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Original Research Paper

General Surgery

PRP APPLICATION ON MID DERMAL BURN WOUND HEALING IN WISTAR RATS

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ABSTRACT

Background : PRP application was reported to help wound healing process in considerable amount of surgeries. This birthed the idea to apply PRP to burn wounds. PRP application helps wound healing by accelerating reepithelialization in wound area.

Materials and Methods : A total of 40 Wistar rats that met the inclusion and exclusion criteria were assigned to 2 random groups. Group 1 (platelet-rich plasma group) was exposed to burn injury and topical platelet-rich plasma was applied. Group 2 (control group) was exposed to burn injury and normal saline. Wound was measured macroscopically and compared on day 0, 3, 7 and 14.

Results : Forty Wistar albino rats were divided into 2 groups of 20 rats each. Group 1 (platelet-rich plasma group) was exposed to burn injury and topical platelet-rich plasma was applied. Group 2 (control group) was exposed to burn injury only. A group of 20 Wistar rats were used as blood donors for platelet-rich plasma. The wound was measured on day 0, group 1 had an average burn area of 501,856 ± 33,793 and group 2 had an average burn area of 482,532 ± 35,242 ($p = 0.085$). On day 3, group 1 had an average burn area of 436,594 ± 41,124 and group 2 had an average burn area of 482.908 ± 41,410 ($p = 0.001$). On day 7, group 1 had an average burn area of 409,549 ± 75,140 and group 2 had an average burn area of 468,676 ± 38,610 ($p < 0.001$). On day 14, group 1 had an average burn area of 304,498 ± 62,715 compared to group 2 with an average burn rate of 433,910 ± 41,583. ($p < 0.001$)

Conclusion : PRP improves wound healing time when measured macroscopically on multiple days compared to the controlled group. A further study on the histological aspect is needed to further understand the mechanism of the wound healing assisted by PRP.

KEYWORDS : Burns, PRP, wound healing, macroscopically, measurement

INTRODUCTION

Burn injury is considered to be one of the major causes of trauma to human body that may lead to death and also disability, with a long period of healing and high health care costs. Different kinds of dressings and therapies have been developed, but most of them are expensive, and the mechanisms underlying these therapies have not been fully documented (Ozcelik et al. 2016). Immediate debridement and skin graft are quite successful in handling burns, but donors for are often insufficient and the patient's condition is often not possible. In such circumstances the use of products that accelerate the process of wound healing can reduce morbidity and mortality (Pallua, 2009).

A mid-dermal burn, as its name suggests, is a burn injury that lies between a superficial dermal burn, which will heal relatively rapidly, and a deep dermal burn, which will not. At the mid-dermal level, the number of surviving epithelial cells capable of reepithelialisation is less due to the deeper burn wound and so rapid spontaneous burn wound healing does not always occur (EMSB). These burns dominate the highest percentage of incidence among other degrees, namely 73%, while the incidence of superficial partial-thickness is 17%, and the remaining 10% are full-thickness burns (Sabarahi , 2010).

Previous research focused on how to improve the overall survival rate. An understanding of the pathophysiology of burns has improved the resuscitation technique that has drastically reduced mortality rate. Latest trend is to focus on lowering the morbidity rate and improving patients' quality of (Venter, 2016).

PRP has been reported to possess the ability to help wound healing in several surgical fields; this derived the idea of applying PRP to burn wound. PRP contains 4-5 times more platelet compare to the baseline count of whole blood. Activated platelet stimulates growth factors release, angiogenesis and proliferation of fibroblast, therefore aids wound healing. PRP functions as haemostatic by forming fibrin clot. (Kazakos, 2009).

PRP increases the physiologic response to a trauma emulating and surpassing the 'normal' deposition of growth factors and proteins in trauma. Advocates of PRP therapy therefore claim benefits include increasing tissue regeneration and lower rate of infection, pain and blood loss (Simon, 2004).

This research is aim to study the effect of PRP on healing of the mid dermal burn injury model.

