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Original Research Paper

General Surgery

COMPARISON BETWEEN HYDROXYROLINE CONDITIONS IN WISTAR RAT GIVEN THE PLATELET-RICH-PLASMA WITH THE CONTROL ON IIB DEGREE BURNS

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KEYWORDS :

INTRODUCTION

1.1 Background

Burns have a direct impact on local and systemic changes in the body that do not occur in most other injuries (Marzoeki, 2006). Burns is a major trauma to the human body that can cause disability and death and also require a long healing time and expensive treatment (Ozcelik et al. 2016).

Burns have a high prevalence rate in modern life. The prevalence of burns in Indonesia is 0.7%. The highest prevalence occurs at the age of 1 year to 4 years of 1.5% (Riskesdas, 2013). Second-degree burns (partial thickness) is damage to the skin that occurs in the epidermis layer and part of the dermis. These burns dominated the highest incidence rate among other degrees, namely 73%, whereas the incidence rate of first degree (superficial partial-thickness) burns was 17%, and the remaining 10% were full-thickness burns (Sabarahi, 2010).

Administration of platelet-rich plasma (PRP) is reported to have assisted the wound healing process in some parts of the surgery, this provides an idea for the application of PRP to burns. PRP is a blood fraction that contains more platelets than normal. After platelet activation, growth factors are released and help the wound healing process. Platelet-rich plasma stimulates angiogenesis and fibroblast proliferation. PRP functions as hemostasis by forming fibrin clots. PRP application helps wound healing by accelerating reepithelialization of the wound area. Dagioglu et al's research in 2018 entitled "Are wounds healing on increased hydroxyproline?" demonstrated the effectiveness of PRP in wound management. This study aimed to see the levels of hydroxyproline in Wistar rats given PRP applications. In that study, it was found that the PRP was significantly proven to be effective in increasing hydroxyproline levels so that it is more effective in healing wounds (Daglioglu, 2018). Therefore, researchers are interested in seeing differences in hydroxyproline levels in Wistar rats with second-degree burns treated with PRP.

1.2 Problem Formulation

Based on the background that has been stated, the problem can be formulated as follows: "Is there a difference in the levels of hydroxyproline in Wistar rats with IIb degree burns treated with PRP and those not given PRP?".

1.3 Hypothesis

There is a difference in hydroxyproline levels in Wistar rats with IIb degree burns treated with PRP when compared to controls (who were not given PRP).

1.4 Aims

1.4.1 General aims

The general objective of this study is to know that PRP can increase or decrease hydroxyproline levels in healing IIb degree burns in Wistar rats.

1.5 Benefits

1.5.1 For knowledge

From the results of this study, it is hoped that the use of PRP can be used in the treatment of burns in humans. This research can also be used for knowledge development or subsequent research.

1.5.2 For researchers

With this research, researchers can apply well-studied medical science, gain insight and ability in clinical trial research, especially those related to PRP and burns, and as a forum to hone thinking skills to become better.

1.5.3 For institution

This research is expected to provide information related to the latest burn treatment techniques and advance the Faculty of Medicine, Universitas Sumatera Utara, especially in the field of research.

LITERATURE REVIEW

Burn Injury

Burns is tissue damage caused by hot fluids, fire, steam, chemicals, electricity, solar radiation, and friction, or friction (Sjamsuhidayat, 2005). Handling and treating burns until now still requires complex treatment and is still a challenge for us (Noer, 2006). Morbidity and disability due to deep burns are still high (Barret, 1996) because deep-degree burns are potentially devastating events due to their effect on skin and other tissues such as blood vessels, nerve vessels, tendons, and bones (Klein, 2007).

