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Original Research Paper

Plastic Surgery

The Role of Topical Vitamin C Administration on Healing Mid-Dermal Burns in Wistar rats

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ABSTRACT

Introduction: A burn is a trauma caused by heat, cold, electric current, chemicals, gas, lightning, and radiation (including sunlight). Water-soluble Vitamin C helps in the process of collagen synthesis and enhances the defense mechanism of the immune system and facilitates wound healing. We aim to see the effect of topical vitamin C administration on healing mid-dermal burns in Wistar rats.

Material Methods: Experimental studies to assess the effect of vitamin C administration on wound healing in second-degree (dermal) burns in Wistar rats. The research design used a simple experimental design (Post Test Only Control Group Design). This research was conducted at the Laboratory of Animal Pharmacy, Faculty of Pharmacy, University of North Sumatra, Medan, in October - November 2018.

Result: The group given topical vitamin C had an average burn area of 304.6 ± 31.5 compared to the control group given normal saline with 433.4 ± 32.2. Based on statistical tests, the value of $p < 0.001$ was concluded, so there were significant differences between the two groups ($p < 0.05$) between topical vitamin C compared with those not given topical vitamin C to increase collagen deposition in mid-dermal burns on the 14th day. The provision of topical vitamin C gives better results than just the administration of saline. On the 14th-day post-burn induction, the burn area in the topical vitamin C group was reduced by 39.4% while in the control group, it was only 10.7%.

Conclusion: Based on the results of research that has been done it can be concluded that Topical vitamin C can accelerate healing and reduction in burn area based on macroscopic observations on day 14 in Wistar rats compared to control groups that are only given normal saline. Based on statistical tests, it was found that p-value <0.001 so that it was concluded that there were significant differences in the average of the two groups ($p < 0.05$) on the increase in collagen deposition in mid dermal burns.

KEYWORDS : Burn, Vitamin C, Burns Healing

INTRODUCTION

A burn is a trauma caused by heat, cold, electric current, chemicals, gas, lightning, and radiation (including sunlight). Significant burns include burns with an area of more than 10% of the total body surface area (TBSA); Special area burns - face, hands, feet, genitalia, perineum, and major joints; Full-thickness degree burns more than 5% of TBSA; electric burns; chemical burns; burns with inhalational trauma; burns that surround the leg or chest area; burns in infants and elderly people; burns in people who have experienced previous medical disorders that require individual management, prolong recovery periods and increase mortality; and burns associated with other trauma.[1]

Based on the depth of the damaged burn tissue, burns are divided into three major classifications namely superficial, mid, and deep burns. The classification is further classified into 5. Burns can be classified based on the degree and depth of the burn.[1] Whereas based on the area of the lesion can be classified into three namely: Minor burns, i.e., degree I burn <10% or second degree <2%; moderate burns, i.e., degree I burn of 10-15% or grade II of 5-10%; Severe burns, i.e., degree II burn> 20% or grade III> 10%. To assess the area of the wound using the "Rule of Nine" method based on the LPTT (Total Body Surface Area). The extent of the burn is determined to determine fluid requirements, drug dosage, and prognosis. The percentage of adults and children is different. In adults, the head has a value of 9%, while the upper extremity has a value of 9% each. For anterior and posterior body parts and lower limbs each has a value of 18%, which includes the thorax, abdomen and back. The last one is the genitalia, with 1%. Whereas in children the percentage of different on the head has a value of 18% and extremity below 14%.[2]

Burn wound healing depends on the depth of the burn. Jackson (1959) described three zones of burn tissue damage: This central zone of coagulation is the central part of a burn with complete coagulative necrosis; the Stasis zone is the edge of the coagulation zone.[3] Circulation is slow in this zone but can recover after adequate initial resuscitation and proper wound care; The outermost zone of this hyperemia is a device for the stasis zone. This is the result of intense vasodilation as seen in the inflammatory phase after trauma. This has finally fully recovered. The healing process in burns depends on the depth of the wound. In epidermal and superficial dermis burns, wound healing occurs primarily. Superficial dermis sores heal from the rest of the hair follicle epithelium found in the superficial dermis. The healing process will take 5-7 days and usually, minimal tissue scars occur. In deep dermal and full-thickness burns, the wound healing process occurs secondary to epithelialization and contraction.[4]

