CHAPTER V

DISCUSSION

In this study, series of experiments were conducted to investigate the therapeutic effects of Aloe vera on burn microcirculation and cytokines production. By using the animal model of second degree or partial thickness burn wound, the results have demonstrated the beneficial effects of Aloe vera gel (300 mg/kg BW) significantly. By means of intravital fluorescent microscopy, histological examination and digital image processing, the crucial roles of Aloe vera in dermal burn wound in rats were discussed as followed.

I. EFFECTS OF ALOE VERA ON BURN WOUND HEALING AREA

Using digital images of burn wounded area taken on each experimental day, the healing area of each rat was eventually calculated and described by percentage of its original burned area (day 0).The results showed that Aloe vera could enhance the wound healing process. This effect of Aloe vera agreed with those of the previous studies which were scientifically proven for its influence on healing of wound (Zawachi, 1974; Danof, 1987; Davis et al., 1988;Visuthikol et al., 1995,).

Another study reported that 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). The active

components, lectin-like compound, polysaccharides, amino acid, ascorbic acid and lignin in Aloe vera (Coats, 1979; Engel et al., 1987; Winter et al., 1981) have been demonstrated to increase and the synthesis of collagen and fibroblasts in a dose-response fashion (Danof, 1987; Davis et al., 1988).

Moreover, our study on histopathologic finding has further confirmed these healing effects of aloe. Especially on day 14, epithelialization and vascularization were significantly demonstrated in the aloe-treated group. Taken together, our results have demonstrated that Aloe vera gel could stimulate wound healing by enhancing the body's ability to mend itself rapidly.

II. EFFECTS OF ALOE VERA ON TISSUE PERFUSION

Over forty years ago, Jackson DM. (1953) described the three functional zones of burn injury: (1) the peripheral *zone of hyperemia*, (2) the intervening *zone of stasis*, and (3) the central *zone of coagulation*. The latter consists of tissue irreversibly damaged via protein denaturation and breakdown of biological at elevated temperatures. The outer zone of hyperemia contains healing tissue that exhibits high metabolic activity. The intervening zone of stasis consists of a layer of tissue 1-2 mm thick characterized by reduced blood flow and progressive tissue necrosis, which can result in additional tissue death within 24-48 hours after the occurrence of the injury. In addition to causing an extension of the initial burn wound area, this process can result in the conversion of a partial- thickness burn into a full-thickness burn, which takes longer to heal and increase the probability of forming hypertrophic scars (Deitch et al; 1983).

By using Laser Doppler Flowmetry, the means value of burn-area tissue perfusion of each group were evaluated. The different patterns of tissue perfusion were observed during the first few minutes after burn in different zone areas. As a result , the response to burn injury with hyperemia was obtained immediately (t=0) as shown in Table 4.2. However, we have observed that in the zone of coagulation connecting to the zone of stasis where edema occurred, there was no flow within 1-2 hours postburn.

As the results shown in Table 4.2 and Figure 4.2, the aloe-treated burn wound rats have significantly increased tissue perfusion compared to burn for all three monitored time points (3, 7 and 14 days). These results are concomitant with those by the intravital-arteriolar-diameter evaluation which will be discussed in detail in the next session.

III. EFFECTS OF ALOE VERA ON ARTERIOLE VASCULAR CHANGES

It is worth to remind that normally, burn wound is characteristically made up of several zones of tissue damage due to different heat transfer. They are zone of coagulation, zone of stasis, zone of hyperemia as previously described.

With regards to our results, the video images of arterioles and leukocytes adhering on postcapillary venules are mostly corrected in the areas of stasis.

On the 3 day postburn, the experimental data demonstrated the increase of arterioles diameter 15-40 μm, not for arterioles diameter 40-70 μm (Table 4.2 and 4.3). According to the ultrastructure of skin microcirculation, capillaries and small arterioles are lined superficial to large arterioles and just

beneath the skin where the inflammation occurs, thus being more affected by thermal injury (as Figure 2.3 demonstrated in the session of review literature).

Since tissue was directly damaged by heat, the local tissue components including blood vessels, extracellular matrix, tissue macrophages and mast cells could be also damaged and/or activated . Some of those thermal trauma activated would turn on their inflammatory reactions. For example mast cells might release those inflammatory mediators including arachidonic acid, which could be produced and switched into prostanoid pathway.

