Treatment with lyophilized-albumin on cutaneous excision lesions

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Summary

This experiment studies the therapeutic effect of a new ointment based on lyophilized albumin extracted from egg on cutaneous excision lesion. The therapeutical assessments of the proposed ointment were done in vivo on the excisions excided on Wistar rat. The wounds were treated topically once a day, along 21 days. Clinical and macroscopic tests, tissue epitalization time wound contraction rate and histopathological examination were evaluated. The analyzes results revealed a significant healing effect of the new lyophized-albumin on the skin excision lesions by stimulation of the regeneration and the maturation of granulation tissue, antiinflammatory effect, collagenation effect at the lesion site resulting an incision-like mark hard to be seen without vicious healing in terms of both the clinic, macroscopic and histological point of view.

Introduction

Because every day the skin is exposed to various physical aggressions this tissue has an important role for controlling the homeostasis and act as a protective barrier of against the harmful environment (Young et al., 2005). However, the healing lesions involves a specialized medical service with high costs (Warden at al., 2005), the new intrution treatments are strongly demanded for the cutaneous wound healing based on tissue regeneration process (Cohen et al., 2005). The many research works done so far expresses the high interest of the scientists for discovery the new medical methods for treating cutaneous lesions (Farinella et al., 1986; Martini et al., 2011; Serafini et al., 2012, Favit et al., 1992; de Mesquita et al., 2010; Aoyagi et al., 2007; Upadhyay et al, 2009; Hafezi et al, 2010; Csupor et al, 2010; Akkol et al, 2011). The wound healing is a complex process which implies some events such as: inflammation, re-epithelization, cutaneous tissue reconstruction and remodeling (Stadelman et al, 1998).

The porpuse of the present sutdy is to evalute the effect of a new ointment based on lyophilized albumin on circular excision. The albumin is a natural polymer extracted from the lyophilized egg white beeing capable to creat a protective film on the skin lesion and could be a good candidate for cutaneous wounds healing.
Materials and methods

Materials and ointment preparation

The vaseline, lanolin and olive oil (Olivae oleum virginae) were purchased from Sigma – Aldrich Ltd.

Ointment base was prepared by mixing the lanolin and vaseline in warm water bath (40 °C) for obtaining of a homogeneous base ointment.

Lyophilized albumin was extracted from the egg was by segregation process of the white egg and the yolk then the samples were freezed in Petri dishes and lyophilized. After the lyophilization process was done the lyophilized albumin was deposited in brown bottles and kept in cold atmosphere away from the moisture. In the ointment base was added 3 g of lyophilized albumin under continuous stirring until homogenisation.

Animals for experiment

The experiment was carried out on adult male rats from Wistar race with a weight of 230 ± 10 g. The animals were hosted in separate cages with a free access to the standard laboratory diet and the cages were placed in a bright room with a constant temperature (22±0.5°C) and humidity (65-70%). The protocol followed the instructions of European Council Directive from November 24th, 1986 (86/609/CEE).

Experimental model of tissue lesions

For this research, the rats were randomly assigned into three equal groups (n = 7 in each group) as follows:

First group: a negative control group was untreated.

Second group: a group treated topically with vaseline only.

Third group: a group treated topically with a new lyophilized albumin-based ointment, respectively.

The rats were intravenous anesthetized with ketamine hydrochloride (100 mg/kg body weight), and after the back region of the animal was clipped and disinfected with 70% alcohol, a circular wound was excised. In this study, the design of the excision was based on a previously described model in Suntar et al., 2010 adapted to our experimental conditions. The circular excision was made on the interscapular region of each rat with a 8 mm of biopsy punch. The wounds were left opened and the treatment was applied daily on the top of the excision, once a day, along 21 days.

Experimental evaluations

Clinical and macroscopic evaluation, period of re-epithelialization, and histopathological examination were performed at days 1, 3, 9 and 21 of treatment.

I. Clinical assessment

The clinical assessments were carried out for studying the following evolutions: edema, inflammatory infiltrate, congestion, formation and the debridement crust and the type of scar.