Basic clinical research on the treatment of moist-based wounds to achieve a moist state has been universally accepted as a standard for various types of wounds. The rational reasons for wound care theory in a humid atmosphere are fibrinolysis, angiogenesis, the incidence of infection, and the formation of growth factors that will speed up the manufacture of active cells.[5] The inflammatory, proliferation, and maturation phases constitute in all three phases of the wound healing process. These three phases are the same for all types of injuries; the only differences is the duration in each phase.[6-8]

Healthy skin contains high concentrations of vitamin C, with levels comparable to other body tissues and well above plasma concentrations, suggesting active accumulation of circulation is possible in the millimolar range. Vitamin C is transported through blood vessels into cells in the dermis layer. Water-soluble Vitamin C helps in the process of collagen synthesis and enhances the defense mechanism of the immune system and facilitates wound healing.[9] Vitamin C is essential during wound healing, it also reduces the expression of pro-inflammatory mediators and increases the expression of various wound healing mediators.[10] Fibroblast cell culture experiments also show that vitamin C can change the gene expression profile in dermal fibroblasts, promote fibroblast proliferation and migration processes that are essential for tissue remodeling and wound healing.[11] Giving Vitamin C in large doses parenterally can cause widespread oxalosis, cardiac arrhythmias, and severe kidney function damage. Large doses can also cause dysuria due to irritation of the distal urethra. Side effects caused by use in large doses (> 1000 mg/day) in the long term can cause dependence on Vitamin C. Large doses can cause diarrhea and renal calculi calcium oxalate can be formed if the urine is acidic.[12]

We aim to see the effect of topical vitamin C administration on healing mid-dermal burns in Wistar rats.

MATERIAL AND METHODS

Experimental studies to assess the effect of vitamin C administration on wound healing in second-degree (dermal) burns in Wistar rats. The research design used is a simple experimental design (Post Test Only Control Group Design). This research was conducted at the Laboratory of Animal Pharmacy, Faculty of Pharmacy, University of North Sumatra, Medan, from October to November 2018.

Wistar rats that had met the inclusion and exclusion criteria of 40 were then kept under similar conditions which were kept in a cage with maintained room temperature and humidity. Randomly, the rats were then grouped into two different treatment groups. Group 1 is an experimental group that was given topical Vitamin C after suffering a burn, while group 2 was a control group that had burns covered with a transparent dressing after administration of saline fluid. All animals were anesthetized through intraperitoneal injection of ketamine hydrochloride. Under sterile conditions, using heated iron is affixed for 35 seconds to the dorsum of the rats that have been sheared for feathers to induce burns. Using the Meeh formula (A = 10 x W2 / 3, where A = area in cm^2, 10 is a constant and W = weight in grams) the surface area of the Wistar rat and burns make up about 1% of the total surface area. Then the size of the burn to be made is 2x2 cm. In group 1, the burn was closed with a transparent dressing after topical Vitamin C application. While in the second group, the burn wound was closed after the administration of saline fluid. After the action, the mice were placed in the same conditions and given the same food. The wound was evaluated for a moment when the burn was induced, days 3, 7 and 14 with transparent dressing opened. Wounds were evaluated using the + Woundesk app by assessing the burn area.

RESULT

From a total of 40 Wistar rat samples included in the study, a normality test was performed first using the Kolmogorov-Smirnov test. Normality test results in all observation periods with values above 0.05, which means that all data are normally distributed then will be analyzed using an independent T-test, while normality test results with values below 0.05, the statistical test used are Mann-Whitney.