Within 72 hours after burns, the wound tissue enters the rejection reaction phase, in which the healthy tissue response destroys the necrotic tissue and cells in the associated lesion area. Usually mixed and extensive, this reaction process consists primarily of three pathogenesis: (1) disintegration of necrotic histiocytes in associated injuries; (2) regeneration of healthy histiocytes from associated lesion areas; (3) microbial infection in associated injuries. In addition to inflammatory reactions, the disintegration of necrotic histiocytes may affect cell liquefaction in the associated injury and very importantly, the accumulation of cell liquefaction results continues with increased tissue injury. Meanwhile, the remaining healthy tissue in the associated injury begins to regenerate on its own when the damaged tissue becomes a destructive substance which is unfavorable for the environment for cell regeneration, thereby inducing serious inflammation. The combination of the two pathogens above disturbs the habitat of the normal flora in the skin and causes microbial destruction in the area

of injury, both of which in turn will make the damage worse and may one day produce systemic injury. This process is known as the rejection injury of necrotic tissues and it is the end of the primary injury to the burn (Xu, 2004).

2.2 Classification

Burns can be classified based on several things, including the cause, the extent of the wound, and the severity of the burn.

2.2.1 Classification based on mechanisms and causes

1. Thermal burn injury

Luka bakar yang biasanya mengenai kulit. Luka bakar ini bisa disebabkan oleh cairan panas, berkontak dengan benda padat panas, terkena lilin atau rokok, terkena zat kimia, dan terkena aliran listrik (WHO, 2008).

2. Inhalation burn injury

Burns are caused by inhalation of hot gases, hot liquids or hazardous products from incomplete combustion processes. Burns are the leading cause of death in burn patients (WHO, 2008).

2.2.2 Classification based on the degree and depth of burns

1. Grade I (superficial) only occurs on the surface of the skin (epidermis). Manifestations of the skin appear reddish, painful, and may reveal a bulla. First-degree burns usually heal within 3 to 6 days and do not cause scarring on remodeling (Barbara *et al.*,2013).

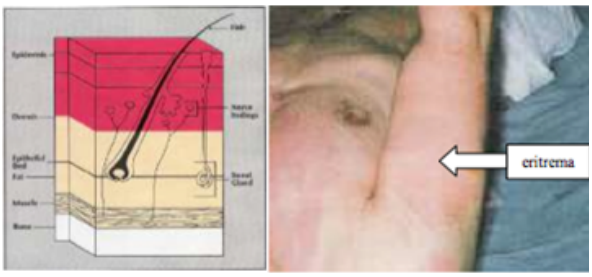


Figure 2.1. Schematic and clinical images of first-degree burns. The skin is intact, reddish in color, no bullae are found, painful (Available from Malick, Carr, 1982; Hettiaratchy, Dziewulski, 2004)

2. Degree II (partial thickness) involves all layers of the epidermis and part of the dermis. The skin will find bulla, reddish color, a little edema, and severe pain. If handled properly, second-degree burns can heal in 7 to 20 days and will leave scarring (Barbara et *al.*, 2013)

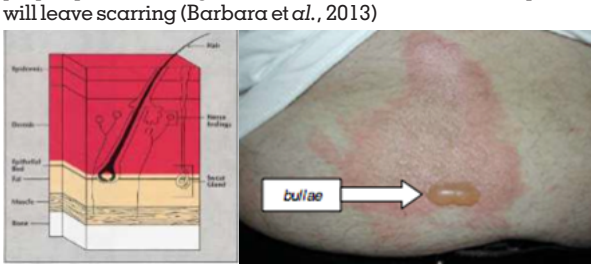


Figure 2. Schematic and clinical drawings of IIa burns. The wound is reddish, looks like a bulla that is very painful (Available from Malick, Carr, 1982; Hettiaratchy, Dziewulski, 2004)

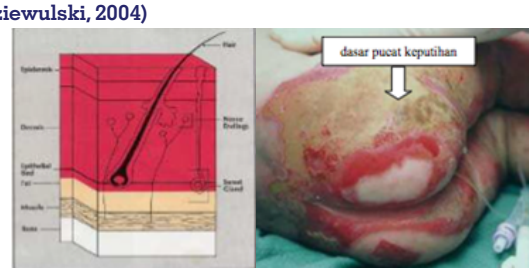


Figure 2. Schematic drawings and clinical drawings of IIb degree burns. The wound with a pale whitish base, looks bullae, feels less painful (Available from Malick, Carr, 1982; Hettiaratchy, Dziewulski, 2004)

1. Degree III (full thickness) involves damage to all layers of the skin, including bone, tendons, nerves, and muscle tissue. The skin will appear dry and there may be a bulla with thin walls, with the appearance of sores that can range from white to bright red to charcoal-like appearance. The pain is usually limited due to the destruction of nerve endings in the dermis. Wound healing occurs very slowly and usually requires a skin donor (Barbara *et al.*,2013).

