Note Laboratory Animal Science

Promotion of Dermal Wound Healing by Polysaccharides Isolated from Phellinus gilvus in rats

Jae-sung BAE1, Kwang-ho JANG1, Seung-chun PARK1 and Hye Kyung JIN1*

1) College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Republic of Korea

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Abstract. The effects of topical administration of polysaccharides isolated from fungus, Phellinus gilvus (PG) on the healing of rat dermal wounds were assessed. In 10 Sprague-Dawley (SD) rats, six 6 mm diameter defects were made with a punch biopsy appliance. After 24 hr, test substances were applied to the defects twice a day: 0.025, 0.25, and 2.5% polysaccharides from PG (PG0.025, 0.25, and 2.5 groups), Madecassol® ointment (MC group), aqueous gel (AG group) and no treatment (control group). Six days postoperatively, the contraction and reepithelialization of the wound surface were assessed. Wound diameter was significantly reduced in all PG groups (P<0.05). Complete epithelialization and macrophages were noted in the PG0.25 group, as compared to the control group. We conclude that polysaccharides isolated from PG have significant dermal wound healing effects, and this investigation suggests the potential clinical application of PG as a wound healing agent.

Key words: Phellinus gilvus, polysaccharides, wound healing.

Wound healing is a fundamental response to tissue injury. The healing process can be related to inflammation leading to epithelialization, formation of granulation tissue, and tissue remodeling [9]. Several natural products have been investigated in the promotion of wound healing [17, 18]. Among them, Madecassol® from qualitative extract of Centella asiatica is a well-known commercial ointment for promoting of dermal wound healing.

Recently, polysaccharides isolated from Phellinus mushrooms have received special attention due to their potent pharmacological activities, including anti-tumor [2, 4, 5, 10], immunostimulating [15], and anti-inflammatory properties [13, 14]. Among these Phellinus mushrooms, PG used in this study has advantages over the other Phellinus mushrooms in that it has a very short growth period (3 months) compared to other Phellinus mushrooms (2-3 years) making it cheaper to produce. In addition, our recent studies have demonstrated significant anti-inflammatory properties of PG such as inhibition of pulmonary inflammation [11] and prevention of intraperitoneal adhesion under infection [1, 3, 6]. We therefore predict that medicinal application of PG has many advantages in medical cost-cutting as well as benefits in clinical application in future. Its anti-inflammatory activities suggest that this natural product may be beneficial in the promotion of dermal wound healing related to inflammation. This is the first report of PG promoting dermal wound healing in vivo. Here we investigated whether polysaccharides isolated from PG can enhance dermal wound healing in rats.

Ten male SD rats (Charles River Korea Inc., Bio Genomics, Korea) weighing 275 to 298 g were acclimated for 1 week before the experiments. They were housed in an atmosphere controlled room (temperature: 22 ± 3°C, relative humidity: 50 ± 10%, air circulating frequency: 13-17 times/hr, artificial light: 300 Lux from 8 am to 8 pm, noise: < 50 db), maintained in clean and sterile polyvinyl cages, and fed with commercial rat feed from Orient Inc., Korea. Food and water were provided ad libitum to the animals. All animal experiments were carried out in accordance with the Guidelines for Animal Care and Use of Kyungpook National University. The fruiting body of PG was kindly provided by Gyeongbuk Agricultural Technology Administration (Daegu, Korea). It was homogenized, extracted under optimal water extraction conditions, distilled water (1:25) at 100°C for 10 hr (unpublished data), and concentrated at 80°C in a rotary evaporator. The recovery procedure of the polysaccharides solution from the fruiting body of PG was performed by the method of Kim et al. [12]. The concentration of 0.025, 0.25, and 2.5% polysaccharides solutions was determined by total sugar content with the anthrone method [7] with glucose as the standard material. These solutions, formulated as a suspension in a small amount of aqueous gel (Biosonic®, AMITIE Co., Korea) and Madecassol® ointment (MC), were from Dongkook Pharmaceutical Co., Korea.

The animals were weighed, the dorsal aspect of the back was clipped and disinfected with 70% alcohol under Xylazine and Ketamine anesthesia (5 and 40 mg/kg, intramuscularly, respectively). Under aseptic conditions, six 6 mm diameter circular defects were made with a punch biopsy appliance. Holes were made 1.25 cm to the right and left of the midline, separated from the test "hole" cranial or caudal to it by 2.5 cm. After the surgery, the skin defects were bandaged with a Tegaderm® (3M Health Care, U.S.A.). Each hole punch was preassigned to a test substance at the time of surgery. The assignment was randomized in such a way that each test substance would be applied at a different wound
location in each animal. This was done to prevent experimental bias on the basis of experimental defects being subjected to different degrees of stretch with animal body movement. At 24 hr postoperatively, test substances were applied to punch-hole defects and to shaved skin 3 mm lateral to those areas. Application of test substances was continued twice a day for the next 5 days. Specific treatments were as follow: 0.025, 0.25, and 2.5% polysaccharides isolated from PG (PGO.025, PGO.25, and PGO.25 groups), Madecassol® (MC group), aqueous gel (AG group) and no treatment (control group). The animals were sacrificed by carbon dioxide asphyxiation at 6 days postoperatively (5 days after initial treatment with test substance).

