ORIGINAL ARTICLE

The Effect of Topical Nano Albumin Administration on Mast Cell Number and Burn Healing Area Reduction

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ABSTRACT

Introduction: Topical application of amino acid compounds that are effective in burns can accelerate burn healing through proliferation, neovascularization, and re-epithelialization. However, research on the benefits of nano albumin is lacking. This animal study aims to examine and determine the effect of topical nano albumin on burn wound healing. **Materials and methods:** This pretest-posttest study examined 27 Wistar rats. Each group (n = 9) received topical application of different substances t.i.d. for seven days. Group-1 (G1) received aquadest, G2 received 1% silver sulfadiazine, and G3 received 0.1 mg topical nano albumin. Topical nano albumin was obtained from the extract of chicken egg albumen. The burn healing was measured by the mast cell number infiltration and burn area reduction. Statistical analysis used the paired t-test, unpaired t-test, one-way ANOVA and post-hoc test. **Results:** The pretest-posttest intervention showed that mast cell number reduction is G1 0.2% (p = 0.936), G2 12.8% (p = 0.006), and G3 27.4% (p = 0.001). The burns healing area reduction is G1 4.4% (p = 0.023), G2 11.1% (p = 0.018), and G3 17.2% (p = 0.011). Furthermore, the comparison of all intergroup mast cell reduction showed significant differences ($p < 0.05$). Meanwhile, the burns healing area reduction of G1 (0.09 ± 0.08 cm) vs. G3 (0.34 ± 0.17 cm) is the only significant result (p = 0.001). **Conclusion:** Topical nano albumin of 0.1 mg t.i.d. for seven days is more effective and significant in reducing mast cell number infiltration and burns healing area compared to 1% silver sulfadiazine and aquadest.
Malaysian Journal of Medicine and Health Sciences (2025) 21(5): 91-94. doi:10.47836/mjmhs.21.5.12

Keywords: Nano albumin, Albumin, Burn, Wound healing, Inflammation

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INTRODUCTION

Burns can be caused by friction, heat, radiation, chemistry, or electricity. It causes tissue damage undergoing wound healing responses through proliferation, neovascularization, and re-epithelialization (1). A multitude of biological mediators of inflammation and growth factors play a complex and prolonged role in wound healing. These mediators include interleukins (IL-1, IL-2, IL-4, IL-8, and IL-10), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and various growth factors (VEGF, transforming growth factor, and epidermal growth factor), interferon-gamma (INF-gamma), tumor necrosis factor (TNF-α and β), and numerous additional immune system cells and extracellular matrix components (2). The presence of angiogenesis is a crucial component of the treatment process that must be done appropriately and successfully as massive mast cell infiltration has the potential to disrupt this process (3,4).

Fluid resuscitation should be started to maintain urine production > 0.5 mL/kg/hour for burns classed as severe (> 20% body surface area). Minor burns can be treated using the "C" of burn: Cooling, Cleaning, Covering, Comfort (5). Among these treatments, 1% silver sulfadiazine, as a common burn wound healing treatment is widely used in patients (6). However, the use of traditional and complementary therapies, as a cost-effective and easy-to-use method with limited side effects, is increasingly studied in various countries. Albumen or egg white has 3.6 g of pure protein. This dose contains all the essential amino acids for the body. Tryptophan is one of the essential amino acids in albumen (7).

Thus, topical application of efficacious amino acid compounds is believed to accelerate burn healing (8–11). However, research on the benefits of nano albumin is lacking. Therefore, this study aims to determine the effect of topical nano albumin administration on mast cell number and burn healing area reduction.