Table 1. Differences in the burn area in the topical vitamin C group and the control group

Day	Extensive Mid-dermal Burns		P-value
	Control (Saline)	Topical Vitamin C	
Day 0	482,83±28,5	502,16±36,2	0,068[a]
3rd day	482,4±29,1	436,7±33,8	<0,001[a]
7th day	468,4±29,8	409,7±32,6	<0,001[a]
14th day	433,4±32,2	304,6±31,5	<0,001[a]

[a]*Independent T-test*

A. Comparison of the area of wounds of the two groups on day 0

The group given topical vitamin C had an average burn area of 502.16 ± 36.2, and the control group had an average burn area of 482.83 ± 28.5. Based on statistical tests, the value of p = 0.068 was obtained, which means there were no significant differences between the topical vitamin C and control groups on burn induction days.

B. Comparison of the area of wounds between the two groups on the 3rd-day evaluation

The group given topical vitamin C had an average burn area of 436.7 ± 33.8 compared to the control group given normal saline had an average burn area of 482.4 ± 29.1. Based on statistical tests, it was found that p-value <0.001 so that it was concluded that there were significant differences between the two groups (p <0.05) between topical vitamin C compared with those not given topical vitamin C to increase collagen deposition in mid-dermal burns today third.

C. Comparison of the area of wounds between the two groups at the 7th-day evaluation

The group given topical vitamin C had an average burn area of 409.7 ± 32.6 compared to the control group given normal saline had an average burn area of 468.4 ± 29.8. Based on statistical tests, it was found that p-value <0.001 so that it was concluded that there were significant differences between the two groups (p <0.05) between topical vitamin C compared with those not given topical vitamin C to increase collagen deposition in mid-dermal burns today seventh.

D. Comparison of the wound area of the two groups at the 14th-day evaluation

The group given topical vitamin C had an average burn area of 304.6 ± 31.5 compared to the control group given normal saline had an average burn area of 433.4 ± 32.2. Based on statistical tests, the value of p <0.001 was concluded so that it was concluded that there were significant differences between the two groups (p <0.05) between topical vitamin C compared with those not given topical vitamin C to increase collagen deposition in mid-dermal burns today. 14th.

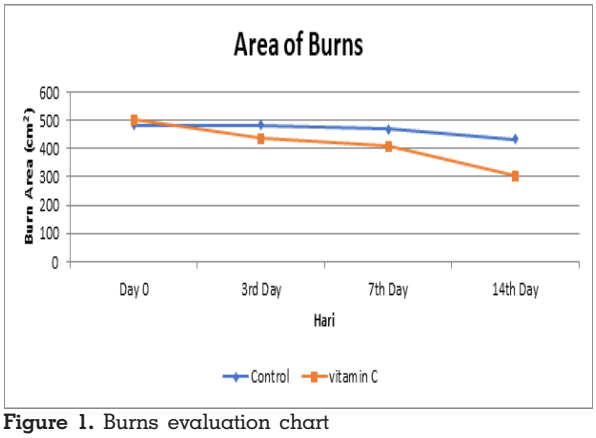

Figure 1. Burns evaluation chart

The provision of topical vitamin C gives better results than just the administration of saline. On the 14th-day post-burn induction, burn area in the topical vitamin C group was reduced by 39.4% while in the control group, it was only 10.7%.

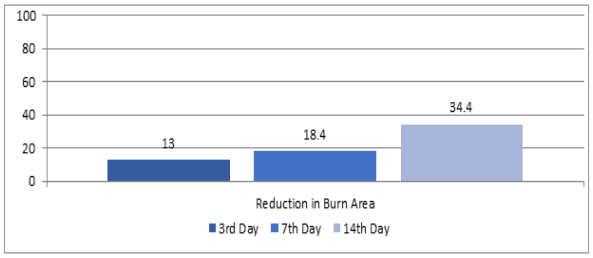

Figure 2. Percentage of reduction in the burn area

In Figure 2, the biggest reduction in burn area on the 14th evaluation was 39.4%, and the smallest on the 3rd-day evaluation was 13%.