The wound surface area was determined by measuring the craniocaudal and lateromedial distances. The average of those two measurements was obtained and used to determine wound diameters. For histopathologic studies, tissue was collected after sacrifice and fixed in 10% buffered formalin. After routine tissue processing, 5 μm thick sections were cut and stained with hematoxylin and eosin. The sections were qualitatively assessed under a light microscope with respect to epithelialization, congestion, edema, infiltration of polymorphonuclear leukocytes and monocytes, necrosis, fibroblastic proliferation, collagen formation and angiogenesis. The degree of reepithelialization was estimated as a % of the incision width reepithelialized in each wound tissue.

All data are presented as the mean ± SD. Statistical analysis among the groups was performed by analysis of variance followed by multiple comparisons and Fisher’s LSD test with the SAS statistical package (release 8.1; SAS Institute Inc., Cary, North Carolina, U.S.A.). Differences were considered to be significant at P<0.05.

Location of the wound had no quantitative effect on contraction of the wound and reepithelialization of tissue in any of the treatments. The most dramatic results were seen in all PG groups, compared to the control or MC group. Wound diameter was smallest in the PGO.25 group (Fig. 1 and Table 1). Predicted wound diameter of the control and each experimental group by measuring craniocaudal and lateromedial distances are shown in Table 1.

Microscopic examination of the wound dermis showed proliferation of fibroblasts, hemorrhage and angiogenesis. The degree of the parameters did not show a significant difference among the groups, but complete epithelialization was noted in the PGO.25 group. Regenerated epithelium

Table 1. Wound diameter predicted by measuring craniocaudal and lateromedial distance (n=10, values are the mean ± SD). Application of test substances to 6 mm wound defects 24 hr postoperatively and twice a day until 6 days postoperatively

<table>
<thead>
<tr>
<th>Groups</th>
<th>Predicted wound diameter (mm)</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.61 ± 1.02</td>
</tr>
<tr>
<td>MC</td>
<td>4.02 ± 0.47</td>
</tr>
<tr>
<td>PGO.025</td>
<td>2.13 ± 0.21*</td>
</tr>
<tr>
<td>PGO.25</td>
<td>1.64 ± 0.22*</td>
</tr>
<tr>
<td>PGO.25</td>
<td>2.12 ± 0.58*</td>
</tr>
<tr>
<td>AG</td>
<td>2.96 ± 0.42</td>
</tr>
</tbody>
</table>

* P<0.05 compared with control was significant.

Control group (no treatment), MC group (Madecassol®), PGO.025, PGO.25, and PGO.25 group (0.025, 0.25, and 2.5% polysaccharides isolated from PG) and AG group (aqueous gel).
showed moderately thick nonkeratinized squamous epithelium with a thick granular layer and hyperkeratosis compared to the adjacent normal epidermis. Dermal papillae are only vaguely formed in regenerated epithelium. In the MC group, only 30% of the incision width was reepithelialized. A crust of necrotic debris with inflammatory cells covered the denuded epidermis and the dermis below showed signs of marked edema (H&E, x 100).

The rate of reepithelialization (%) of the control group was 52 ± 2.5. The rates for the PG0.25 (93 ± 2.7), PG2.5 (83 ± 2.7) and PG0.025 (62 ± 2.8) groups were significantly higher than that in the control and AG (34 ± 4.2) group (P<0.05) (Fig. 3). There was no statistically significant difference in the rates of reepithelialization of the AG and MC groups.

The PG used in this study is a fungus belonging to the Hymenochaetaceae basidiomycetes and found mainly in tropical areas of America and Africa [8]. In Korea, it is commonly referred to as Sangwhang and has been used as folk medicine for a variety of human diseases, such as diabetes, cancer and toxification. The results of the present study revealed that polysaccharides at all concentration (0.025, 0.25, and 2.5%) isolated from PG have significant wound healing activity as shown by the results of contraction and reepithelialization. This is the first report on PG promoting dermal wound healing in vivo and suggests a potential therapeutic role for PG as an adjuvant for the treatment of skin wounds.

We have previously demonstrated that polysaccharides isolated from PG inhibit pulmonary inflammation [11] and intraperitoneal adhesion related to intraperitoneal inflammation [1, 3, 6]. These effects of PG related to anti-inflammatory activities might be beneficial in the treatment of dermal wound healing. In the studies, we showed that PG is a potent macrophage stimulator that enhances macrophage cytotoxicity and phagocytic capacity. Macrophages play a key role in wound repair. Leibovich et al. [16] reported that healing is delayed when wound macrophages are depleted. Therefore we think that dermal wound healing may be promoted by modulating the macrophage activity of polysaccharides isolated from PG.

In summary, polysaccharides isolated from PG are a potentially useful agent in the treatment of dermal wounds. Additional studies regarding its mechanism of action will further reveal its usefulness and limitations.

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**Fig. 2.** Histopathologic appearance of a wound on the rats 7 days post-punch biopsy. The wounds showed dermal proliferation of fibroblasts and mononuclear inflammatory cells with angiogenesis. The PG0.25 group (A) had complete reepithelialization with an increased number of epithelial cell layers, a thick granular layer, hyperkeratosis and parakeratosis. Reepithelialization was only 30% in the MC group (B) and 60% in the control group (C). A crust covered the denuded epidermis and the dermis below showed signs of marked edema (H&E, x 100).

**Fig. 3.** The rate of reepithelialization (%) of control and experimental wounds (n=10, values are the mean ± SD). The rate of reepithelialization (%) of PG0.25 was significantly higher than that in all groups (P<0.05). * P<0.05 versus all groups.
REFERENCES