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KEY WORDS: Platelet Rich Plasma; PRP; Burn; Wound Healing; Partial Thickness Burn Injury

EFFECT OF TOPICAL PLATELET RICH PLASMA ON BURN HEALING AFTER PARTIAL THICKNESS BURN INJURY

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ABSTRACT

Background: PRP application helps heal the wound and fastens reepithelialization in wound area. Platelets play an important role in hemostasis and wound healing after tissue damage. After trauma, platelets will activate fibrinogen and form fibrin clot that works as hemostatic agents and tissue adhesive. PRP increases collagen type I expression, MMP-1 and mRNA in human's fibroblast. PRP induces the synthesis of new collagen through fibroblasts. By adding PRP or PPP in cell culture will help fat stem cells and human's dermal fibroblasts proliferation.

Materials and Method: Forty Wistar albino rats were divided into 3 groups of 10 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. Group 3 (blood donor group) was used as blood donors for platelet-rich plasma. The skin tissue was harvested on the fourteenth day and the rats were killed on the same day. Tissue's fibroblast, PMNL, vessels proliferation and collagen were examined histologically.

Result: Histopathologically, there were significantly higher fibroblast, vessels proliferation and collagen levels ($P>0.05$) in the platelet-rich plasma group than in the control group. There is no significant difference in PMNL in both group statistically.

Conclusion: Platelet-rich plasma seems to improve burn healing in this experimental burn injury model. As an initial conclusion, it appears that platelet-rich plasma can be used in humans, although further studies should be performed with this type of treatment.

INTRODUCTION

Various wound dressing techniques have been used to treat burns. Plenty of burn dressing products are costly and exorbitant for the underprivileged patients in many countries. Wound care, including burns, is conducted to prevent wound degradation by providing favorable wound healing process. Closed wound care is assumed to be the best way to inhibit evaporation. Moist dressing will facilitate healing process.[1,2]

The selection of wound dressing technique is extremely important in burn wound care. A decent wound dressing will minimize the number of complications and consequently increases the healing rate and decreases the risk of hyperpigmentation and the formation of hypertrophic scar.[3] 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 are blood fractions that contain concentrated platelets. After platelets activation, growth factors will be released to aid in wound healing. PRP stimulates angiogenesis and fibroblast proliferation, functioning as haemostatic agent by forming fibrin clots. Application of PRP helps heal the wound and faster reepithelialization in wound areas. Research by Kazakos et al showed the effectiveness of PRP in acute wounds and burn wounds.[5] Adding PRP or PPP in cell culture will help fat stem cells and human's dermal fibroblasts proliferation.

Second degree burn (partial thickness) damages the epidermis and partial dermis of the skin. It is the highest in prevalence compared to the other degree which is 73%, while first degree (superficial partial-thickness) is 17% and the rest of 10% will be third degree burn wound (full thickness).[7]

PRP is blood fragments that contains higher amount of platelets. After platelet activation, growth factors will be released to aid in wound healing. It stimulates angiogenesis and fibroblast proliferation and works as haemostatic agent by forming fibrin clots. PRP application helps wound healing by accelerating reepithelialization in wound area. Study by Kazakos shows PRP's effectivity in acute wound and burn wound management.[5]

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. Histopathology examinations for wound healing were assessed by light microscope with hematoxylin-eosin staining, and collagen deposition was assessed by light microscope with Masson's 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 donor group. 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

Forty samples were obtained from the two groups, 20 samples from the control group and 20 samples from the experimental group.

Table 1. Analysis fibroblast score between experimental and control group

Group	Fibroblast Score					p-value
	None	Focal	Mild	Moderate	Significant	
Control	3	3	7	2	5	0.030*
Exp. group	1	4	6	9	0	

*Mann Whitney test

In the experimental group, most cases are classified in moderate group (45%), whereas in the control group, most cases are classified in mild group (35%). From chi-square analysis, we found significant differences in fibroblast score between control and patient group (p=0.03).