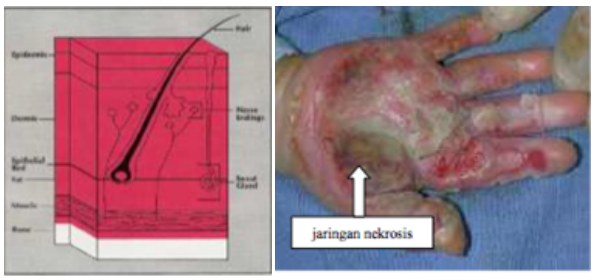


Figure 2. 4. Schematic drawings and clinical drawings of III burns. Necrotic skin appears. Basic blackish wound. No pain. Sometimes the tissue appears under the skin such as tendons, muscles, bones. (Available from Malick, Carr, 1982; Hettiaratchy, Dziewulski, 2004)

2.3 Burns Healing Phase

Burn healing depends on the depth of the burn. Jackson (1959) describes three zones of burn tissue damage (Arturson, 1996):

1. This central coagulation zone is the central portion of the burn with complete coagulative necrosis.
2. The stasis zone is the edge of the coagulation zone. Circulation is sluggish in this zone but can recover after adequate initial resuscitation and proper wound care.

This outer zone of hyperemia is a device for the stasis zone. This is the result of intense vasodilation as seen in the inflammatory phase after trauma. It is finally fully recovered.

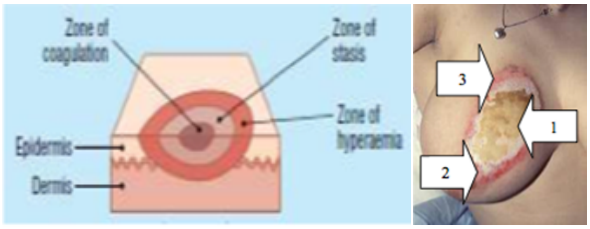


Figure 2. 5. Schematic figures and clinical manifestations of injury zones in burns: 1. Coagulation zone; 2. Stasis zone; 3. Hyperemia zone (Available from Hettiaratchy, Dziewulski, 2004. ABC of burns)

At first and second degree minor burns, spontaneous healing is the main objective. Second-degree minor burns heal from the epithelium of the residual hair follicles, which are abundant in the superficial dermis. Healing is complete within 5-7 days and scarring is almost less. In second-degree deep and third-degree burns, secondary healing, which involves epithelization and contraction, inflammation (reactive), proliferation (reparative), and maturation (renovation) are the three phases of wound healing. This process is the same for all types of wounds, the difference is the duration in each stage.

2.3.1 Inflammation phase

This phase is the same in all traumatic wounds immediately after injury until day 5, the body's inflammatory response that begins with blood vessels and cellular components (Werner S, 2003). The main objectives of this phase are hemostasis, loss of dead tissue, and prevention of colonization and infection by pathogenic microbial agents (Gurtner, 2007). Clinically,

inflammation appears with symptoms of erythema, swelling, warmth, and pain, 'rubor et tumor cum calore et dolore. This phase usually ends on the 4th day after the injury (Kerstein MD, 1997).

a. **Vascular response:** Immediately after the burn there is local vasodilation with extravasation of the third room fluid. In extensive burns, the increase in capillary permeability can be generalized to the large extravasation of plasma fluid and requires replacement.

b. **Cellular response:** Neutrophils and monocytes are the first cells to migrate at the site of inflammation. Then the neutrophils begin to decline and are replaced by macrophages. This cell migration is initiated by chemotactic factors such as calicrein and fibrin peptides released from the coagulation process and substances released from mast cells such as tumor necrosis factor, histamine, proteases, leukotrienes, and cytokines. The cellular response helps in phagocytosis and the cleansing of dead tissue and toxins secreted by the burn tissue.

