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ORIGINAL ARTICLE

Psalmotoxin-1 Venom on the Inflammatory Response and Burn Healing Process in the Experimental Burn Model

Mehmet Yiğit Akgün[1], Mehmet Akgül[2]

[1]Department of Neurosurgery, Koc University Hospital, Istanbul, Türkiye
[2]Department of Neurosurgery, Yuksek Ihtisas Hospital, Kirikkale, Türkiye

Abstract

Introduction: Burn wounds caused by monopolar or bipolar cautery, which are common in surgical practice, can lead to serious complications in the post-operative period. We observed that psalmotoxin-1 (Pctx1) induces an inflammatory response during the burn-healing process and creates an effective wound-healing process. It also triggered the development of granulation tissue. Thanks to this venom, complications that may occur can be prevented in the early period.

Methods: In this experimental study, 18 healthy, 300–350 g weighted, adult (aged>5 months) male Wistar rats were randomly assigned to 2 groups. Group 1 (n=9) was the control group. Group 2 (n=9) was burned and treated with PCTx1 0,1cc/kg IP. Post-recovery burn areas were evaluated by immunohistochemical staining. All samples were classified in terms of tissue repair response (TRR) values (inflammation, fibrosis, neovascularization, and epithelization) and other examination criteria of bacterial colonization and bleeding.

Results: PCTx1 helped the wound healing process and when the inflammation, fibrosis, epithelization values of neovascularization, and other TRR values were examined, they were found to be significantly different compared to the control group 1 ($p<0.05$). In addition, the bacterial colonization and bleeding values of control group 1 were found to be significantly higher than group 2 (PcTx1) ($p<0.05$). According to histopathological examination, more granulation tissue and neovascularization were observed in the epidermis and wound area in Group 2 than in Group 1. In Group 2 (burn group treated with PcTx1), intense healing was observed characterized by the formation of well-organized granulation tissue in the epidermis and dermis. PcTx1 was also observed to be more effective by accelerating inflammation.

Discussion and Conclusion: Using PcTx1 in the wound healing process of burns, completing the remodeling phase of wound healing. We observe that this venom has the potential to be considered among the treatment options for such injuries in the future.

Keywords: Burn; healing process; inflammatory response; psalmotoxin 1; venom.

Burn wounds can cause chronic disabilities, serious cosmetic problems, and severe psychological symptoms[1]. Not only burns that may occur in daily practice but also burns that can be seen after surgery can cause serious complications. Burn wounds caused by monopolar or bipolar cautery seriously affect the healing of surgical incisions. Many factors such as the size and depth of the burn can affect morbidity and mortality[2]. The wound-healing process in burns consists of tissue inflammation, granulation, and remodeling, which is a complex process[3,4].

Skin disruption usually causes fluid loss, hypothermia, infection, scarring, deterioration of the immune system, and alteration of the body image[5-7]. Serious mortality rates are found in major skin injuries that cause all these factors to

Correspondence: Mehmet Yiğit Akgün, M.D. Department of Neurosurgery, Koc University Hospital, Istanbul, Türkiye
Phone: +90 850 250 82 50 **E-mail:** myigitakgun@gmail.com
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be seen together. The death rate from burns has decreased in the last decade; however, when more than 70% of the body surface is damaged or burned, it is still high[8,9].

In particular, post-surgical burns may cause repetitive operations due to superficial or deep infections. As a result of the infection, progressive problems can be seen until the removal of the instrumentation systems, which are frequently used in neurosurgery practice. In addition, cerebrospinal fluid fistula and abscess formations can cause meningitis and lead to serious neurological deficits. Oxygen-free radicals contribute to delaying the wound repair process. Oxidative stress has a role in tissue damage and causes delay of the recovery. It is thought that early antioxidant therapies in the maintenance treatment of burn wounds strengthen cellular antioxidant defense mechanisms, thus preventing delays in the burn healing process caused by free oxygen radicals[10-13].