Table 2. Analysis PMN score between experimental and control group

Group	PMN Score					p-value
	None	Focal	Mild	Moderate	Significant	
Control	7	6	3	0	4	0.063*
Exp. group	3	3	5	4	5	

*Mann Whitney test

Average score for PMN group was 2 (0-4). In the experimental group, most cases have mild and significant PMN score (25%), whereas in the control group, most cases have significant PMN score (35%). From Mann Whitney analysis, we found no significant differences in PMN score between control and patient group (p>0.05).

Table 3. Analysis vessel proliferation score between experimental and control group

Group	Vessel Proliferation Score					p-value
	None	Focal	Mild	Moderate	Significant	
Control	7	5	3	3	2	0.017*
Exp. group	0	3	3	6	8	

*Mann Whitney test

In the experimental group, most cases have significant vessel proliferation score (40%), whereas in the control group, most cases have none vessel proliferation score (35%). From Mann-Whitney analysis, we found significant differences in vessel proliferation score between control and patient group (p=0.017).

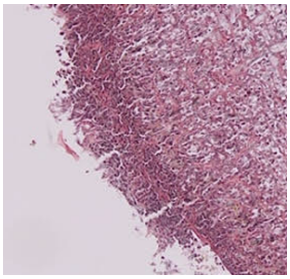

Figure 1. Photomicrograph of control group burn wound with standard Hematoxylin eosin, 100x. Note that mild fibroblast, PMNL and vessels proliferation and its arrangement throughout the wound healing process.

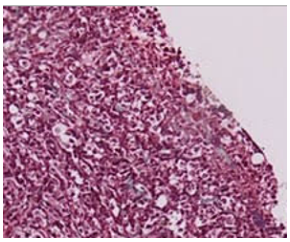

Figure 2. Photomicrograph of PRP treated burn wound with standard Hematoxylin eosin, 100x. Note that mild PMNL and vessels proliferation and its arrangement throughout the wound healing process.

Table 4. Analysis collagen score between experimental and control group

Group	Collagen Score					p-value
	None	Focal	Mild	Moderate	Significant	
Control	1	11	5	1	2	0.001*
Exp. group	1	2	1	8	8	

*Mann Whitney test

In the experimental group, most cases have moderate (40%) and significant (40%) collagen score, whereas in the control group, most cases have focal collagen score (55%). From chi-square test analysis, we found significant differences in collagen score between control and patient group (p=0.006).

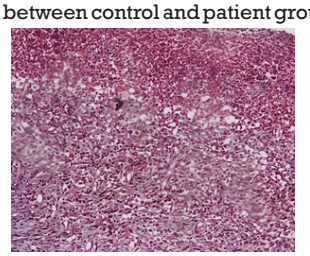

Figure 3. Photomicrograph of control group burn wound with Trichrome Masson's staining, 100x. Note that focal collagen deposition and its arrangement throughout wound healing process.

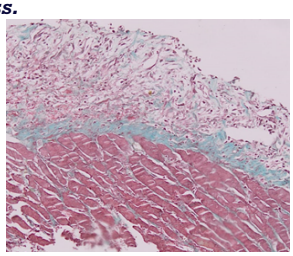

Figure 4. Photomicrograph of control group burn wound with Trichrome Masson's staining, 100x. Note that mild collagen deposition and its arrangement throughout wound healing process.

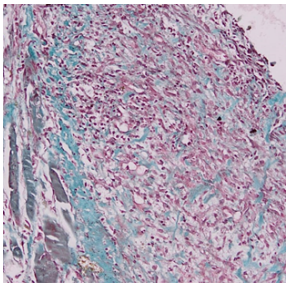

Figure 5. Photomicrograph of PRP treated burn wound with Trichrome Masson's staining, 100x. Note that moderate collagen deposition and its arrangement throughout wound healing process.

