Lab Anim Res 2012: 28(1), 17-21
http://dx.doi.org/10.5625/lar.2012.28.1.17

Laboratory Animal Research
http://submission.kalas.or.kr

A comparative study of the effects of topical application of *Aloe vera*, *thyroid hormone* and *silver sulfadiazine* on skin wounds in *Wistar* rats

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Many research studies report the healing effects of *Aloe Vera*, *thyroid hormone* cream and *silver sulfadiazine*. However, the effects of these therapeutic agents are not well understood and have not been compared in one study. This study aimed at investigating the effects of topical application of an *Aloe vera* gel, a *thyroid hormone* cream and a *silver sulfadiazine* cream on the healing of skin wounds surgically induced in *Wistar* rats for determining the treatment of choice. In a randomized controlled trial, twelve male rats, aged 120 days and with a mean weight of 250 to 300 g, were divided randomly into 5 groups based on drug treatments: *Aloe vera* gel (AV), *thyroid hormone* cream (TC), *silver sulfadiazine 1%* (S), vehicle (V) and control. To evaluate the efficacy of each treatment technique, a biomechanical approach was used to assess tensile stress after 14 days of treatment. Tensile stress was significantly improved in the *Aloe vera* gel group as compared with the other four groups ($P \leq 0.05$). While the other treatment options resulted in better healing than the control group, this difference was not significant. We conclude that *Aloe vera* topical application accelerated the healing process more than *thyroid hormone*, *silver sulfadiazine* and vehicle in surgically induced incisions in rats.

Key words: Wound healing, *Aloe vera*, *thyroid hormone*, *silver sulfadiazine*, incision, topical application

Received 17 December 2011; Revised version received 1 February 2012; Accepted 21 February 2012

Skin wounds, with a prevalence of approximately 10% among hospitalized patients and 20% among bedridden patients treated at home, are considered the second most common cause of work absenteeism, pose high morbidity, and impose high financial costs [1,2]. However, the therapeutic effects of various treatment options, such as antibacterial agents and herbal medicines, have been extensively studied on different types of skin wounds [3,4].

Aloe vera, a tropical cactus of the lily family, is widely known for its therapeutic effects and has been used as a natural remedy for centuries [5]. Topical administration of *Aloe vera,* as a curative agent is reported to be highly effective in accelerating the healing process of wounds and burns [6]. *Aloe vera* gel or the mucilaginous portion of *Aloe vera* consists of several pharmacologically active ingredients which stimulate fibroblasts during the formation of cicatricial tissue, enhancing the deposition of collagen fibers in the extracellular matrix [7], as well as increasing collagen concentration and tensile strength [8,9].

Thyroid hormone is another agent that contributes to optimal epidermal cell proliferation [10-13]. An increase in *thyroid hormone* delivered in drinking water

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improved the quality of wound healing in euthyroid rats leaving less scar tissue [14,15]. Several reports recommend thyroid therapy for hypothyroid patients undergoing radiation-induced neck fistulae [16-18]. This agent stimulates keratinocyte proliferation, epidermal formation, dermal thickening, and hair growth [12,19,20]. *Thyroid hormone* is also a collagen stimulator that leads to skin thickening [21,22]. However, the effects of *thyroid hormone* on wound healing are not well understood or supported [23,24].

Silver sulfadiazine (SSD) is a topical anti-infective agent used to prevent and treat infections of wounds [3]. SSD is widely applied for deep partial-thickness and full-thickness injuries despite the fact that this agent has cytotoxic effects on fibroblasts and keratinocytes in vitro and leads to retardation of wound healing in vivo. Although it is the most extensively used topical agent for the management of wounds, little is known about the effects of SSD as an antimicrobial agent on surgically induced skin incisions [25-28].

The present study was conducted to investigate and compare the effects of topical application of an *Aloe vera* gel, a *thyroid hormone* cream and a *silver sulfadiazine* cream on the healing process of skin wounds surgically induced in *Wistar* rats.

Materials and Methods

Twelve *Wistar* strain male rats, aged 120 days and with a mean weight of 250 to 300 g, were obtained from the breeding center of the Anatomical and Biological Sciences Center, Shahid Beheshti Medical University (SBMU) Tehran, Iran. The animals were maintained under natural light and humidity conditions (temperature 22ºC; 12-h light/dark cycles) in individual cages cleaned daily, fed once a day, and offered water *ad libitum*. The study was approved by the Institutional Medical Ethics Committee of Shahid Beheshti Medical University.