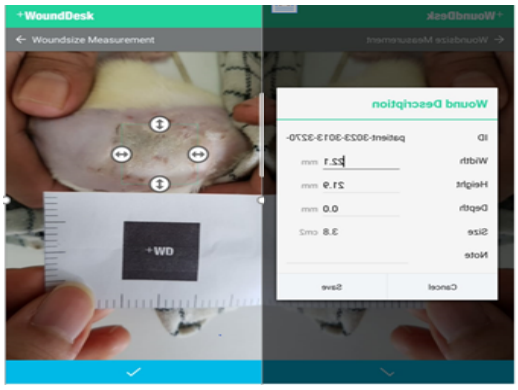

Figure 3. The use of wound desks in wound assessment

DISCUSSION

In this study showed that there were significant differences in the average two groups ($p < 0.05$) between topical vitamin C compared with those not given topical vitamin C to increase collagen deposition in mid-dermal burns so that the burn area was reduced more better day by day compared to the control group. Statistically, it can be concluded that on day 3 with a p-value <0.001 there is a significant difference between the two groups between groups given topical vitamin C compared with those not given topical vitamin C. On the 7th-day and the 14th-day also showed the results of statistical tests with a value of $p < 0.001$ so that it was concluded that there were significant differences in the average of the two groups between these two groups. The result is in line with research conducted by Kazakos et al. (2009) which shows the effectiveness of topical vitamin C administration in assisting wound healing by accelerating reepithelization in areas of acute wounds and burns, also in research conducted by Aracena et al. Reported that topical vitamin C administration in 10 patients with eye burns accelerate reepithelialization of the eyelids and cornea. In a study conducted by Umit Ozcelik et al. (2016) who used 30 Wistar rats divided into three groups, the group of mice that had been deliberately burnt and given topical vitamin C showed similar results where faster healing of burns was seen, compared to the group that was not given topical vitamin C. In a study conducted by Venter et al. (2015) on 60 Wistar rats that were previously given streptozocin to cause diabetes mellitus induced mid-dermal burns were then divided into two groups in which half were the control group and the rest were given topical vitamin C, healing of burns in day 21 macroscopically and microscopically showed significant differences. In a study conducted by Marck, RE (2014) states that topical vitamin C helps heal burns in experimental animals even in mid-dermal burns. Better wound healing was also found in the research of Lyras et al. (2010).

However, this result differs slightly from the research obtained by Roos E. Marck et al. (2016) which states the addition of topical vitamin C in the treatment of burns does not result in better graft repair and epithelialization this study also could not show better scar quality well. There were no statistically significant differences between the average take rate or the average epithelialization rate on days 5-7 between the area treated with topical vitamin C and the control area. However, the area of the wound treated by topical vitamin C showed more or better and equal epithelialization on days 5-7 compared to the area treated standard.

The anti-inflammatory effect of vitamin C on TG animals does not delay tissue repair but rather shortens the healing time concerning CG. A decrease in pH and oxygen pressure in skin lesions induces angiogenesis (Diegelmann et al., 1981; Knighton et al., 1981), which was confirmed in TG animals from our study. Therefore, the topical application of vitamin C in wounds maintains the integrity of blood vessel walls (Azulay et al., 2003), increases the number of neovessels and increases blood supply to the wound, which increases the proliferation and viability of cells involved in the healing process. The newly formed blood vessels show typical anatomical structures in the confocal microscopic examination and appear to be integrated into robust vascular architecture (Kirsten et al., 2004).

CONCLUSION

Based on the results of research that has been done can be concluded that topical vitamin C can accelerate healing and reduction in burn area based on macroscopic observations on day 14 in Wistar rats compared to control groups that are only given normal saline.

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