Effect of the Combination of Aloe vera, Nitroglycerin, and L-NAME on Wound Healing in the Rat Excisional Model

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ABSTRACT

Purpose: Many systemic and topical therapeutic agents such as growth hormone, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF) have been used as vulnerary agents. However, the role of nitric oxide (NO) as a wound-healing stimulant has been received with mixed reviews. NO is a potent vasodilator that is thought to be an endothelium-dependent relaxing factor, and a regulator of blood pressure and regional blood flow. It affects vascular smooth muscle proliferation and inhibits platelet aggregation and leukocyte adhesion. Therefore we compared the effects of several topical substances that have similar or reverse properties. Methods: Using the excisional rat wound model, we evaluated the topical effects of Dermaide Aloe (D-Aloe, Dermaide Research Corp, Palos Heights, IL), nitroglycerin, Aquaphor® (Beuersdorf, Inc., Norwalk, CT) alone, with D-Aloe with nitroglycerin, 2%, and L-NAME (NO inhibitor) with Aquaphor®, and L-NAME with Aquaphor® and D-Aloe for a 21-day period. All wounds were measured by planimetry at 1, 7, 10, 13, 16, 18, and 21 days. Results: At day 1, all wounds had an average wound size of 2.27 cm² (SD = 0.372) with no significant difference in wound size among the groups. Topically applied D-Aloe appeared to promote wound healing faster than the remaining other topicals (p < .05, Student-Newman-Keuls and Dunn’s Method) over the study period. However, topicals combined with D-Aloe, the vehicle Aquaphor®, and L-NAME improved the wound healing process when compared with nitroglycerin alone (p < .05). Conclusions: D-Aloe appears to have a wound-healing advancement factor that can reverse the effects of petrolatum- and nitroglycerin-based products as observed in the remaining groups when compared with nitroglycerin alone. It appears that D-Aloe’s effect of preventing dermal ischemia by reversing the effects of thromboxane synthetase (TxA2) may act synergistically with NO or could be an oxygen radical scavenger.

INTRODUCTION

The demonstration in 1987 of the formation of nitric oxide (NO) by an enzyme in vascular endothelial cells opened what can now be considered a new era in biological research; NO, which accounts for the biological properties of endothelium-derived relaxing factor

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Presented in part at the 2nd Joint Meeting of the Wound Healing Society and the European Tissue Repair Society, May 1996, Boston, Massachusetts.
(EDRF), is the endogenous stimulator of the soluble guanylate cyclase. In addition, NO is an effector molecule released by macrophages and other cells after immunological activation (Moncada et al., 1989).

NO is synthesized from the amino acid L-arginine by an enzyme called NO synthase. In 1990, it was apparent that there were at least two types of NO synthase. One is constitutive, a cytosolic Ca++/calmodulin dependent and only releases NO for short periods of time in response to a receptor or a physical stimulation. The NO released by this enzymatic reaction acts as a transduction mechanism that has several underlying physiological responses. The counterpart enzyme is induced after macrophage or endothelial activation and a variety of other cells triggered by cytokines, and once expressed, synthesizes NO for longer time sequences. Furthermore, this enzyme is cytosolic, Ca+++-independent and requires tetrahydrobiopterin concomitantly with other cofactors, which is inhibited by glucocorticoids (Palmer, 1993). Until now the only established role for NO is as a cytotoxic molecule attacking invading microorganisms and tumor cells. It is likely, however, that the release of NO via this enzyme has other biological consequences including pathological vasodilation and tissue ischemia (Dicket AL, et al., 1994).

Last year at the Wound Healing Society we presented data that provided evidence that Nω-nitro-L-arginine methyl ester (L-NAME) enhanced wound healing as a NO inhibitor (Heggers et al., 1996). This study confirms that topical application of NO inhibitors will enhance the healing process either as an O radical scavenger or a thromboxane synthetase (TxA₂) inhibitor.

