The Effects of Sericin Cream on Wound Healing in Rats

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Sericin has good hydrophilic properties, compatibility, and biodegradation, it can be used as a wound-healing agent. We evaluated the effects of sericin on wound healing and wound size reduction using rats by generating two full-thickness skin wounds on the dorsum. Group 1 animals were treated with Betadine® on left-side (control) wounds and, with 8% sericin cream on right-side (treated) wounds. Group 2, cream base (formula control) and 8% sericin cream (treated) were topically applied to left- and right-side wounds respectively. Sericin-treated wounds had much smaller inflammatory reactions, and wound-size reduction was much greater than in the control throughout the inspection period. Mean time in days for 90% healing from sericin-treated wounds was also much less than for cream base-treated wounds. Histological examination after 15 d of treatment with 8% sericin cream revealed complete healing, no ulceration, and an increase in collagen as compared to cream base-treated wounds, which showed some ulceration and acute inflammatory exudative materials.

Key words: wound healing; sericin; histopathological inspection; wound-size reduction

Materials and Methods

Materials. Sericin powder was kindly supplied by Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. Petrolatum, mineral oil, lanolin, glycerin, bisabolol, propylparaben, and methylparaben were purchased from Sigma (Singapore), and were used without further purification. Betadine® (a 10% povidone-iodine solution) was purchased from a local drug store in Bangkok. All other chemicals were of extra-pure reagent grade and were used as received.

Animals. Eight week-old male Sprague-Dawley rats purchased from National Laboratory Animal Center of Mahidol University, Thailand, weighing 250 ± 5 g, were used in the experiments. Each rat was caged alone at 25 ± 2 °C and subjected to a 12:12 h light–dark cycle.

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(standard fluorescent light), and allowed Chow and water
*ad libitum*. The rats were acclimatized for 1 week before
use. They were maintained according to the “Guide for
the Care and Use of Laboratory Animals” established by
National Laboratory Animal Center of Mahidol Uni-
versity, Thailand.

*Sericin cream preparation.* White petrolatum, mineral
oil, lanolin, glycerin, bisabolol, propylparaben, and
methylparaben were used to formulate a sericin cream
base. For 8% sericin cream, sericin powder was
dissolved in warm water and then mixed with other
ingredients during cream-forming process.

*In vivo animal tests.* There were 18 rats in total, which
were divided into two groups of nine each. Two full-
thickness skin wounds in each rat were prepared by
excision (1.5 × 1.5 cm) on the dorsal of each rat under
aseptic surgery. In group 1, Betadine® was topically
applied on left-side wounds (control wounds) and 8% sericin cream was topically applied on right-side wounds
(treated wounds). Group 2, cream base (formula control
wounds) and 8% sericin cream (treated wounds) were
topically applied to left-side and right-side wounds
respectively. Surgery was performed with the animal
anesthetized with 30 mg/kg intramuscular injection of
Zoletel, and Baytril® (enrofloxacin, Bayer, Germany),
an anti-bacterial agent, at 10 mg/kg was also injected
subcutaneously. The skin over the dorsal area was
shaved completely and application fields were outlined
with a marking pen just prior to skin excision. Rimadyle®
(carprofen, Pfizer, USA) at 5 mg/kg was injected subcu-
taneously every 24 h for 5 d as pain reducer. All wounds were cleaned daily with sterile
normal saline solution. Betadine®, cream base or sericin
cream was applied to wounds daily after cleaning. All
creams were applied evenly in sufficient amounts to
cover all wound areas. The rats of each group were
scrutinized for 15 d after application, during which the
wound surfaces were observed. Body weights and skin
irritations were observed daily. This protocol has been
approved by Mahidol University Animal Care and Use
Committee (MU-ACUC).

*Wound-size measurements.* All wounds were measured
for area with a stereomicroscope (Carl Zeiss,
Germany, Primo Star model, 0.3 × 0.65 objective lens),
and photographed with Moticam 2300® at 1,024 × 768
pixels. Motic Images Plus 2.0 ML was used to analyze
the data.

*Histological evaluations.* After 5, 10, and 15 d, three
rats in each group were terminated and skin samples
were taken. The central portion of underlying tissue was
taken and fixed in 10% buffered formalin. Each speci-
men was embedded in a paraffin block and thin sections
(3 μm) were prepared, and stained with hematoxylin-
eosin, and Masson’s trichrome method. Wound-healing
effects were examined histologically under a light
microscope, an Olympus BX 50, using low-power
magnification, at 200X and 100X.

*Statistical analysis.* All data were expressed as the
mean ± SD. Evaluation of statistical significance was
determined by paired and unpaired Student’s *t*-test. A
*p* < 0.01 was considered significant.

*Results and Discussion*

The molecular weight of sericin powder was deter-
mined using prestained SDS–PAGE standards broad
range and Bio-Rad® as markers. The results indicate
that sericin powder contains peptides with molecular weights
ranging from 90 to 125 kDa, as shown in Fig. 1. A study
by Gamo *et al.* found that sericin is a complex mixture
of 5–6 polypeptides differing widely in size (40–
400 kDa).13) Normally, sericin solution without heat
treatment exhibits distinct bands of three main sericin
components at > 250, 180, and 100 kDa under SDS–
PAGE,14) but sericin extracted by autoclaving method
may show broader bands due to the mixture of different
molecular weights peptides.