Tarantulas inject their prey with toxins to neutralize the other animals. These toxins alter the work of the ion channels which allow passing of the potassium and other ions in the cell membrane[14]. PcTxl is "a 40-residue peptide, isolated from the venom of a Trinidad Chevron tarantula, *Psalmopeous cambridgei*"[15,16]. Psalmotoxin binds "acid-sensing ion channels (ASIC)"[14]. PcTx-1, a specific ASIC1a blocker, is considered as a potential therapeutic strategy for burn healing process.

ASICs belong to degenerin/epithelial Na+ channel superfamily, and they are proton-gated ion channels and act as extracellular pH sensors. ASICs are closely associated with inflammations. It is found that ASIC expression increases in inflammatory conditions, and non-steroidal anti-inflammatory drugs can attenuate this response. Pharmacological blockade of ASIC1a or deletion of ASIC1 gene rescues the neurons and the myelin from damage in the mouse model[17].

In the present study, we investigated whether there was efficacy of PCTx1 venom on the healing process of burn. The experimentally induced burn model was developed and rats were used for this purpose comparing with the controls.

Materials and Methods

The present study was continued in our University Animal Research Laboratory with (December 27, 2016, dated and 16/98 numbered) numbered approval of the Local Animal Research Ethics Committee. All animals were cared for according to the principles of the National Academy of Sciences.

In vivo Rat Model

Eighteen healthy, adult (aged>5 months) male Wistar rats, 300–350 g weighted, were randomly divided into 2 groups. The number of rats was calculated using the degree of freedom sampling method. According to that formula, 18 rats were the maximum sample size for our research that could significantly impact our final data analysis result. In each of the groups, there were 9 rats:

- Group 1 (Control group): Burned and untreated rats
- Group 2 (Study group): Burned and treated with psalmotoxin (PCTx1) 0,1cc/kg IP, for once.

During the entire study, the rats were kept in the University Animal Research Laboratory. Room temperature was 25°C±1.9°C and room humidity was 52%±6%. The rats were given a standard diet and water. All animals were euthanized following explanation. The room lights were on a 12:12 light:dark cycle.

Experimental Burn Model in Rats

All rats were anesthetized with "a 2:1 mixture of ketamine hydrochloride100 mg/mL (Pfizer, Luleburgaz, Türkiye): xylazine hydrochloride 20 mg/mL (Bayer AG, Leverkusen, Germany) 0.75 mL/kg im." After the dorsum of the rats was shaved under deep sedation, this region was cleaned with povidone iodine. A simple full-thickness burn model exists: a 2×2 cm brass plate is held for 2 min in the flame of a Bunsen burner and, subsequently, pressed against the prepared skin of the rat for 10 seconds[18,19].

In our model, the metal probe is placed in 100°C water for 10 s, and then, it was placed on the dorsum of the rats without applying pressure. According to the desired burn rate, the metal probe was placed in the dorsum of the rats (Fig. 1). After an average of 10 s of waiting, the PcTx1 venom,

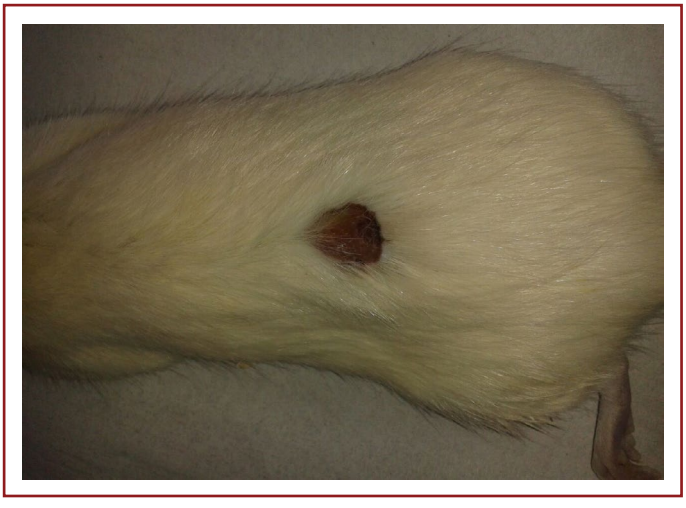

Figure 1. Burn model created on rat dorsum.

which we thought would speed up the healing after thermal injury, was administered intraperitoneally to the rats at 0.1 cc/kg (4 nmol/kg body weight) in the study group (Group 2). In control group 1, no treatment was applied. PcTx1 was dissolved and diluted in ddH_2O. The choice of dose of PcTx1 was based on the Ref[20].

