biological activity of barbaloin and aloe extracts was found to inhibit histamine release from mast cell (Nakagomi et al. 1984).

Moreover aloe vera might be aspirin-like agent and also blocked prostaglandin synthesis (Davis et al.,1986). Lectin aloctin A in aloe vera inhibited biosynthesis of PGE_2 (Saito et al., 1982). Aloe vera not only acted as a TXA_2 inhibitor but also maintained a homeostasis within the vascular endothelium as well as the surrounding tissue (Heggers et al. 1993). Thus it is possible that aloe vera reduced arteriolar vasodilation through its antibradykinin, antihistamin and antiprostaglandin activities.

2. Postcapillary venular permeability and leukocyte adhesion

Postcapillary venular permeability and leukocyte adhesion of aloe-treated burn wound-rats were not difference form those of burn wound-rats and NSS-treated burn wound-rats, reflecting role of aloe vera in activation of lipoxygenase pathway. The metabolites of lipoxygenase pathway, LTC_4, LTD_4, LTE_4 cause intense vasoconstriction and the increased permeability,

while LTB_4 causes adhesion of leukocyte and is a powerful chemotactic agent (Robbins, 1994).

Hart et al. (1988, 1989, 1990) found that high molecular polysaccharide in aloe vera had immunomodulatory activity by depleting classical and alternative compounds of complement system. Low molecular constituents of aqueous aloe gel inhibited the release of reactive oxygen species (ROS) including superoxide anion (O_2^-) and H_2O_2 by PMA-stimulated human PMN, while the intraphagosomal activity of ROS was unaffected. Sabech et al. (1993, 1996) found antioxidant such as glutathione peroxidase (GSH) and superoxide dismutase (SOD) in aloe gel.

The exclusion of antioxidants such as SOD, GSH, and Catalase (CAT) from contact points of PMN with the endothelium was a significant factor regulating endothelial permeability following PMN activation (Siflinger-Birnboim, 1996). According to the present study, it is proposed that the adhesion of leukocyte with endothelium may be an important factor contributing to increased endothelial permeability.

On day 14

3. Arteriole vascular changes

On the fourteenth day the present study demonstrated that daily application of aloe vera significantly enhanced the vasodilation of the second- and third-order arterioles and also depleted the increase of permeability and leukocyte adhesion.

This finding that aloe vera markedly enhanced the vasodilation of the second- and third-order arterioles indicates that aloe vera not only increased PGE_2, PGI_2, PGD_2, but also inhibited TXA_2 (Afzal et al., 1991 ; Cera et al., 1980 ; Heggers et al., 1993), leading to reduction of vasoconstriction and preservation of the dermal microvasculature (Cera et al, 1980 ; Heggers and Robson, 1982). Afzal et al. (1991) demonstrated that aloe vera contained cyclooxygenase enzyme and could convert arachidonic acid into different prostanoids i.e., PGE_2, PGD_2, $PGF_{2\alpha}$ and TXA_2. It was suggested that PGD_2 might be a link between arachidonic acid and nitric oxide release (Warren et al., 1994), suggesting that PGD_2 and NO could account for enhancement of vasodilation in this experiment. In the present study, aloe vera reduced vasodilation on the seventh day after burn why it enhanced vasodilation on the fourteenth day after burn. Dora et al. (1997) showed that during vasoconstriction a signal could originate in smooth muscle cells and act on the endothelium to cause synthesis of endothelium-derived relaxing factor, which proposed that the rise in smooth muscle Ca^{2+} generated a diffusion gradient that drove Ca^{2+} through myoendothelial cell junction and into the endothelial cell, thereby initiating the synthesis of NO.

4. Postcapillary venular permeability and leukocyte adhesion

On the fourteenth day the results of the presents study found that aloe vera depleted the increased permeability and leukocyte adhesion. These findings can be explained by immunomodulatory activity of aloe vera (Hart et al., 1988). In addition, Davis et al. (1994) suggested that aloe vera could inhibit leukocyte recruitment. It is possible that active substances in aloe vera may prevent endothelial dysfunctions after burn.

Hypothesis for the effects of aloe vera on the second degree burn model

As the overall results of this investigation, we would like to purpose thje posible mechanisms of aloe vera showed in figure 5.1. Such that the machanisms of aloe vera could be both actions of antiinflammation and wound healing. As an antiinflammatory agent, our results have showed that aloe vera could inhibit the abnormalities of vascular diameter changes, vascular permeability, and leukocyte adhesion. However, these inhibitory mechanisms could not be confirmed at this point. Aloe vera has been known as a herbal medicine that composes of various types of sterols. And these sterols have been proposed as an antiinflammatory agents. We believe that these various kinds of active ingredients could maintain the homeostasis of endothelial functions(EC). Besides, aloe vera has been reviewed as a growth factor for fibroblasts. Therefore, aloe vera not only act as an antiinflammatory agent but also as a wound healing agent. Gibberin and auxin, the others components founded in aloe vera, has been reported as a promotor of protein synthesis.