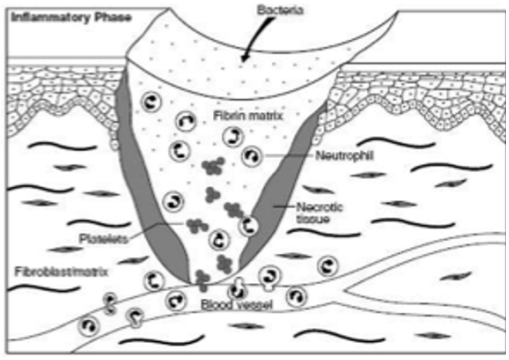


Figure 2. 6. Inflammation phase (Available from Gurtner, 2007. Grabb and Smith's Plastic Surgery. 6th ed.)

2.3.2 Proliferation phase

The proliferation phase begins 4 days after injury and ends on day 21 for acute wounds, according to the size of the wound and the patient's health condition. This phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, wound contraction, and epithelialization. Clinically, there is a reddish colored tissue like gravel.

The cells that serve as the foundation are fibroblasts that secrete collagen where skin regeneration occurs. Fibroblasts are specifically responsible for wound contraction. The cells that function as patches are the pericyte that regenerates the outermost layer of the capillaries and endothelial cells that produce the inside. this process is called angiogenesis. In the final stage of epithelialization is wound contracture due to the differentiation of keratinocytes into the outermost layer or stratum corneum.

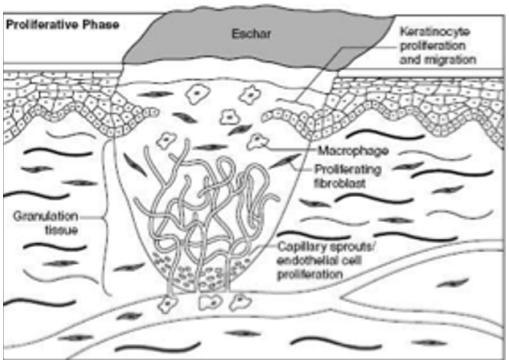


Figure 2. 7. Proliferation phase (Available from Gurtner, 2007. Grabb and Smith's Plastic Surgery. 6th ed.)

2.3.3 Remodelling phase

The Remodeling phase is the third phase of healing in which maturation of the graft or scar occurs. In this final project, the wound healing phase initially includes the placement of fibrous structural proteins, namely collagen and elastin around the epithelium, endothelium, and smooth muscle as the extracellular matrix. Then in this phase of resolution the extracellular matrix remodeling into scar tissue and fibroblasts into the myofibroblast phenotype which is responsible for scar contraction.

At the level of two deep dermal and full-thickness burns remaining for self-healing from this resolution, the phase is prolonged and takes years and is responsible for hypertrophic scarring and contractures. The hyperpigmentation in minor burns is due to the overactive response of melanocytes and the hypopigmentation seen in deep burns is due to the destruction of melanocytes from the skin appendages. In the grafted area, once innervation begins, it grows with nerves altering the control of melanocytes which usually leads to hyperpigmentation in dark-skinned individuals and hypopigmentation in white individuals.

The balance between the synthesis process and collagen degradation occurs in this phase. Excess collagen is degraded by the collagenase enzyme and then absorbed. The rest will shrink according to the existing tension. The result of this phase is scar tissue that is pale, thin, limp, and easy to move from the base (Bisono, Pusponegoro, 1997).

Collagen is initially arranged irregularly, so it requires lysyl hydroxylase to convert lysine to hydroxylysine which is thought to be responsible for cross-linking between collagen. This cross-linking causes the tensile strength to occur so that the wound is not easily torn again. Tensile strength will increase rapidly in the first 6 weeks, then will increase slowly over 1-2 years. In general, the tensile strength of the skin and fascia will never reach 100%, but only about 80% of normal (Marzoeki, 1993; Schultz, 2007).