To prevent bias, every treatment was performed under the same environment. We would swab and suture the wound each time we finished assessing the wounds on the specific day.

Histopathological Assessment

Post-recovery burn areas were evaluated by immunohistochemical staining. The aim here is to investigate the efficacy of the PcTx 1 venom, which we think accelerates the healing of various grades of burn injury after thermal injury[21].

In the study, burn specimens were taken in both groups. Samples were fixed in 4% formalin solution. After 2 days, tissue samples were washed with water. Before the embedding in the paraffin, tissue samples were soaked in ethanol (60%, 70%, 80%, 90%, and 100%) and xylene for 1 h. Cross-sections were obtained and hematoxylin and eosin staining was used for the histopathological examinations.

All samples were examined and classified semiquantitatively between 0 and 3 in terms of TRR values (inflammation, fibrosis, neovascularization, and epithelization)[22] and other examination criteria of bacterial colonization and bleeding.

The number of bacteria was obtained by taking a culture of the wound bed in each treatment group every day. The swab was soaked in normal saline and 1 mL aliquots were planted on blood agar and MacConkey agar.

Statistical Analysis

The data obtained were analyzed using IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY). Shapiro–Wilk test was used as a normal distribution test; if the data were normally distributed, we used the ANOVA test to analyze the mean differences in each group, but if the data were not normally distributed, Mann–Whitney U-test was used. A $p<0.05$ was considered for significance.

Results

Histopathological assessment for TRR values of the groups was shown in Table 1. Epithelization values ($p<0.05$) and other TRR values of the inflammation, fibrosis, and neovascularization in study group 2 were significantly higher than those control group 1 ($p<0.05$) (Table 1). Bacterial colonization and bleeding values of the control group 1 were significantly higher than those of the study group 2 ($p<0.05$) (Table 1).

In control group 1, necrosis and degeneration in the epidermis, incomplete healing attempt, characterized by the thin epidermis (Fig. 2), and well-organized granulation tissue formation in the dermis (Fig. 3) were observed in the wound area.

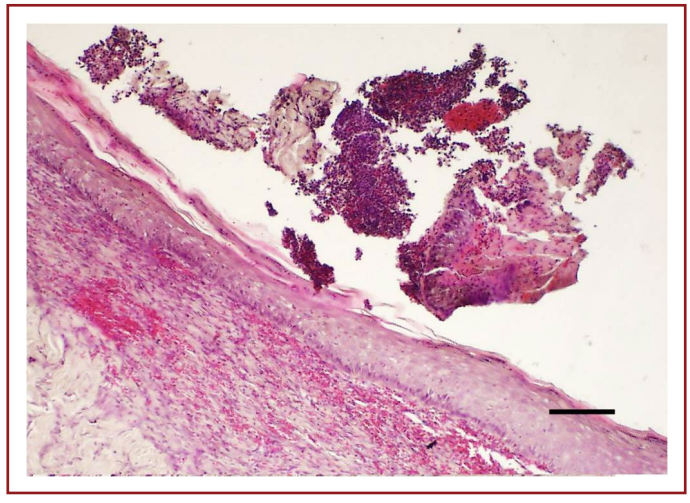

Figure 2. Necrotic and degenerative epidermis is shown in the control group. Arrow shows focal necrotic cell debris (Hematoxylin-eosin, Bar= 320 µm).

Table 1. Histopathological assessment values of the groups

TRR values	Group 1 (control-Burn) (n=9)			Group 2 (Burn-PCTx1) (n=9)			p*
	Median	Min	Max	Median	Min	Max	
Cellular Inflammation	1.5	0	3	2.5	1	4	0.041
Neovascularization	1.5	0	3	2.5	1	4	0.041
Epithelization	1	0	2	3	2	4	0.024
Fibrosis	1	0	2	3	2	4	0.024
Other examination criteria							
Bacterial colonization	1.5	0	3	0.5	0	1	0.036
Bleeding	1.5	0	3	2.5	1	4	0.006

*p value shows the results of Mann–Whitney U-test; *p<0.05 was considered for significance; *TRR: Tissue repair response; PCTx1: Psalmotoxin 1.