MATERIALS AND METHODS

Study design

This 7-day randomized pretest-posttest animal study with a control group design was performed from October to November 2022 in Sebelas Maret Laboratory, Indonesia. Because in vitro studies are limited and it is not always possible to test nanotechnology-based products on burn sufferers directly, animal models play a crucial role in laboratory investigations into burn wound healing. By providing a deeper understanding of the processes and mechanisms related to wounds, these models have the potential to significantly advance the treatment of burn injuries (12). We used 2.5 to 3-month-old male Wistar rats weighing 250-300 g. They were kept indoors under suitable conditions at 22±2°C, 40-60% humidity, and a 12-hour photoperiod cycle. Rats were fed pellets and water ad libitum. According to Federer's sample size formula, we divided 27 rats into Group-1 (G1) receiving aquadest or distilled water (H_2O), G2 receiving 1% silver sulfadiazine, and G3 receiving 0.1 mg topical nano albumin t.i.d. for seven days. The albumen extract from chicken eggs was used to create topical nano albumin.

Data collection

All 27 mice were anesthetized with ketamine 60 mg/bodyweight intramuscularly on day 1. The thigh hair was shaved and disinfected with ethanol. Next, the skin was burned for 8 seconds with a 2 cm diameter coin that had been heated for 3 minutes. Mast cell number (Figure 1A) and burn area measurements (Figure 2A) in the burn area were carried out at the end of day 1. G1-G3 received each determined therapy for seven days. On day 7 of post-intervention, we remeasured the mast cell (Figure 1B) and the burn area reduction (Figure 2B).

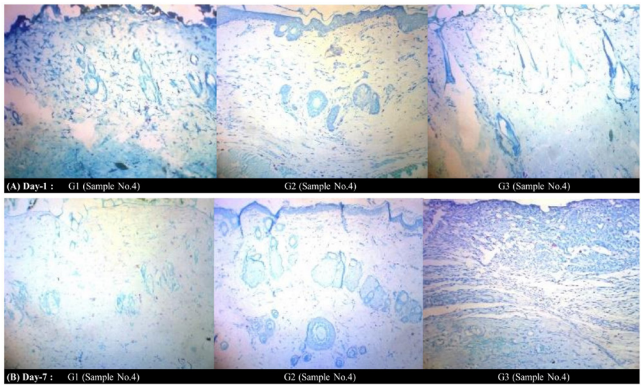

Figure 1: (A) The histological view (X400, Hematoxylin and Eosin staining) of the burn area among three groups showed Deep homogenization in the dermis, coagulation necrosis in the epidermis, nuclear elongation in the epidermis, dark-staining epidermal nucleus, intraepidermal separation, subepidermal (dermo-epidermal) separation, and nuclear elongation in the epithelial hair follicle. Based on the calculation, there were 45 mast cells in G1, 48 in G2, and 56 in G3. (B) 7-day following intervention, a proliferation of matrix and epithelial cells, and culminates in the creation of scar tissue, which is identified by the deposition of a highly ordered collagen matrix. Based on the calculation, there were 47 mast cells in G1, 44 in G2, and 40 in G3. G1, Group 1 receives aquadest or distilled water (H_2O); G2, Group 2 receives 1% silver sulfadiazine; G3, Group 3 receives 0.1 mg topical nano albumin.