II. Histopathological examination

In the end of the experiments, in order to determine the evolution of epithelization process and the histopathological examination a specimen sample of the healed tissue was removed under anesthesia and using a 3 mm punch biopsy. The collected samples were inserted in 10% buffered formalin for at least 24 hours, progressively dehydrated in solutions containing an increasing percentage of ethanol (60, 80, 90, and 98%, v/v), which then rinsed with amyl alcohol, embedded in paraffin under vacuum condition. Further, the skin tissue was cut with 5 μm in thickness, deparaffinized, and stained with Hematoxylin–Eosin (HE) and Szekely (Sz).

III. Re-epithelization period

The lesion closure was considered the final stage of the wound healing and the terminus point of the epithelization process. The days required to complete this process were defined as epithelization period and was determined using macroscopic observation in the days 1, 2, 3, 6, 9, 12 and 21, respectively of the treatment and stored as digital pictures.

IV. Wound contraction rate

Wound contraction rate (WCR) was calculated using the following formula:

\[
WCR = \frac{A_0 - A_t}{A_0} \times 100\%
\]

where the A0 is the initial wound area (50.27 mm2), At is the wound area expressed in mm2 using SigmaScan software (SigmaScan
Ro 5.0) and calculated in the days: 0, 6, 9 and 12, respectively of the treatment.

V. Statistical analysis

Obtained data from excision model were analyzed by one-way ANOVA software analyzed with one-way ANOVA followed by Bonferroni post-test. The analysis of statistics was performed using SPSS 15, where p<0.05 was considered statistically significant.

Results and discussions

Macroscopic evaluation

Macroscopic evaluation of the epidermal wounds treated with the lyophilized-albumin demonstrated the efficacy of the treatment based on the new proposed ointment, Figure 1. In this study, the complete healing for the excision lesions was found after 9 days of treatment.

Epithelization period

Epithelization period was shorter, about 12 days, for the group treated with lyophilized-albumin ointment compared with the one treated with ointment base only (18 days) or the other group untreated for which the complete healing occurred after 21 days.

Fig.1. Macroscopic pictures for healing evolution of the cutaneous wounds during the lyophilized albumin treatment.

Wound Area

Lesion area was measured every day during the treatment. The experimental determination of the area is displayed in Table 1 for day 0, 6, 9, and 12.

Table 1. Excision Area.

<table>
<thead>
<tr>
<th>Group</th>
<th>Excision Area (mm²)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Lyophilized-Albumin Ointment</td>
<td>64.00 (SD)</td>
</tr>
<tr>
<td>Base-Ointment</td>
<td>64.00 (SD)</td>
</tr>
<tr>
<td>Negative Control</td>
<td>64.00 (SD)</td>
</tr>
</tbody>
</table>

Wound Contraction Rate

No important changes concerning the wound contraction was observed in the first three days of the treatment (as these are the days when inflammatory processes take place). The cellular proliferation began after day 3, and significant reduction in the wound areas was achieved at days 6 and 9 (p<0.001). There can be noted that the lyophilized albumin ointment presented favorable effects since the day 6 of the experiment and the WCR was 64.06 ± 0.45 for the group treated with lyophilized albumin ointment versus 26.74 ± 1.23 for the group treated with base ointment while for the negative control group was about 5.78 ± 1.79 (Table 2).

In the day 9 of the treatment the results were evidently positive for the lyophilized albumin ointment group, the WCR being 97.14 ± 0.14 versus 33.79 ± 1.28 (the wound closure for the group treated with the ointment base) or versus 14.85 ± 1.7 for the not-treated group. The epithelization process was completed after 12 days in the case of the proposed lesion model. Surface lesion re-epithelization with a new epithelium supposed the emigration and proliferation of keratinocytes from the margins of the lesion (Stadelman et al, 1998) involving elements like: subcell exchange, extracellular matrix, integrin receptors and growth factors (Soo et al., 2000).

Histopathology tests

Fresh excision was characterized by 2-3/hpf lymphocytes and fibroblasts with slight edema, one capillary vessel and a focal fibrosis.

Table 2. Wound Contraction Rate.
Fig. 2. Images of histological tissue section for fresh excision on the rat skin: (a) Slight dermal edema, HEx100; (b) Congestion, HEx100; (c) Congestion, HEx100.