MATERIALS AND METHODS

Study Design

This study was conducted at the Pharmacy laboratory in Pharmacy Faculty in Sumatera Utara University with the acknowledgement from the Experimental Research Ethical Committee of Sumatera Utara University Faculty of Pharmacy. Sixty male Wistar albino rats weighing 250-300 grams were used. All rats were brought to the Pharmacy laboratory 2 weeks before the experiment and kept in individual cages under controlled temperature (22°C), humidity and lighting conditions (12-hour day, 12-hour night). All experimental subjects received standard rat chow and water.

Rats were randomly and equally divided into 2 groups. Each group consisted of 20 rats. Group 1 rats (study group; n=20) were exposed to PRP application after inflicted with burn injury; group 2 rats (control group; n=20) were exposed

normal saline for its burns; and a group of 20 Wistar rats were used for blood donor group for the preparation of PRP.

Experimental design

All animals were anesthesized by an intraperitoneal injection of 50 mg/kg ketamine hydrochloride. Under sterile conditions, a 2x2 cm iron plate was kept in boiling water and touched to the rats shaved dorsum for 35 seconds with its own weight to induce a partial thickness burn injury. The 35-second duration was based on results of a preliminary study. Partial-thickness burn injury was confirmed with histopathologic analyses. In group 1, the burn site was covered with transparent dressing after application of topical PRP. In group 2, the burn site was covered with transparent dressing after the application of saline. After experimentation, all rats were kept in special cages under controlled temperature, humidity and lighting conditions. All rats were fed with standard rat chow and water. The skin is then harvested on the fourteenth day after the burn injury. Tissue specimens were fixed in 10% formaldehyde solution, and then embedded in paraffin. Histopathologic examinations for wound healing were assessed by light microscope with hematoxylin-eosin staining, and collagen deposition was assessed by light microscope with trichrome staining in 100 magnification areas.

All experimental subjects were killed after the administration of anesthesia (60 mg/kg intraperitoneal ketamine) on the fourteenth day after burn injury.

Preparation of platelet-rich plasma

Platelet-rich plasma was derived after 20 step configuration of blood taken from the rats in group 3. With the first soft, short spin (1500 rpm, 10 minute), the plasma fraction was separated from the red blood cells. Then, the plasma fraction was separated with a hard, long spin (2000 rpm, 15 minutes) into the PRP and the plasma-poor platelets. After this, the PRP was combined with 10% calcium chloride to activate platelets to produce sufficient clot formation that could be applied on the burn site.

RESULTS

In this study, there are 40 wistar rats that met the requirements. All rats were divided into 2 groups, 20 samples in group 1 (experimental) and 20 samples in group 2 (control). On day 0, group 1 had an average burn area of 501,856 ± 33,793 cm^2 and the control group had an average burn area of 482,532 ± 35,242 cm^2. Based on statistical tests, P value is 0.085. There is no significant difference between the PRP group and controls on the day of induction of burns.

Table 1. The difference in the extent of burns in the PRP group and the control group

Day	Extent of burns IIb		p
	Control (Saline)	PRP	
Day, (X±S.D) Day-0	482,532 ±35,242	501,856±33,793	0,085[a]
Day-3	482,908±41,410	436,594±41,124	0,001[a]
Day-7	468,676±38,610	409,549±75,140	<0,001[b]
Day-14	433,910±41,583	304,498±62,715	<0,001[b]

[a]*Independent T-test*
[b]*Mann-Whitney*

On day 3, group 1 had an average burn area of 436.594 ± 41.124 cm^2 and group 2 had larger burn area compared to group 1 having an average burn area of 482.908 ± 41.410 cm^2. Based on the statistical test, the P value was 0.001 meaning there was a significant difference between the 2 groups.

On day 7, group 1 had an average burn area of 409,549 ± 75,140 cm^2 and group 2 had and average burn area of 468,676 ± 38,610 cm^2 which means the control group had a larger wound area. The P value was 0.001, which means there was a significant difference between the 2 groups.