**MATERIALS AND METHODS**

**Topical therapeutic agents**

Topical therapeutic agents used included Dermaide-Aloe (D-Aloe) 50 gm/100 (50%, Dermaide Research Corp, Palos Heights, IL; Fig. 1). (Robson et al., 1982; Heggers et al., 1995; Heggers et al., 1996); nitroglycerin, 2 gm/100 (2%, Fougera & Co., Melville, NY); Aquaphor® (petrolatum, mineral oil, mineral wax, wool wax, alcohol, Beuersdorf Inc, Norwalk, CT), vehicle control; and nitro-L-arginine methyl ester hydrochloride (L-NAME) 74 mM (Sigma Chemical Co, St. Louis, MO) in Aquaphor®.

**Animal model**

The acute excisional wound model was used as previously described (Hokanson et al., 1991). Sprague-Dawley rats after appropriate anesthesia received four 1.5-cm dorsal defects through the skin and panniculus carnosus: two proximal and two distal (Fig. 1). This study was conducted in compliance with our institution’s Animal Care and Use Committee under ACUC protocol #92-05-026.

All animals were treatedTopically every 3 days for 21 days with 4 gm of the following compounds: 1) D-Aloe alone; 2) nitroglycerin alone (1.1); 3) Aquaphor® alone; 4) D-Aloe plus nitroglycerin; 5) L-NAME in Aquaphor®; 6) L-NAME in Aquaphor® plus D-Aloe; and 7) untreated control.

All treatment modalities were based on a volume-volume basis. All test groups included 6 animals each to ensure statistical significance. Wound closure rate was assessed by serial planimetry at 1, 7, 10, 13, 16, 18, 19, and 21 days. Wound half-lives and overall healing rates were calculated by regressing the log of the areas of all wounds over time. After healing, the breaking strength of each resultant scar was determined using the Instron tensiometer model 4201 (Instron Corp, Canton, MA).

**STATISTICAL ANALYSIS**

All wound area measurements were expressed as a percentage of the initial wound size. These serial area measurements were then plotted against time. For each rat that survived beyond 50% wound closure, an exponential decay curve was fitted to this data by a nonlinear, least-squares technique. From this continuous function the time taken for each animal’s wound to reach 50% of its initial size was calculated. These wound half-lives were compared using the Kruskal-Wallis one-way analysis of variance and by Scheffe’s technique for multiple comparisons. All analyses were performed using the Number Cruncher Statistical System on a personal computer. Analysis of absolute area measurements
by simple linear regression showed that initial wound area made no significant difference to half-life calculations (Hokanson et al., 1991).

**RESULTS**

Wound healing

At day 1 all wounds had an average size of 2.27 cm² (SD ±0.372) with no significant difference in wound size among the groups. While the initial defect is 1.5 cm², due to the elasticity of the defect the size increases. Topical applied D-Aloe appeared to promote wound healing faster than the remaining other topicals ($p < .05$), student-Newman-Keul’s and Dunn’s method over the study period (Table 1). However, topicals combined with D-Aloe, the vehicle Aquaphor®, and L-NAME improved the healing process when compared with nitro-

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TABLE 1. WOUND HEALING OVER TIME OF TOPICALLY APPLIED DERMAIDE ALOE, NITROGLYCERIN, AND L-ARGININE IN THE RAT EXCISIONAL MODEL