Day 3: The group treated with ointment based on lyophilized albumin on excision showed a granulation tissue with a mild inflammatory infiltrate which affected the hypodermis only. For the base ointment group was observed the inflammatory infiltrate localized at hypoderm and the intresticial regions of muscle tissue. For untreated group the wounds were characterized by the histology with an inflammatory infiltrate, edema as well as an inflamation of the muscle fragment.

Day 9: In this day of the experiment the animals subjected to the lyophilized albumin creme presented wounds with remarkable healing decribed by the granulation mature tissue while for the rats treated with base ointment only had a dermal acanthosis and non-treated group showed a large lymphoplasmacytic inflammatory infiltrate. These results can conduct to the conclusion that the novel proposed ointment based on albumin extracted from the white egg could have an important antiinflammatory effect on the excision lesions as well as on the normal regeneration tissue process free from defects of the cutaneous tissue architecture.

Day 21: This day is characterized by the presence of the mature granulation tissue with a normal collagenation for the excision lesions treated with lyophylized-albumin creme. This fact can be understood in terms of the first stage of collagenation when could be stimulated by the albumin which is adjusted later on by the reparatory process. Usually, if the collagen production continually increase the hyper collagenation will generate the fibrous tissue with keloid scars. This does not happen in our experiment which means that albumin can manage efficiently this process. On the other hand, in the base-ointment group was observed an inflammatory infiltrate in hypoderm while in the case of negative control group the inflammation infiltrate went down deeply including the striated muscle.

Fig. 3. Images of histological tissue section for excisions on the day 3: (a) Ulceration, hypoderm, HEx200; (b) Ulceration, hypoderm, details, HEx400; (c) Rich inflammatory infiltrate, HEx200; (d) Interstitial inflammatory infiltrate, HEx100; (e) Granulation tissue with inflammatory infiltrate with the point of interest in hypoderm, HEx100; (f) Inflammatory infiltrate in hypoderm, details, HEx200.

Although, in the literature can be found some studies made on the natural products for healing effects on the cutaneous wounds with favorable results there are no investigations on lyophilized albumin formulation (de Mesquita et al., 2010; Aoyagi et al., 2007; Upadhyay et al., 2009; Hafezi et al., 2010; Csupor et al., 2010; Akkol et al., 2011). Generally, the cutaneous lesion treatment especially, when the wound is accompanied by the significant lose of the tissue, the healing ends when the wound is completly closed regardless the origin of the trauma. Sometimes, the doctors use the skin graft to fill up the lack of the tissue for reducing the multiple risky complications such as the lose of the liquid, electrolytes and proteins, and the bacteria colonization and finally, the inflammations issue. This method helps the reconstruction of the affected tissue in terms of the functional and aesthetic aspects because ensure the healing of the wound and preventing the hypertrophy (Xu et al., 2004). Our proposed ointment are tested for the first time in cutaneous wounds healing.
However, our treatment studied in this research proposed a topically application of the new ointment using lyophilized albumin which acted as a wound dressing on the lesion and in the perilesional site.

![Negative Control Group](image1)

**Fig. 4.** Images of histological tissue section for excisions on the day 9:
(a) Lymphoplasmacytic inflammatory infiltrate, HEx100; (b) Lymphoplasmacytic inflammatory infiltrate, HEx100; (c) Epidermis acanthosis, HEx100; (d) Epidermis acanthosis, HEx200; (e) Mature granulation tissue, HEx100.

![Base Ointment Group](image2)

![Lyophilized-Albumin Ointment Group](image3)

**Fig. 5.** Images of histological tissue section for excisions on the day 21:
(a) Inflammatory in striated muscle, HEx100; (b) Inflammatory infiltrate in hypoderm, HEx100; (c) Inflammatory infiltrate in hypoderm, details, HEx200; (d) Mature granulation tissue, dermal collagenation, HEx100.

**Conclusions**

The novel lyophilized-albumin ointment showed an important effect on the stimulation and regeneration process of granulation tissue, an antiinflammatory and the normal collagenation effect on the excision lesions resulting an incision-like shape hardly seen characterized by the non-vicious healing from clinical and macroscopic point of view as well as histology.

**References**


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