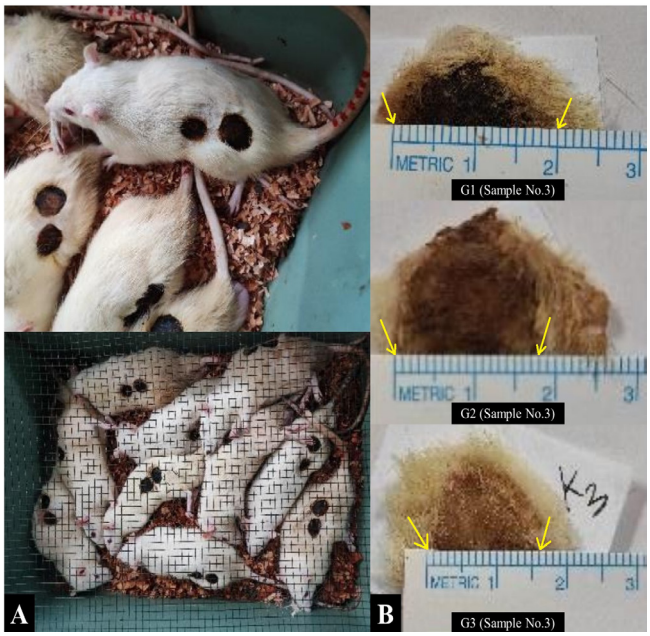

Figure 2: (A) All rats' thigh area (2 cm of diameter) was burned on day 1. (B) On day 7, the measurement of burn area reduction (the darker region) showed that G1's burn area was reduced to 2.0 cm, G2 1.8 cm, and G3 1.6 cm. G1, Group 1 receives aquadest or distilled water (H_2O); G2, Group 2 receives 1% silver sulfadiazine; G3, Group 3 receives 0.1 mg topical nano albumin.

Data analysis

The paired t-test determined the mean comparison of the pretest-posttest burn area and mast cell reduction of each group. The unpaired t-test analyzed the mean comparison of burn area and mast cell reduction between G1-to-G3. Furthermore, one-way ANOVA and post-hoc tests were used to determine the most significant mean reduction among those intervention groups. We used IBM SPSS 22 for Windows to conduct statistical analysis with a significance level of $p<0.05$.

Ethical Clearance

Ethical clearance for conducting this study was obtained from the Ethics Committee for Research Involving Human Subjects (JKEUPM) of Universiti Putra Malaysia [Reference no: UPM/TNCPI/RMC/1.4.18.1(JKEUPM)/F2].

RESULTS

In this study, after the normality test showed normal distribution, the statistical test used a parametric test. The pretest-posttest intervention showed that mast cell number reduction is G1 0.2% ($p = 0.936$), G2 12.8% ($p = 0.006$), and G3 27.4% ($p = 0.001$). Moreover, the comparison of all intergroup mast cell reduction showed significant differences ($p < 0.05$) (Table I). The burns healing area reduction are G1 4.4% ($p = 0.023$), G2 11.1% ($p = 0.018$), and G3 17.2% ($p = 0.011$). However, the burns healing area reduction of G1 (0.09 ± 0.08 cm) vs. G3 (0.34 ± 0.17 cm) is the only significant result ($p = 0.001$) (Table II).

Table I: Mast cell number pretest-posttest mean±SD comparison for each group, followed by its inter-group comparison of mast cell number reduction.

Group	Pretest	Posttest	Reduction (%)	p-value[a]
G1 (n = 9)	50.78 ± 7.69	50.67 ± 7.86	0.11 ± 4.01 (0.2)	0.936
G2 (n = 9)	50.33 ± 6.82	43.89 ± 5.46	6.44 ± 5.17 (12.8)	0.006
G3 (n = 9)	51.11 ± 6.07	37.11 ± 9.36	14.00 ± 5.17 (27.4)	0.001
p-value[b]	0.972	0.004	0.001[c]	
Inter-Group Comparison		**Reduction Comparison**		**p-value[a]**
Post-Hoc	G1 vs. G2	0.11 ± 4.01 vs. 6.44 ± 5.17		0.016
	G1 vs. G3	0.11 ± 4.01 vs. 14.00 ± 5.17		0.001
	G2 vs. G3	6.44 ± 5.17 vs. 14.00 ± 5.17		0.010

G1, Group 1 receives aquadest or distilled water (H2O); G2, Group 2 receives 1% silver sulfadiazine; G3, Group 3 receives 0.1 mg topical nano albumin; SD, standard deviation.
[a] Paired t-test
[b] One-way ANOVA
[c] This significant result is continued by post-hoc

Table II: Burns healing area (cm of diameter) pretest-posttest mean±SD comparison for each group, followed by its inter-group comparison of burns healing area reduction.