Prostaglandins and thromboxanes are the main metabolites of arachidonic acid. Recently, their release in response to burn has been demonstrated. The two stable metabolites are prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The other two unstable metabolites of arachidonic acid, prostacyclin (PGI_2) and thromboxane A_2 (TAX_2) have been shown to have mare potent action than the stable forms. The fact is that both PGE_2 and PGI_2 are vasodilators whereas $PGF_{2\alpha}$ and TAX_2 are vasoconstrictors. As such they all provide for the homeostasis called 'Yin-Yang' hypothesis for vasomotion. It is believed that the consequence of thermal trauma could cause an imbalance of this 'Yin-Yang' mechanism. Confirmation that thromboxane plays a key role in progressive dermal ischemia was found in the histologic antibody-staining studies (Hegger et al., 1980). Therefore, several studies have tried and developed their experiment using antithromboxane inhibitors in order to prevent progressive dermal ischemia and to increase ultimate amount of skin salvage.

We believe in the imbalance of 'Yin-Yang' mechanism by thermal trauma together with the inflammatory response, which can explain for the spatial and temporal different patterns of arteriolar diameter changes.

On 3 day postburn, the present study demonstrated that daily application of Aloe vera could reduce the arteriolar vasodilation (Table 4.3). A number of investigations have demonstrated that many active substances of Aloe vera had antiinflammatory activities; however, the direct effects of Aloe vera on arteriolar changes, postcapillary venular permeability and leukocyte adhesion have not yet been studied. Aloe vera contained bradykininase (Fujita and Shosuke, 1976) and carboxypeptidase (Fujita et al., 1979) which could hydrolyze bradykinin (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 PGI_2 (Saito et al., 1982). Thus it is possible that Aloe vera reduced arteriolar vasodilation through its antibradykinin, antihistamine and antiprostaglandin activities.

On 7 and 14 day postburn the experimental results of arteriolar diameters ,especially in those of size 15-40 μm , demonstrated that daily application of Aloe vera could inversely enhanced the vasodilation of arterioles.

In other words, Aloe vera helped prevent the progression of dermal ischemia. Various studies have demonstrated that Aloe vera did not only

suppress inflammatory mediators, bradykinin and histamine, but also was able to increase PGE_2, PGI_2, PGD_2, and inhibit TXA_2 (Afzal et al., 1991; Cera et al., 1980; Hegger et al., 1993). Therefore it is possible to reduce to vasoconstriction and preservation of the dermal microvasculature (Cera et al., 1980; Heggers and Robson, 1982). In addition, it was suggested that PGD_2 might be a link between arachidonic acid and nitric oxide release (Warren et al., 1994). Such PGD_2 and NO could account for the enhancement of vasodilation in this experiment. In the present study, Aloe vera reduced vasodilation on the 3 day postburn meanwhile it enhanced vasodilation on the 7 and 14 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, initiating the synthesis of NO.

From our findings for this part of intravital-arteriolar diameter measurements, it might be conceptually concluded that Aloe vera not only acted as a TXA_2 inhibitor but also maintained the homeostasis of vascular endothelium as well as the surrounding tissue.

IV. EFFECT OF ALOE VERA ON LEUKOCYTE ADHESION

Leukocytes, particularly polymorphonuclear neutrophils (PMNs), are central mediators of microvascular endothelial injury in many acute pathologic processes (Boykin et al., 1980; Harlan, 1987; Movat ,1987; Weiss, 1989). PMNs have been identified as possibly contributing to the microvascular occlusion seen following burn injury both systemically and

locally. Deich et al. have demonstrated an increase in PMN activation when PMNs are exposed to burn blister fluid in vitro (Deitch et al., 1990). Nelson et al. have demonstrated an increase in the surface expression of CR3 expression (CD11b/CD18) on circulating PMNs following burn injury (Nelson R et al.,1986). PMN-mediated injury is dependent in part on PMN adherence to the vascular endothelial cell surface and PMN-PMN aggregation in the microvasculature. PMN-EC adherence results in the formation of a microenvironment between the PMN and the EC (Harlan, 1987).

In the lipoxygenase pathway of arachidonic acid, 5-lipooxygenase which is the predominant 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 size and subsequently to the perivascular interstitium (Headvist et al., 1994).