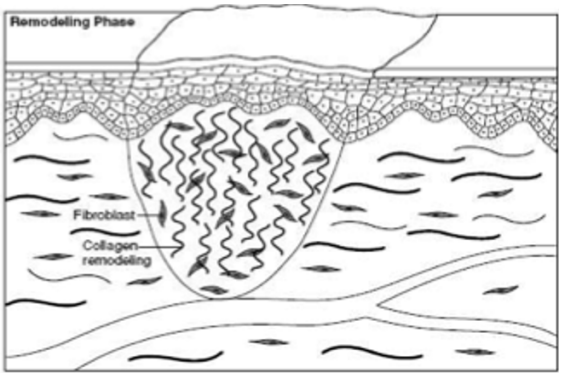


Figure 2. 8. Remodeling phase (Available from Gurtner, 2007. Grabb and Smith's Plastic Surgery. 6th ed.)

2.4 Platelet-rich plasma (PRP)

Platelets, which are one of the smallest and lightest parts of blood cells, normally circulate in the blood at 150,000-450,000 platelets/ml of blood. By definition, platelet-rich plasma (PRP) should contain platelets at a higher concentration than baseline. There are several parameters needed to determine the amount of PRP such as the platelet concentration above the baseline, the presence of leukocytes, whether there is PRP clotting, and whether PRP requires exogenous activation or not. These several parameters distinguish platelet-poor plasma (PPP) and PRP. Graziani et al (2006) stated that the optimal concentration of PRP is 2.5 times higher than the baseline (Graziani, 2006). According to the method, the basic procedure for making PRP is by centrifugation. Variations in relative centrifugal force (RCF), temperature, and time are

quite important indicators in the centrifugation process. The RCF variation can be seen in Table 2.1.

Table 2. 1. Variation in the results of relative centrifugation force on whole blood cells

Run	Parameters			Outputs	
	RCF (× g)	Centrifugation time (minutes)	Temperature (°C)	Yield (fold)	Recovery (%)
1	240	8	8	2.5	66.6
2	360	8	8	0.9	30.4
3	240	16	8	1.3	52.7
4	360	16	8	0.5	19.5
5	240	8	16	2.6	69.3
6	360	8	16	1.1	33.2
7	240	16	16	1.6	10.4
8	360	16	16	1.8	73.7
9	200	12	12	2.4	82.7
10	400	12	12	0.6	24.2
11	300	5	12	5.2	87.7
12	300	19	12	0.7	28.2
13	300	12	5	1.6	62.7
14	300	12	19	0.8	30.3
15	300	12	12	1.8	73.6
16	300	12	12	1.3	53.7
17	300	12	12	1.7	69.1

RCF relative centrifugal force.

In a study conducted by Amable et al in 2013, explained the variation in RCF results (Table 2.1), the best results in the first centrifugation of whole blood cells were in the 11th condition (300xg, 5 minutes, 12^{0}C) which is the only condition with lowest centrifugation time (Amable, 2013).

Tabel 2. 2. Variasi hasil pengaruh suhu pada sentrifugasi pertama

	12°C	18°C	*P* value
PRP1 (300 × *g*, 5 minutes)			
Platelet recovery (%)	90.6 ± 21.7	87.7 ± 22.8	1.0000
Platelet yield (fold)	3.1 ± 0.7	2.9 ± 0.6	0.5066
PRP1 volume (%)	26.7 ± 0.7	27.6 ± 1.9	0.7000

Meanwhile, according to variations in temperature results (table 2.2), temperature variations are considered not very influential because there is no significant difference in the final PRP-1 results, both at 120C and 180C (room temperature) (Cassie, 2011; Muller 2002).

After using the agreed method in making PRP-1, whole blood (WB) will produce 3 fractions, namely the lower layer containing erythrocytes/red blood cells with half the volume, the middle layer containing leukocytes/white blood cells (buffy coat), and the uppermost layer containing plasma, platelets, and a small number of white blood cells. The top layer is called platelet-rich plasma-1 (PRP-1). Each layer can be seen in Figure 2.9 below.