On day 14, group 1 had an average burn area of 304,498 ± 62,715 cm^2 and group 2 had and average burn area of 433,910 ± 41,583 cm^2 which means the control group had a larger wound area. The P value was < 0.001, which means there was a significant difference between the 2 groups.

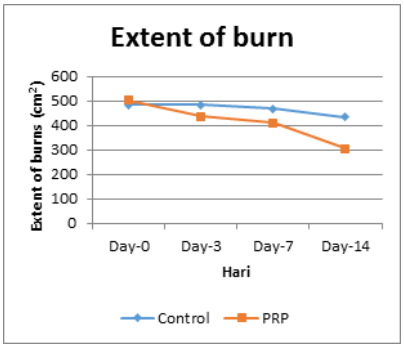

Figure 1. Burn chart evaluation

DISCUSSION

This study proved that topical PRP application in mid dermal burn wound in Wistar rats accelerates the healing of burn wound when measured on the 3rd, 7th and 14th days.

Immediately after trauma, platelets activate fibrinogen and form fibrin clot that functions as tissue sealant. Platelets act as chemotactic and induce proliferation of fibroblasts, endothelial cells and progenitor cells that regulate wound healing process. PRP stimulates the release of growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-b1 (TGF-b1), platelet-derived epidermal growth factor (EGF), platelet-derived angiogenesis factor platelet factor 4 (PF4) and platelets - activating factor (PAF). Management of burns can vary according to the cause. Despite the fact that skin damage occurs in all burns, local and systemic management are not the same for each burn (Tiwari, 2012). Burn wound treated with PR showed that inflammation subsided faster and had a more stable epithelial formation. Patients treated with PRP have a higher quality of wound without hypertrophy or contracture scars (Unal M, 2017). The findings from the study of Shu-Hung Huang (2018) provides the evidence that PRP with its anti-inflammatory effects can be used as an alternative therapeutic method to treat burns. Bernuzi et al (2010) concluded that the concentration of PRP affected the wound healing ability, 5% accelerates wound healing while 20% did not provide wound healing ability as the 5% did.

In this study it was shown that there were significant differences between the two groups (p <0.05), experiment group has better result in wound healing compare to the controlled group. The differences started to show on the third day, with p value = 0.001. There was also a significant differences on day 7 and day 14 between the 2 groups, experimental group shows better wound healing at time of measurement compare to the control group p value was <0.001. This is supported by the research by Kazakos et al. (2009) which shows the effectiveness of PRP in aiding wound healing by accelerating reepitalization in acute wound and burn areas, also in a study conducted by Aracena et al. reported that PRP administration in 10 burn patients in the eye accelerates reepithelization of the eyelids and cornea. In a study by Umit Ozcelik et al (2016) on 30 Wistar rats divided into 3 groups, Wistar rats were intentionally burnt, one group was given PRP and showed similar results in which faster healing was seen in PRP group. In a study conducted by Venter et al. (2015) in 60 diabetic Wistar rats induced by Streptozosin

injections were later burnt intentionally on their back then divided into 2 groups, the wound were examined macroscopically and microscopically on day 21 showed significant differences where PRP group show faster wound healing. Marck RE (2014) conducted a research on experimental animals showed a faster burn wound healing on deep dermal burn in a group where PRP was applied. Better wound healing is also found in the study of Lyras et al (2010).
On the contrary, Roos E. Marck et al (2016) stated that the addition of PRP in the treatment of burn did not result in better graft repair and epithelialization, nor could this study show a better quality of scars. There were no significant differences statistically between the mean retrieval rates or the average epithelialization rate on the 5th -7th day, between PRP treated and control areas. However, the wound area treated using PRP showed better epithelialization on the 5th -7th day compared to the standard treated area.

CONCLUSION

PRP accelerates wound healing when measured 14 days post burn induction compared to the control group. Based on statistical tests p value <0.001 was obtained, so there was a significant differences in burn wound area between control and PRP group.

CONFLICT OF INTEREST

The group of mice that received PRP had better rates of IIB degree burns.

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