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Aloe And Other Topical Antibacterial Agents In Wound Healing

By John P. Heggers, Ph.D. & Wendell Winters, Ph.D.

Unlike any other wound, the burn is a non-uniform injury in which some tissues are partially or completely damaged, while other tissues suffer minimal damage. The latter will heal without any therapeutic treatment, while the former will become permanently damaged, creating a granulating wound if not appropriately treated.

Infection also plays a major role in the conversion of this wound.¹ Many of the topical agents used are to control burn wound infections. However, there are other products that have multivaried effects on the burn wound. Some of the major properties attributed to Aloe vera include its ability to:

- a. penetrate tissue
- b. anesthetize the tissue
- c. allay bacterial, fungal & viral growth
- d. act as an anti-inflammatory
- e. dilate capillaries & enhance blood flow²

Heggers and his co-workers³ showed that topical application of anti-eicosanoids, more specifically anti-thromboxane agents, could reverse progressive tissue necrosis in the partially damaged tissue. Topical application of an Aloe compound resulted in healing patterns comparable to the anti-thromboxane agents. Robson and his colleagues² also showed that such an Aloe compound had anti-bacterial properties as well.

Therefore, topical application of anti-microbials and other chemo-therapeutic agents is essential in order to restore the normal healing process and prevent infection. Halsted has been quoted as saying, "A wound which has been irrigated with solutions of carbolic acid, corrosive sublimate, or other disinfectant labors under the disadvantage of a more less extensive area of superficial necrosis"³. McCauley and his colleagues⁴ have show that both silver sulfadiazine and Sulfamylon® are toxic to fibroblasts in tissue culture at concentrations of 0.005% and 0.1%, respectively. Leitch, et al⁵ recently presented data that silver sulfadiazine, Sulfamylon® and silver sulfadiazine with chlorohexadine significantly retarded wound healing in the acute wound model.



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Since application of topical chemotherapeutic agents is essential in the prevention of infection and enhancement of wound healing, we examined Aloe's role in accelerating wound healing or reversing the wound retardant effect of silver sulfadiazine as well as the influence of Bactroban® and clindamycin on the healing process.

In order to be assured that Aloe contained active components which are essential in the healing process we employed a Polyacrylamide gel electrophoresis (PAGE) and cell growth assays to determine the presence of active components. Acrylamide-bis acrylamide 37.5:1 (Fisher Biotech Houston), tris-HCl (Sigma St. Louis), sodium lauryl sulfate (SDS), N,N,N',N'-tetramethylethylenediamine (TEMED), ammonium persulfate (Bio-Rad (Richmond), rainbow weight marker (Amersham Arlington Heights were purchased from the respective vendors). Electrophoresis was performed using a 12.5% separating gel and a 4% stacking gel run in a Bio-Rad Protean II Xi vertical electrophoresis cell system at 30mA constant current⁶. The gel was stained by silver stain kit method, and the kit was purchased from Bio-Rad.⁷

Cells were grown in Dulbecco's Minimal Medium (DMEM) supplemented with 10% heat-inactivated horse serum, 5% fetal calf serum, 50 units penicillin, 0.05% mg/ml streptomycin 1mM L-glutamine and 1mM sodium pyruvate. The rat adrenal cultured cells were prepared following incubation at 37° C in 5% CO₂. Cell concentration and viability were determined by hemacytometer counts and dye exclusion with 0.04% trypan blue.

Cells at 5×10^4 cell/well were plated into 96-well flat-bottom plates and maintained 24 hours at standard conditions in adherence studies. The media was removed before the addition of the Aloe-DMEM mixture. After 72 hours of incubation, 10ul of MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) solution (5mg/ml) was added to each well. The formazan crystals, formed only in viable cells after four hours at 37° C, were dissolved by addition of 100ul of acid-isopropanol solution. The plates were read by a Micro ELISA reader (MR 580 Dynatec) at 570nm (630nm reference wavelength, calibration setting of 1.99).