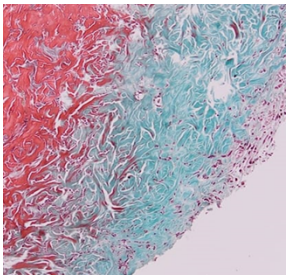

Figure 6. Photomicrograph of PRP treated burn wound with Trichrome Masson's staining, 100x. Note that significant collagen deposition and its arrangement throughout wound healing process.

DISCUSSION

Desired properties of wound cover materials that has the ability of forming a barrier against fluid loss and microorganisms, supporting cell proliferation in wound healing, and allowing vessel proliferation, keratinocyte adhesion, and differentiation; unfortunately, no such material currently exists. It has been demonstrated that PRP increases wound healing in acute trauma wounds, chronic non-healing wounds, and incisional wounds, and is effective in soft and hard tissue reconstructions.[5,9,13,14] It has been shown that the inflammatory phase is reduced, and prolonged inflammation (which often leads to bacterial infections and scar formation) does not occur in wounds treated with PRP.[14] Hao et al. showed that using PRP with acellular xenogeneic dermal matrix for treatment of deep second-degree burns decreased infection rate and increased wound healing.[15]

The most important growth factors in PRP are TGF-b and PDGF. They affect every step of wound healing by triggering cell growth and differentiation. Several in vivo and in vitro studies showed that all cells in the wound healing process are sensitive to the growth factors.[5] Epidermal growth factor is a chemotactic factor for fibroblasts, and topical application of EGF increases epidermal regeneration and strength of wound tension.[5] Endothelial cells are sensitive to bFGF and VEGF.[16] Transforming growth factor-b triggers collagen synthesis and quickens maturation of collagen in the early period of wound healing.[9] In addition, using TGF-b and PDGF together increases collagen deposition more than using TGF-b alone.[9] PRP increases epithelial cell differentiation and organization of collagen bunches.[9] Also, PRP enhances tissue incorporation of biological mesh.[17] In our study group, inflammatory cell infiltration (PMNL) was statistically higher in the study group. This result supports Hao et al. study that using PRP with acellular xenogeneic dermal matrix for treatment of deep second-degree burns decreased infection rate and increased wound healing.[16] This efficacy was because the inflammatory phase was reduced and prolonged inflammation (which often leads to bacterial infections and scar formation) does not occur in wounds treated with PRP.[9]

There are several studies suggesting that addition of PRP to the graft site enhances wound healing, promoting epithelization and angiogenesis in split-thickness skin grafts and donor sites.[8,9] Comparing to our study, we found that vessel proliferation and collagen deposition scores were higher in the study group with significant differences. Endothelial cells are sensitive to bFGF and VEGF.[6] Vessel proliferation is triggered by VEGF, PDGF, and bFGF.[10] Platelet-derived endothelial cell growth factor stimulates fibroblast and smooth muscle cell migration and proliferation, increases collagen deposition, and is also a chemotactic factor for neutrophils and monocytes.[4] Vessel proliferation is triggered by VEGF, PDGF, and bFGF.[10] Collagen, fibronectin, and glycosaminoglycan synthesis from fibroblasts is stimulated by TGF-b.[4]

Studies suggesting that addition of PRP to the graft site enhances wound healing, promoting epithelization and angiogenesis in split-thickness skin grafts and donor sites should be piloted.[18,19] Klosová et al. demonstrated that the viscoelastic properties of scars treated with the combination of split- thickness skin grafting (STSG) plus autologous platelet concentrate return more rapidly to the plateau state than areas treated with STSG only.[21] In a recent study, PRP increased the speed of repair of the extracellular matrix and its components in deep second-degree burn wounds.

CONCLUSION

There were significant differences in fibroblast development, collagen production, and vessel proliferations between PRP-treated group and control group but there were no significant differences in PMN proliferation statistically, even though PMN can be found less in control group. We conclude that platelet-rich plasma is effective in improving burn wound healing in this experimental burn injury model. Use of platelet-rich plasma can be an option for patients who have extensive burn areas.

Declaration of interest

The authors report no conflicts of interest.

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