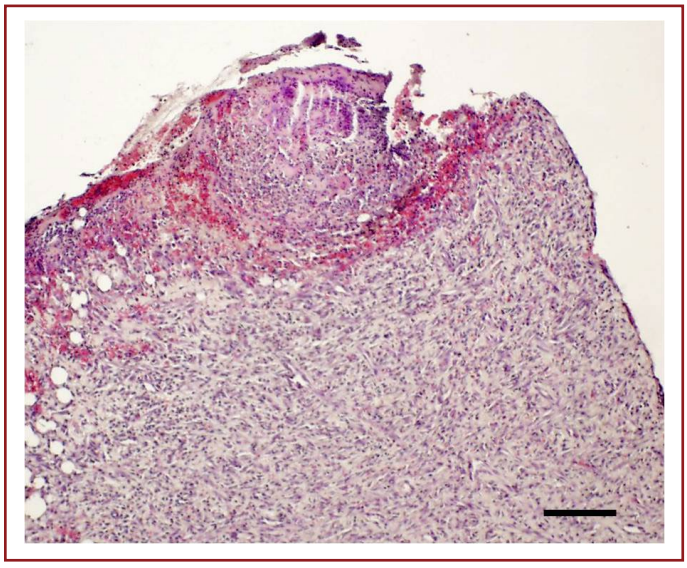

Figure 3. Granulation tissue and prominent neovascularization (arrows) are shown in the control group. Newly formed epidermis is covering the wound area (Hematoxylin-eosin, Bar= 80 µm.

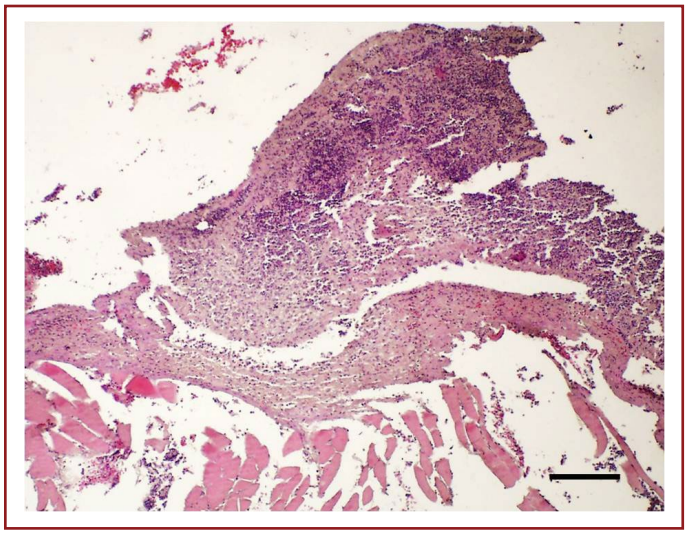

Figure 4. In the study group, newly formed epidermis and some necrotic cells still present on the surface. Note that multifocal hemorrhages in the new capillaries (Hematoxylin-eosin, Bar= 320 µm).

In the study group 2 (burn treated by PcTx1), complete healing attempt, characterized by thin epidermis (Fig. 4), well-organized granulation tissue formation in dermis, and prominent neovascularization were observed in the wound area (Fig. 5).