The acute model was used as previously described by Heggors, et al.⁸ Appropriately anesthetized Sprague Dawley rats, two proximal and two distal, received four 1.5 cm² dorsal defects through the skin and panniculus carnosus. This study was conducted in compliance with UTMB's Animal Care and Use Committee under ACUC protocol #92-05-026. (Fig. 1)



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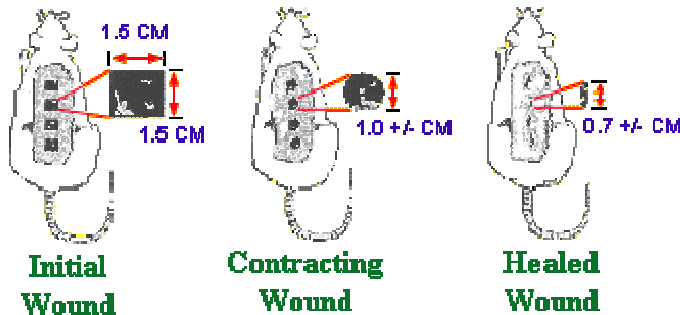


Figure 1 Schematic representation of the acute wound healing model.

The skin defects were treated three times a day for 14 days with Aloe vera gel (n=10), 2% mupirocin ointment (n=10), 1% clindamycin cream (n=10), 1% silver sulfadiazine alone (n=10), 1% silver sulfadiazine cream + Aloe (n=10). An untreated group served as control (n=10). Wound closure rate was assessed by serial planimetry. Following healing, the breaking strength of each resultant scar was determined using an Instron tensiometer model #4201 (Instron Corp, Canton, MA). Wound half-lives and overall healing rates were calculated by regressing the log of the areas of all wounds over time.

Results

The SDS PAGE analysis revealed a high molecular weight polypeptide in Aloe vera 1:1 gel #5.

The rat adrenal cultured cells in the presence of Aloe vera gel #5 showed a 26% increase in growth activity when compared to the control (*Fig. 2*). Therefore, we utilized the Aloe vera gel #5 for our in vivo assay.

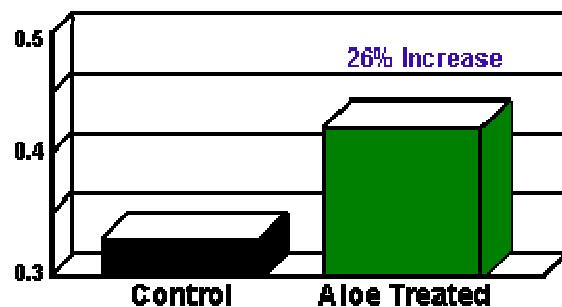


Figure 2 Tissue culture response to Aloe 1:1 gel compared to control (untreated).

Acute Wound Healing

Topical application of each therapeutic agent had a profound effect on the healing process. Overall healing rates of all the treated groups were significantly different as compared to the control group ($p < 0.05$). The Aloe group had the shortest half-life, and healed faster than the control group (*Table I*). All the other treated groups had longer half-lives compared to the control group. While silver sulfadiazine with Aloe



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significantly increased the breaking strength ($2.000 + 0.504$) of the healed wound, Aloe alone was slightly stronger than the control silver sulfadiazine.

Table I

Fractional Area & Healing Rates of Wounds Treated With Topical Antibacterials

Group	Days (n)	Fraction Of Initial Wound Area Throughout + SD	Overall Healing Rate (Slope +SD)	1/2 Life
1. Control	480	0.289 + 0.385	0.1477 (0.0027)	6.38
2. Aloe	360	0.279 + 0.364	0.1657 (0.0027)	6.14*
3. SSD	480	0.368 + 0.420	0.1800(0.0050)	8.56
4. SSD + Aloe	480	0.277 + 0.392	0.1339 (0.0030)	6.94
5. Bactroban®	480	0.332 + 0.414	0.1300 (0.0026)	8.74
6. Clindamycin	324	0.396 + 0.482	0.1711 (0.0037)	8.30