Group	Pretest (cm)	Posttest (cm)	Reduction (cm) (%)	p-value[a]
G1 (n = 9)	2.00 ± 0.00	1.91 ± 0.08	0.09 ± 0.08 (4.4)	0.023
G2 (n = 9)	2.00 ± 0.00	1.78 ± 0.17	0.22 ± 0.17 (11.1)	0.018
G3 (n = 9)	2.00 ± 0.00	1.66 ± 0.17	0.34 ± 0.17 (17.2)	0.011
p-value[b]	1.000	0.005	0.005[c]	
Inter-Group Comparison		**Reduction Comparison (cm)**		**p-value[a]**
Post-Hoc	G1 vs. G2	0.09 ± 0.08 vs. 0.22 ± 0.17		0.068
	G1 vs. G3	0.09 ± 0.08 vs. 0.34 ± 0.17		0.001
	G2 vs. G3	0.22 ± 0.17 vs. 0.34 ± 0.17		0.093

G1, Group 1 receives aquadest or distilled water (H2O); G2, Group 2 receives 1% silver sulfadiazine; G3, Group 3 receives 0.1 mg topical nano albumin; SD, standard deviation.
[a] Paired t-test
[b] One-way ANOVA
[c] This significant result is continued by post-hoc

DISCUSSION

A lot of interest has been generated by the development of particles based on nanotechnology, mostly for use in pharmaceutical and biomedical applications to treat disorders, including wound healing. A substance's surface area and surface area to volume ratio both significantly increase when it is reduced to a nanometric size, resulting in advanced and advantageous physicochemical features (13). Consequently, due to their inherent qualities or by transporting and distributing therapeutic substances within the wound bead, topical nano albumin can aid in the healing of burns (14).

With 50% of the total plasma protein in human plasma, albumin is the most prevalent type of plasma protein. The extravascular compartment, which includes the extracellular matrix under the skin, contains two-thirds of the body's total albumin (15). It is a 585 amino acid protein that is tiny (66 kD). Beyond its principal role of preserving the plasma oncotic pressure, it performs a multitude of vital tasks, including binding to ligands, binding to metal cations, possessing antioxidant qualities, and scavenging reactive oxygen and nitrogen species (16).

There is a significant increase in capillary permeability within the burn wound's microcirculation after thermal injury to the epidermis (2). Following burn injury, there is an instantaneous rise in permeability and large mast cell infiltration, which seems to peak after 8 hours and lasts for at least 48 hours (17). The average half-life of albumin is 15 days. Considering that topical nano albumin is given for seven days in this investigation, these observations are pertinent to burn patients. The outcomes on the seventh day were explained by their characteristics (18). In comparison to day 1, there was a notable reduction in the mast cell infiltrate in the G3; hair follicles were present, and the wound area showed signs of reepithelization, despite the epidermis still becoming thicker. In the G1 only slight and moderate inflammatory infiltrates were observed.

Moreover, we recommended to increase the sample size in future studies to increase statistical power and strengthen the generalizability of the results. Using more subjects may provide more representative and valid results. No Further In Vivo Testing: It is recommended to conduct further clinical trials in humans to validate these findings. Human studies are needed to confirm the efficacy and safety of nano albumin on burn wound healing, considering that the biological response of humans may be different from animals.

CONCLUSION

These findings represent the first in vitro investigation of the substantial and beneficial effects of topical nano albumin treatment at a dose of 0.1 mg t.i.d. for seven days on mast cells and the area burn area reduction. Based on macroscopic and histological investigation, as well as comparison with 1% silver sulfadiazine and

aquadest, it showed more significant anti-inflammatory and wound-healing properties.

ACKNOWLEDGEMENT

The authors are grateful to all research colleagues in Dr. Moewardi Hospital and Sebelas Maret University for providing feedback and technical support. The research dataset is available on reasonable request, email: handayuganitafuri@gmail.com or faizal9m@student.uns.ac.id.

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