On day 0, the rats were anesthetized by the intra-muscular injection of 50 mg/kg ketamine hydrochloride (ROTEX, made in Germany) and 5 mg/kg diazepam (TPICO, made in Iran). Their dorsal hair was shaved, and their skin was cleaned with povidone-iodine. The rats were equally divided into experimental and control groups randomly (six rats in each group). Four incisional wounds measuring 20 mm in length were made in the dorsolateral regions of each experimental rat and one incisional wound in each control rat. All the incisions were performed in the skin and subcutaneous cell tissue using a scalpel. The depth of the surgical incisions was controlled by removing the epithelial tissue to the extent that the dorsal muscular fascia was exposed. The wounds were closed with sutures of 3-0 silk.

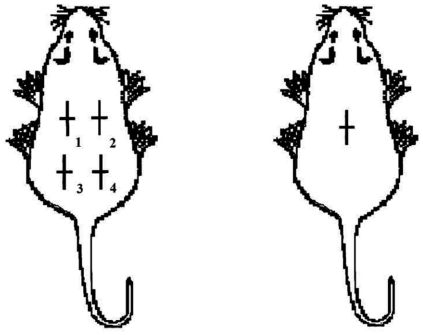

Figure 1. Diagram of the wound healing model.

The wounds in the experimental rats were treated with: *Aloe vera* gel (AV), *thyroid hormone* cream (TC), *silver sulfadiazine* 1% (S), or vehicle (V). All the therapies were initiated on day 0 and repeated daily for 14 consecutive days. The wounds in the control group received no medication (Figure 1).

Preparation and administration of *Aloe vera* gel, *thyroid* cream and *silver sulfadiazine* cream

An *Aloe vera* plant was obtained from a medical plant garden and sent to the Laboratory of Anatomical and Biological Sciences Center, Shahid Beheshti Medical University (SBMU) for the extraction of *Aloe vera* gel. After washing the leaves thoroughly, the base, apex and margins were cut off carefully to facilitate the slicing of the *Aloe vera* leaves, revealing the transparent mucilage. The transparent mucilage was carefully removed into beakers and further processed in a blender. A greenish gel-like liquid was obtained which was further refined through the use of a strainer. We also prepared a topical T3 cream by dissolving 150-ng of T3 (Sigma, made in Spain) in 20 μL of ethanol and then mixed it into the vehicle [29,30]. *Silver sulfadiazine* was purchased from Sobhan Daru Co. as a synthetic cream.

Collection and preparation of wound samples

The tensile strength of the wounds was measured in both the experimental and control groups on day 14 after surgically inducing the wounds. We cut standardized

5mm-wide skin strips perpendicularly to the incisions with a double-bladed cutting instrument. The specimens were placed in 0.9 percent saline after necropsy and were evaluated within one hour. From each wound, we obtained one strip specimen and placed it in a material testing machine (Zwick, made in Germany) using two griping clamps. The distance between the edges of the clamps was 5 mm and the specimens were loaded uniaxially while the deformation rate was kept constant at 15 mm a minute so that failure and complete load-deformation curves were recorded by transducers coupled to bridges. From these curves, we analyzed the parameters of (1) ultimate tensile stress (newtons/square millimeter), which was derived from the ultimate load divided by the original unstrained cross-sectional area; (2) ultimate tensile strength (Fmax newtons), the load that causes a structure to fail; and (3) the area under the load-deformation curve (newton millimeters), the amount of work done by the deforming load.

Data analysis

Data were summarised as mean±SEM. We used a one-way analysis of variance and t tests to compare the data related to ultimate tensile stress, Fmax, and area under load-deformation curve. All the analyses were performed using a SPSS System on a personal computer. A probability (P) of <0.05 was considered significant.

Results

Since an increase in the synthesis of collagen occurs in the wound healing process, we used a biomechanical method to examine and compare among the treatments for tensile stress, Fmax, and area under load-deformation curve (Table 1).

As Table 1 indicates, the tensile stress was not significantly different among TC, AV and S. Averaged ultimate tensile stress was greater in the AV, TC and S groups than in the V and control groups, but this difference was not statistically significant.

Fmax was statistically different between AV and the other groups (P<0.05). No difference in Fmax was observed among the TC, S, V, and control groups.

The area under the load-deformation curve was significantly greater in the AV group than in the other groups and was not significantly greater in the S and TC groups than in the V and control groups.

Discussion

Skin wound healing is a dynamic response to injury that has three overlapping phases: inflammation, granulation tissue formation and remodeling. During the wound healing process, especially the transition from granulation tissue to scar tissue formation, collagen remodeling occurs, that is, the degradation of collagen with the formation of larger collagen bundles and an increase in the number of intermolecular cross-linkages. This process is controlled by several proteolytic enzymes called matrix metalloproteinases that are discharged by fibroblasts, macroghages, epidermal cells and endothelial cells. The tensile strength of a wound can be related to its collagen formation and maturation. On the other hand, the strength of the repaired wound tissue is the result of the remodeling of collagen and the formation of stable intra-and inter-molecular cross-linkages [31].