Discussion

Wound healing involves "clotting, inflammation, synthesis of matrix, angiogenesis, fibroplasia, epithelization, contraction, and remodeling." Growth factors regulate tissue repair and control cell growth[23-26]. Fluids around the wound

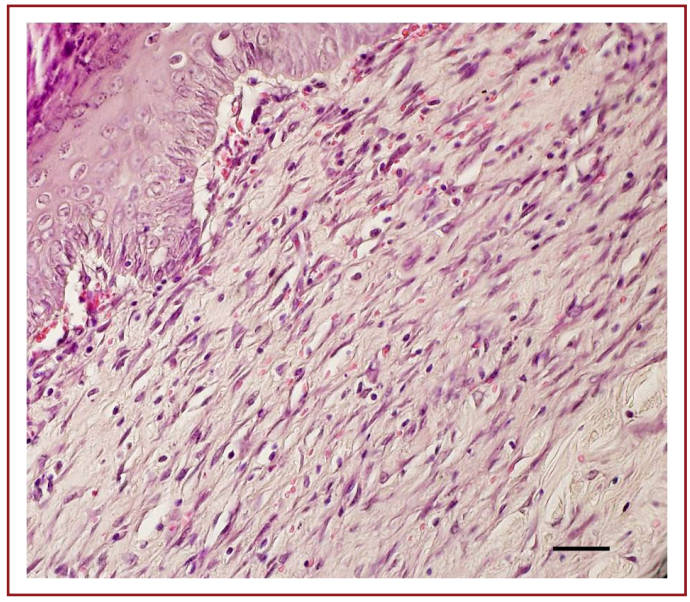

Figure 5. In the study group, well-organized granulation tissue is shown. The necrotic cells are also shown (Hematoxylin-eosin, Bar= 280 µm).

are important growth factor aids that support the wound healing process[27]. Growth factors are associated with certain high-affinity receptors on the cell surface to support cell growth. Growth factors have a strong effect on the wound repair process, even in small amounts in the wound area[9,26]. Cell proliferation and synthesis of several major peptide growth factors include "epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, and transforming growth factor-beta" in the extracellular matrix[9,23,28,29].

In this study, we investigated the effectiveness of PCTx1 venom in the healing process of the burn. Our results showed that PCTx1 helps the wound-healing process. Inflammation, fibrosis, epithelization values of neovascularization, and other TRR values were found to be significantly higher in study group compared to the control group. In addition, bacterial colonization and bleeding values of Group 1 were significantly higher than Group 2. According to histopathological examination, granulation tissue and significant neovascularization were observed in the wound area in burn group treated with PcTx1. Incomplete healing attempts, characterized by granulation tissue formation in the thin epidermis and dermis, were observed in control group 1. We have observed that PcTx1 provides a better burn recovery.

In burns, wound healing process is composed of remodeling, inflammation, and granulation[10]. Wound healing was completed very well in the group in which PcTx1 was used in our study. In addition, the inflammation and granulation

phases of the healing process as well as the remodeling phase have been completed.

Formation of scars, inflammation, proliferation, and remodeling are detected in burns[30-32]. In the repairing process, fibroblasts, collagen restoration, and angiogenesis occur. Collagen production leads to the increase of the wound strength[33,34]. In the remodeling, collagen synthesis, and degradation balance go on[30-32]. Contraction in the wound which is related to fibroblast function causes wound size reduction[13,30-32].

There is a centered granulation tissue in the contraction area which is composed of collagen, capillaries, macrophages, and fibroblasts[35]. In the skin, endothelial cells, smooth muscle cells, and fibroblasts play a role in the healing process[13,30,36]. In our study, we observed that PcTx1 induced an inflammatory response during the burn healing process. During the PcTx1 burn treatment process, also triggered the development of granulation tissue and the remodeling phase of wound healing was also completed.

Conclusion

We observed that PcTx1 induces an inflammatory response during the burn healing process and creates a faster effective wound healing process. It also triggered the development of granulation tissue. Randomized, controlled, prospective studies, and larger cohort studies are needed. Thus, we observe that this venom has the potential to be considered among the treatment options for such injuries in the future.

Ethics Committee Approval: The present study was continued in our University Animal Research Laboratory with (December 27, 2016, dated and 16/98 numbered) numbered approval of the Local Animal Research Ethics Committee.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: M.Y.A., M.H.A.; Design: M.H.A.; Supervision: M.Y.A., M.H.A.; Materials: M.H.A.; Data Collection: M.H.A.; Analysis: M.Y.A., M.H.A.; Literature Search: M.Y.A., M.H.A.; Writing: M.Y.A., M.H.A.; Critical Review: M.Y.A.

Conflict of Interest: None declared.

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