Regarding toxicity, 8% sericin cream, as well as
cream base, showed no any of the toxicity in rats. All
rats gained weight during the period of observation. The
average weight during the period of observation in both
groups did not differ significantly at the end of the study.
No rats showed signs of anorexia, phlegmatic or
titubant. Table 1 shows the average weight, body-weight
gain, food consumption, and food efficiency ratio of rats
in both groups during the experimental period. There
was no statistically significant difference between body
weights of rats in either group. Moreover, there was no
rash or redness of the skin in sericin-treated wounds as
compared to control wounds. These results indicate
that sericin cream is safe and biocompatible. Besides,
sericin-treated wounds healed almost completely with-
out any allergic rash while wounds not treated with
sericin still showed some inflammation.

The inflammatory reactions of the skin in sericin-
treated wounds tended to be much smaller than those in
the control throughout the inspection period. Figures 2
and 3 (A–D) show wound sizes from a group-2 rat
treated with cream base and sericin cream on days 0, 5,
10, 15. During the first few days after operation, there
was inflammation of wounds in all cases, but the wounds
improved substantially from day 10 after treatment. All
wound-area measurements were expressed as per-
centages of initial wound size. Table 2 data represent the
percent of wound-size reduction of wounds in rats in
both groups, recorded from post-wounding on days 5, 10,
and 15. These results indicate that sericin-treated
wounds in group-2 rats showed a statistically signifi-
cant difference in percent of wound-size reduction as
compared to cream base-treated wounds (*p* < 0.01),
especially at 90% healing/d, but there was no statisti-
cally significant difference in percent of wound-size reduction in control and sericin-treated wounds in group-1 rats. This might have been due to the antiseptic properties of Betadine®, which help to promote healing;¹⁵ these properties are not strongly present in sericin cream. Besides, the sample size of rats in each group was quite small. Further experiment will be performed with larger numbers of rats to eliminate individual variables.

The time taken for each animal’s wound to be reduced by 50% and 90% of its initial size was also calculated. Ninety percent healing was chosen as our end-point, since healing of the remainder of the wound to completion is generally variable and dependent upon other factors such as animal interference. Time to achieve 50% and 90% wound healing is shown in Fig. 4. Mean time in days for 90% healing from sericin-treated wounds was much less than from cream base-treated wounds (11 vs 15 days).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>Average initial body weight, g (n = 18)</td>
<td>249.60 ± 3.63</td>
<td>245.00 ± 5.54</td>
</tr>
<tr>
<td>Average body weight gain/d, g/d</td>
<td>5.42 ± 0.58</td>
<td>5.20 ± 0.33</td>
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<tr>
<td>Average food consumption, g/d</td>
<td>22.53 ± 1.74</td>
<td>24.09 ± 1.81</td>
</tr>
<tr>
<td>Average food efficiency ratio</td>
<td>0.231 ± 0.02</td>
<td>0.225 ± 0.03</td>
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*Food efficiency ratio = body weight gain/food consumption

*Significant difference at p = 0.01 by the Mann-Whitney U Nonparametric Test
Histological examinations of a wound from a group-1 rat on day 15 after treatment are shown in Figs. 5 and 6. As illustrated in Fig. 5, the left-side wound (control, treated with Betadine®) was not fully epithelialized, and some ulcers were present. Unevenness of epidermis near the ulcer was observed, collagen decreased, and there was greater inflammation than in the right-side wound or sericin-treated. Masson’s trichrome stains collagen and yields a blue color, while the red color represents cytoplasm, red blood cells, and muscle. The pattern of staining intensity corresponds to the relative quantity of collagen fiber deposit, which reflects the process of synthesis and degradation and remodeling as well as the timing of the lesion. Persistent injury causing inflammation is another factor that interferes with collagen formation and deposition. More than 70% of wounds treated with sericin cream showed complete healing from day 10 of treatment. Figure 6 represents histological results from a right-side wound treated with sericin cream. The surface of epidermis became even. These results show complete healing, no ulceration, and an increase in collagen.

Masson’s trichrome stain of healed scars, which stains blue on collagen fibers as a major component of connective tissue, shows dense collagen in sericin-treated wounds. Collagen is the most common protein in animals and ultimately provides the tensile strength of healing in wounds.10,16 Also, sericin promotes epidermis growth, as shown by full recovery of the epidermis to its normal thickness in all sericin-treated wounds.

To see the effects of cream base on wound healing, cream base and 8% sericin cream were applied on left and right-side wounds respectively. Figures 7 and 8 show histological results for skin samples of a group-2 rat after 15 d of treatment with cream base and 8% sericin cream, on left and right-side wounds respectively. Left-side wounds (Fig. 7) showed some ulceration and more inflammation than did right-side wounds.
Unevenness of the epidermis near ulcers was also observed on the cream base-treated side. Sericin-treated wounds, on the other hand, showed an increase in collagen.

The histology at 100X of left and right-side wounds of a group-2 rat is shown in Fig. 9. The sericin-treated wound showed complete healing (Fig. 9A), while the cream base-treated wound displayed some ulceration and acute inflammatory exudative material covering the ulcers (Fig. 9B).

Conclusion

All these results suggest that sericin has wound-healing effects without causing allergic reactions, but the molecular weight of this protein fraction might affect its healing property. Further investigation should be done of the absorption and wound-healing effects from different molecular weights of sericin.

Acknowledgments

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References