This study examined and compared the effects of *Aloe vera* leaf gel, *thyroid hormone* cream, and *silver sulfadiazine* cream on the healing of skin incisional wounds in rats by biomechanical methods to assay the tensile stress of the wounds. According to the results of the study, Fmax and the area under the load-deformation curve significantly increased in the wounds treated with *Aloe vera* as compared with the wounds treated with *thyroid hormone* and *silver sulfadiazine,* suggesting that *Aloe vera* was more effective in healing the wounds, which has been observed in other studies. A possible explanation for this increase is that the *Aloe* leaf gel extract infiltrates into the wounds, contributes to the increase of fibroblasts, macrophages, and epidermal cells activities, subsequently facilitates enzyme activity

Table 1. Comparison of the means of biomechanical properties of incision wounds 14 days after surgery

Parameter	Aloe vera	Thyroid hormone	Silver sulfadiazine	Vehicle	Control
Tensile stress	3.66±0.61	3.00±0.25	3.33±0.33	2.83±0.30	2.16±0.30
Tensile strength	9.50±0.84*	6.16±0.60	6.00±0.51	6.16±0.70	4.50±0.88
Area under load-deformation curve	56.50±6.93*	27.83±5.36	34.16±3.75	23.83±3.80	27.33±5.92

(*) shows significant differences.

during collagen remodeling, and possibly even aids in the formation of cross linkages as the collagen matures. In other words, Aloe vera may have a direct effect on the wound healing process as a whole, which is manifested as an increase in Fmax and the area under the load-deformation curve, suggesting an improvement in the tensile strength of the wounds. The results are also in agreement with those of Chithra *et al.* that attributed this improvement in tensile strength to an increase in the aldehyde groups of collagen fibres responsible for forming cross-linkages [8]. Subramanian *et al.* also confirmed the effect of *Aloe vera* on increasing wound contraction and collagen synthesis and attributed this to the mannose-6-phosphate known to be present in *Aloe vera* leaf gel [32]. Mannose-containing products have been shown to increase macrophage activity and therefore stimulate fibroblast activity and collagen synthesis [33,34].

The results indicate that *thyroid hormone* did not have any significant effect on the healing of the incisional wounds. Thus, the results do not provide evidence for Safer *et al.* who found that topical triiodothyronine stimulated epidermal proliferation, dermal thickening, and hair growth [12,19]. One possible explanation for this discrepancy is the difference in the type of wound between the two studies; in the present study incisional wounds were under investigation while in the other, excisional wounds. An incision is a cut made into the tissue, mainly for operation, therefore topical medication must infiltrate into the tissue to accelerate the healing process. On the other hand, an excision is a removal skin tissue and topical medication is put on the wound directly, which may contribute more to the healing process. Another possible explanation is the difference in the methods of assessing wound healing: Safer *et al.* used a histological approach to assess whether topical triiodothyronine stimulates epidermal proliferation, dermal thickening, and hair growth while in the present study we drew on a biomechanical assessment method to explain the physical characteristics of the skin tissue.

The findings of the current study indicate that SSD was not significantly effective on skin incisions, providing more evidence for the studies conducted by McCauley *et al.* and Cooper *et al.*, in that they explained the inhibitory and cytotoxic effects of SSD and mafenide acetate on human keratinocyte and fibroblast growth [25-28]. Leitch *et al.* also found inhibitory effects of antimicrobial agents on wound contraction in an acute rat wound model [35]. Muller *et al.* compared the impact of SSD with or without *Aloe vera*, nystatin with and without SSD, and placebo on time to achieve 50% and 90% wound healing in excisions of *Sprague-Dawley* rats. No difference in the acceleration of wound healing was observed between the control and SSD treatment lesions. In addition, the combination of *Aloe vera* with SSD has been suggested to improve wound healing [36]. Such inhibitory effects may be related to the cytotoxic characteristics of SSD on cells synthesizing collagen, proteoglycan, and other products.

The results of this study indicate that topical application of *Aloe vera* can lead to significantly rapid wound healing and stronger repaired tissue. We can conclude that *Aloe vera* can effectively be applied as a topical treatment to accelerate the wound healing process of surgically induced incisional wounds in rats as compared with other topical applications such as*: thyroid hormone*, *silver sulfadiazine*, and vehicle. We also recommend further studies using histological assessment for further approval.

Acknowledgments

The financial support provided by Shahid Beheshti Medical University is greatly appreciated.

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