<table>
<thead>
<tr>
<th>Topical Group</th>
<th>1</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>13</th>
<th>16</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermaide Aloe</td>
<td>2.34*</td>
<td>2.81</td>
<td>1.76</td>
<td>0.711a</td>
<td>0.305**</td>
<td>0.126b</td>
<td>0.002c</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>2.27</td>
<td>2.676</td>
<td>1.89</td>
<td>1.071</td>
<td>0.508</td>
<td>0.225</td>
<td>0.05</td>
</tr>
<tr>
<td>Aquaphor®</td>
<td>2.22</td>
<td>2.72</td>
<td>1.62**</td>
<td>0.846a</td>
<td>0.518</td>
<td>0.177</td>
<td>0.019</td>
</tr>
<tr>
<td>D-Aloe+ nitroglycerin</td>
<td>2.34</td>
<td>2.71</td>
<td>2.17</td>
<td>1.078</td>
<td>0.547</td>
<td>0.262</td>
<td>0.014</td>
</tr>
<tr>
<td>Aquaphor®+ L-NAME+</td>
<td>2.32</td>
<td>2.561</td>
<td>1.80**</td>
<td>0.926a</td>
<td>0.390</td>
<td>0.203</td>
<td>0.013</td>
</tr>
<tr>
<td>L-NAME+</td>
<td>2.15</td>
<td>3.09</td>
<td>2.07</td>
<td>0.807a</td>
<td>0.371**</td>
<td>0.206</td>
<td>0.014</td>
</tr>
<tr>
<td>Aquaphor®+ D-Aloe</td>
<td>2.22</td>
<td>2.77</td>
<td>2.77</td>
<td>2.32</td>
<td>1.071</td>
<td>0.246</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Significant compared with groups 2, 3, and 6 ($p < .05$)
**Significant compared with groups 2, 3, and 5 ($p < .05$)
*Significant compared with groups 2 and 4 ($p < .05$)
+Significant compared with groups 2 and 3 ($p < .05$)
+Nonsignificant between all treatment groups
+CSignificant compared with groups (p < .05)
glycerin alone and nitroglycerin plus D-Aloe ($p \leq .05$).

**Breaking strength**

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference ($p = .114$) (Fig. 2). However when group 6 is compared with the untreated control group (group 7) there is a significant increase in breaking strength ($p = < .05$). The increase in breaking strength of group 1, compared with the other groups, while not significant, still appears stronger than most with the exception of group 7.

**DISCUSSION**

We have been exploring the beneficial effects of *Aloe vera* for the past 14 years (Heggers et al., 1985; Heggers et al., 1996; Robson et al., 1982). Its healing properties have been noted in the Holy Bible and Ebers Papyrus. Extracts from the *Aloe vera* plant have been shown to have beneficial heterogenous properties, these include an ability to penetrate tissue; anesthetize tissue; preclude bacterial, fungal and viral growth; act as an anti-inflammatory; and dilate capillaries and enhance blood flow (Robson et al., 1982).

Because the application of topical chemotherapeutic agents is an essential component in the control and prevention of infection, the effects of these agents on the healing process was assessed (Heggers et al., 1991; Leitch et al., 1993).

Concomitantly D-Aloe's role in reversing the wound retardant effect of Aquaphor®, nitroglycerin, was also compared with L-NAME because wound healing is the ultimate goal for all wounds whether infected or not.

In previous studies L-NAME accelerated the healing process in our animal model (Fig. 1). We then compared a variety of compounds that would mimic NO, and the vehicle, the compounds were used separately and in combination.

D-Aloe alone and in combination significantly accelerated the healing process compared with individual products (Table 1). Whereas there was no significant difference overall in the breaking strength of the treated wound, only one exception existed and that was the L-NAME, Aquaphor®, *Aloe vera* combination when compared with the untreated control (Fig. 2).

L-NAME's contribution to the healing process is to limit the amount of oxygen radicals generated by endogenous NO, thus reducing the potential for tissue ischemia. However, D-Aloe inhibits TxA₂, thus preventing vasoconstriction and preventing dermal ischemia. It is likely, however, that the elimination of NO and TxA₂ with L-NAME/D-Aloe combination may also have other biological consequences such as control of cytokine release. Further studies are necessary to clarify what additional mechanisms of control are exhibited by such a combination.

**ACKNOWLEDGMENTS**

The authors wish to acknowledge Ms. Cassie Maness for her devoted and conscientious ef-
forts in the preparation of this manuscript. We would also like to acknowledge our Graphic Arts Department: Ms. Sandra Baxter, our medical Illustrator, who provided timely and precise illustrations of the data, and Mr. Lewis Miltutin, Jr, and Ms. Tina Garcia for their photographic expertise. This research was funded in part by the Shriners Burns Institute, the Division of Plastic Surgery, and the Division of Reproductive Sciences, Department of Obstetrics and Gynecology, UTMB Galveston, TX.

REFERENCES


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