In our experiment, the exhibition of marked enhancement of leukocyte adhesion and transmigration was observed through intravital fluoresence microscope by using acridine orange labeled leukocytes and observed as shown in Figure 4.8-4.10. These findings agree with agreed with those of previous histological studies (Robbins, 1994; Garcia et al., 1986; Bizios et al., 1988). The results of digital-image analysis for leukocyte adhering (per cent/ 100 μm, Table 4.5) demonstrated the sustained adhesion in both groups of

BURN, BURN-NSS for all three monitor time points. With regards to the typical pathophysiology of burn wound (as previously mentioned in Chapter II), the subsequence of events occurred during 12-24 hours postburn was called the period of transformation. During that period, the adhesive molecules on the free surfaces of endothelial cells, platelets and leukocytes are expressed. This leads to leukocyte margination, extravasation and migration to those injured parenchymal cells and also microorganisms as shown in Figure 2.5. As we have mentioned that the early phase of burn (0-72 hours), the products of vasoactive amine and kinin systems modulate the inflammatory response. The release of proinflammatory mediators by tissue macrophages, mast cells, or other tissue cells such as damaged fibroblasts are initiated. Therefore, soluble mediators like histamine, interleukin-1 and TNF-α are obtained. By means of these mediators, they induce the expression of endothelial-leukocyte interactions. As soon as leukocytes have arrived at the site of burn, they release mediators which control the later phase of so called 'accumulation' and activation of other cells.

Interestingly, our results showed in Table 4.5 and Figure 4.10d that daily treatment of Aloe vera (300 mg/kg BW) could reduce the endothelial-leukocyte interaction significantly on day 14. Hart et al., 1988 and David et al., 1999) also demonstrated such effects of Aloe vera on leukocyte recruitment. As the results of this leukocyte recruitment evaluation, it is possible to say that Aloe vera might have some active ingredients which are able to inhibit or suppress those more serial sequence of the enhancement of endothelial-leukocyte interactions. Therefore, we have made further evaluation for the effects of Aloe vera on those levels of cytokines, for which there is quite a few of evidence for. It is possible that active substances in Aloe vera may prevent endothelia dysfunctions after burn.

V. EFFECT OF ALOE VERA ON CYTOKINE PRODUCTION

The pathophysiogical events following thermal injury are not only limited to the local effects of heat at the burn area but are also superimposed by the acute inflammatory reactions. As if those inflammatory responses are further challenged by infection, the overproduction of chemical mediators, causing endothelial-leukocyte interaction and overproduction of cytokines might be contribute to systemic effects. Thus in patients with major burn as such, systemic effects might turn to multiple organ dysfunction distress syndrome, which may develop further into progressive organ failure and death. According to these importance of cytokines, therefore, we are much interested to study on the effect of Aloe vera on plasma cytokines levels as which developed at different time points after burn. Cytokines are involved in both inflammation and immunity as shown previously in Figure 2.6. There are complex interactions among different forms of cytokines in thermal injury. For example, transiently increased circulating levels of TNF-α in burns occur and are associated with poor prognosis. It is also suggested that TNF-α might play an important role in the development of multiple organ failure after thermal injury (Liu et al., 1993,1995). Inversely, it has been postulated that IL-6 response for IL-1 and TNF-α suppression. Moreover, Deveci and his co-workers explained their experimental results on full-thickness burn wound rat model that IL-6 may be inhibits the severity of the inflammatory response in the early period of thermal injury. Especially, they reported that high level of IL-6 decreased the levels of TNF-α.

Results obtained in our study showed that there was a marked increase in serum TNF-α and IL-6 on 3 days postburn. In the present study, we observed that treatment with Aloe vera on burn wound could prevent the elevation of TNF-α and IL- 6 in all 3 monitor time points. Interestingly, the recent study of Blumenfeld et al. (2000) demonstrated that the wound healing

process in the deep partial-thickness skin burn guinea-pigs. The major carbohydrate substance of Aloe vera called 'acemannan' has been indicated for their action on immunological system.

HYPOTHESIS FOR THE EFFECTS OF ALOE VERA ON THE SECOND BURN WOUND MODEL

As the overall results of this study, we would like to propose the possible mechanisms of Aloe vera shown in Figure 5.1. Such that the mechanisms of Aloe vera could be both actions of antiflammation and wound healing. As an antiflammatory agent, our results have showed that Aloe vera could retard the abnormalities of vascular diameter changes and leukocyte adhesion. Regarding the experimental results of TNF-α and IL-6 levels, Aloe vera-treated burn showed a significant decrease of them. However, it might not be proper to make the assumption that Aloe vera help to inhibit these cytokine productions since we have not performed our evaluation on day 1-2. The levels of both TNF-α and IL-6 might have be already activated and then have turned to decrease rapidly via Aloe vera.

Therefore, on day 3-14 both of them already decreased to a lesser extent than the other groups of BURN and BURN-NSS. The idea is that Aloe vera might have an active ingredient that promotes wound healing process rapidly via the activation of cascade-response of cytokines. Therefore, IL-1 (primary phase) would decrease faster and let IL-6 (intermediate phase) to increase in stead and faster. Then, IL-8 and IL-10 which are the tertiary phase of cytokines could be produced faster. Finally, it means that Aloe vera could help heal the burn wound speedily.