As an overall conclusion, we have made the hypothesis that since aloe vera composed of a combination of active components, therefore, the actions of both antiinflammation and wound healing could be observed in our study.

In the future, aloe vera might be a great therapeutic agent used for burn wound patients.

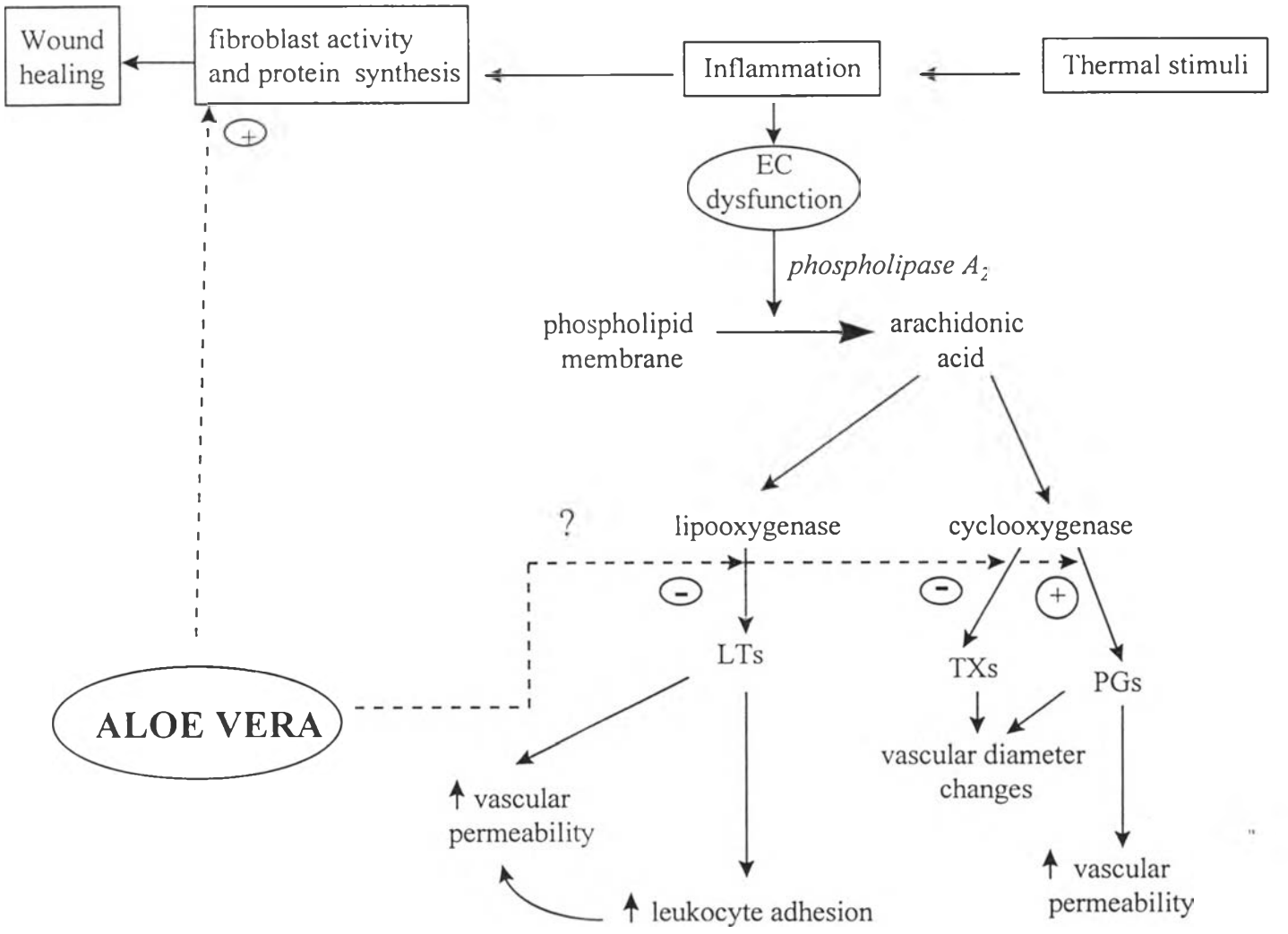

Figure 5.1 The purposed mechanisms of aloe vera as an antiinflammatory agents and wound healing agents