Table II

Breaking Strength of Healed Wounds

Group	Breaking Strength (KG) + S	(n)
1. Control	1.461 + 0.421	30
2. Aloe	1.640 + 0.533	29
3. SSD	1.521 + 0.432	28
4. SSD + Aloe	2.000 + 0.504*	28
5. Bactroban®	1.845 + 0.421	24
6. Clindamycin	1.621 + 0.404	15

*SSD + Aloe breaking strength is significant ($p = <0.05$)

*All half-life days are significant ($p = <0.05$)

Topical Aloe significantly enhances the rate of wound healing, and, when combined with silver sulfadiazine, it apparently reverses the wound retardant effect of silver sulfadiazine. Clindamycin and mupirocin significantly delayed wound closure as did silver sulfadiazine, while the breaking strength for the three topical agents appears stronger or comparable to the control. (*Table II*)



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Conclusions

Topical application of a variety of cytokines to open wounds has revolutionized the process of wound healing. Hayward, et al⁹ provided evidence that the basic Fibroblast Growth Factor (bFGF) reverses bacterial retardation of wound contraction in a chronic granulating wound.

Carney and his co-workers¹⁰ showed that exogenous delivery of synthetic Thrombin Receptor-activating peptides enhanced the healing process and neovascularization of an incisional wound. In a clinical trial Bishop, et al¹¹ evaluated two potential wound healing agents in a blinded trial for the treatment of venous status ulcers. Contrary to previous *in vitro* and *in vivo* studies by McCauley, et al⁴ and Leitch, et al⁵ the Bishop study showed that silver sulfadiazine was significantly more therapeutic in healing the venous status ulcer when compared to a biologically active tripeptide copper complex or a placebo. These results suggest that a silver sulfadiazine cream may facilitate healing in wounds that heal by epithelialization. Robson and co-workers,^{12,13} in two separate publications, reported on the efficacy and safety of platelet-derived growth factor B-B and bFGF in chronic pressure sore ulcers.

Our previous studies have provided evidence that Aloe vera may contain a growth factor like substance.^{2,3,4} Recently, Winters¹⁴ presented data regarding a growth stimulating as well as a growth suppressant substance in Aloe gel. PAGE analysis revealed the presence of a polypeptide species possibly responsible for these activities. By immunoblot technique, the Aloe substances were found to contain Na⁺/K⁺ATPase and Con A activities. These mitogenic lectin-like substances in Aloe have been previously described.

Therefore, with this foundation of knowledge regarding the exogenous administration of cytokines and Aloe substances in the process of wound healing, we closely examined Aloe vera gel 1:1 (#5) to provide further evidence of its wound-healing potential compared to other chemotherapeutic agents.

McCauley, et al⁴ showed that silver sulfadiazine in tissue culture was toxic to fibroblasts and keratinocytes, and Leitch and his co-workers⁵ showed that it retarded wound healing *in vivo*. We wondered if Aloe would respond as bFGF did in reversing the retardation of



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wound healing⁹ and if the treated, healed wound was stronger or weaker than the control wound.

The Aloe-alone treated wounds healed faster and had a half-life of 6.14 days which was significantly ($p < 0.05$) shorter when compared to the other groups (*Table 2*). The silver sulfadiazine and Aloe group, while it healed significantly faster ($p = < 0.05$) than the Bactroban® silver sulfadiazine, clindamycin groups, had a half-life of 6.94 which was slightly longer than the control wound (half-life 6.38).

The breaking strength of the Aloe-treated wound (1.640) was significantly less ($p < 0.05$) than the silver sulfadiazine + Aloe group (2.000) (*Table II*). The control breaking strength was 1.461, less than the Aloe-treated wounds, while the silver sulfadiazine + Aloe treated wounds were significantly ($p < 0.05$) greater than all other groups. The Bactroban® - clindamycin- and silver sulfadiazine-treated wounds were apparently stronger than the controls, but the healing time was significantly ($p < 0.05$) retarded. This study further substantiates the fact that Aloe contains a growth promoting factor that enhances the healing process and the breaking strength of these healed wounds. Aloe can also reverse the wound healing retardant effect of silver sulfadiazine, a topical antimicrobial used to treat and control burn wound sepsis.