Aloe vera has been known as a herbal medicine that is composed of various types of sterols and those sterols have been proposed as an antiinflammatory agents. We believe that such various kinds of active ingredients could maintain the homeostasis of endothelial cells and their surrounding cells. 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. Gibberrelin and auxin, the others components found in Aloe vera, have been reported as a promotor of protein synthesis.

As an overall conclusion, we have made the hypothesis that since Aloe vera is 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.

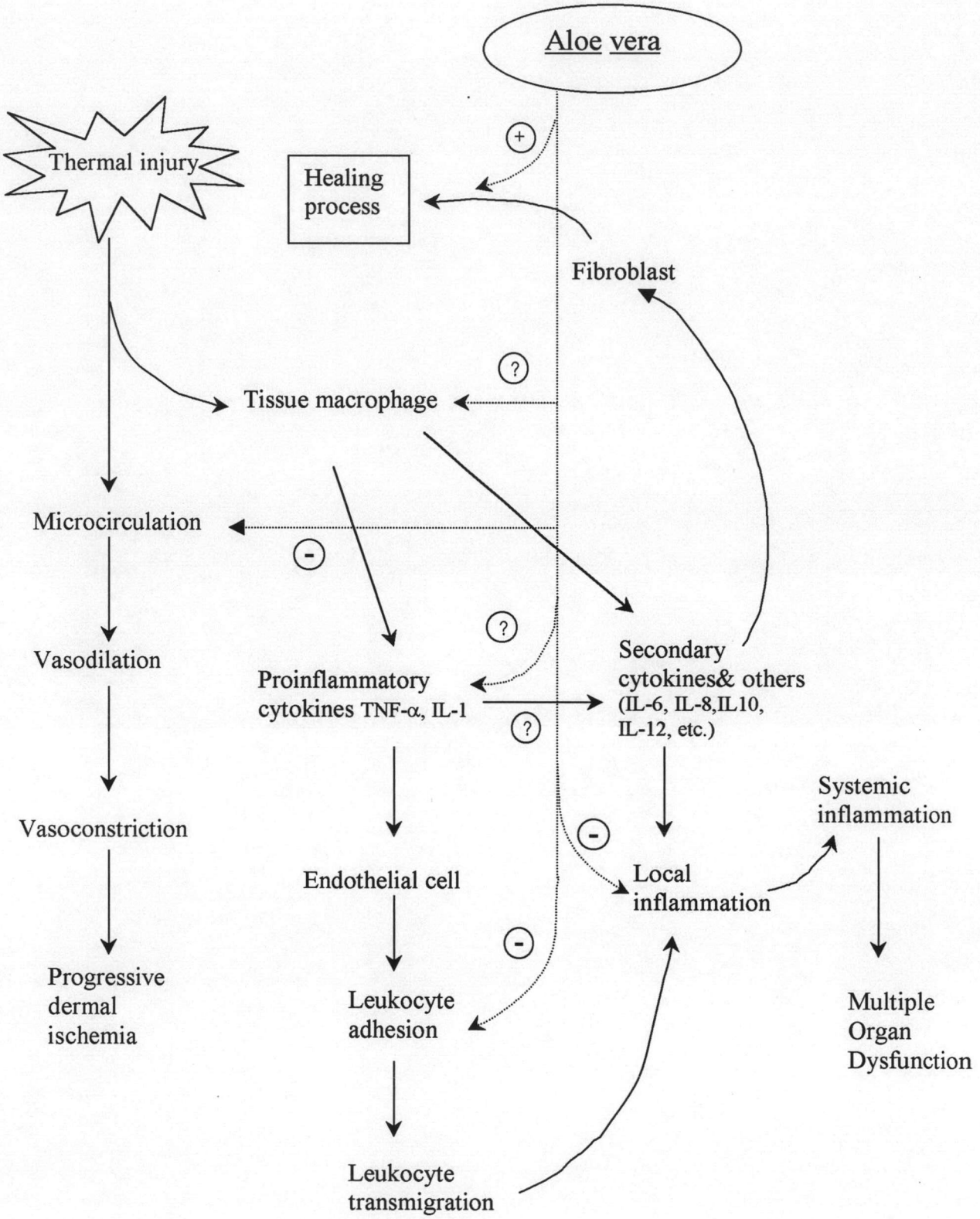

Figure 5.1 *The purposed mechanisms of Aloe vera as an inflammatory agents and wound healing agents*