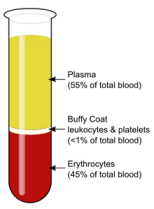


Figure 2. . Blood components

Due to the difference in content in each layer, the method of taking PRP-1 must use a pipette/micro pet to prevent the mixing of red blood cells and buffy coat. With the presence of leukocytes in PRP-1, several studies suggest that high levels of leukocyte concentration in PRP are directly related to catabolic gene expression which results in disruption of the healing process of the tissue. (Sundman, 2011).

2.5 Platelet-rich plasma mechanism of action (PRP)

PRP functions as a tissue sealant and drug delivery system, utilizing platelets initiating wound repair by releasing GF which works locally through α-granule degranulations (Marx, 2004; Lacci, 2010).

This active secretion of GF is initiated by the blood clotting process and begins within 10 minutes after clotting. More than 95% of pre synthetic GF is secreted within 1 hour, therefore PRP should be prepared by adding an anticoagulant and should be used on grafts, flaps, or wounds within 10 minutes of initiation of clotting. The use of PRP must remain sterile and the platelet concentrate will be viable for up to 8 hours under anticoagulation and placed in a sterile container. When the clotting process activates platelets, the GF secreted through the cell membrane binds rapidly to the outer surface of the cell membrane to graft, flap, or wound via transmembrane receptors. Platelets that are damaged or become non-viable in PRP processing will not secrete bioactive GF and give disappointing results (Marx, 2004).

Marx et al's study demonstrated that mature mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells, and epidermal cells express cell membrane receptors for GF in PRP. The transmembrane receptors induce the activation of endogenous internal signaling proteins, leading to the expression of normal (open) gene sequences in cells, for example, cell proliferation, matrix formation, collagen synthesis, and others. GF never enters the cell or nucleus, so PRP is not mutagenic and PRP works by stimulating faster wound healing (Marx, 2004). PRP suppresses the release of cytokines and limits inflammation, interacts with macrophages to improve tissue healing and regeneration, triggers new capillary growth, and accelerates epithelialization in chronic wounds (Pietrzak, 2005). Platelets contained in PRP play a role in the host defense mechanism in the wound area by producing signal proteins that attract macrophages. Previous PRP studies demonstrated antimicrobial activity against Escherichia coli, Staphylococcus aureus, including methicillin-resistant Staphylococcus aureus, Candida albicans, and Cryptococcus neoformans (Lacci, 2010). After the initial release of GF contained in PRP, platelets will synthesize and secrete another GF during their 7 days of life. (Marx, 2004).

2.6 Wound healing with PRP

To learn more about the benefits of PRP, it must be understood that the body's response to injury consists of 3 phases, namely inflammation, proliferation, and remodeling. The inflammatory phase is preceded by platelet aggregation resulting in hemostasis. Besides, platelets also secrete thromboxane and serotonin which stimulate hemostasis by vasoconstriction. Also, platelets secrete histamine which stimulates polymorphonuclear (PMN) and monocytes to the wound site. Furthermore, chemotactic growth factors will recruit endothelial cells to make new blood vessels (angiogenesis), as well as stimulated fibroblasts to form an extracellular matrix so that the wound will quickly close (Kaur, 2011). Various cytokines and growth factors influence wound healing and maturation. Cytokines play a role in cell recruitment for proliferation and differentiation. Growth factor derived from platelets or PDGF comes out of alpha granules and functions in the recruitment and activation of immune

cells and fibroblasts. An example of a product that has been used and approved by the FDA is the β-chain isomer form of PDGF (PDGF-BB) which is clinically proven to accelerate healing, including in chronic diabetic neuropathy wounds. Besides, platelets also secrete TGF-β, which stimulates fibroblast maturation, migration, and extracellular matrix synthesis. Meanwhile, other growth factors, namely EGF, and VEGF are released by fibroblasts, endothelial cells, and immune cells to increase the acceleration of wound healing. The wound healing process is a process that is well organized and consists of a collection of complex events which include cell interactions between cells and cells matrix and growth factors as signals that regulate the process. The growth factor is a compound that functions to stimulate cell growth, proliferation, healing, and differentiation. The role of growth factors is not as new cells that replace previous cells, but as signaling molecules between cells so that cells are stimulated for growth, proliferation, healing, and differentiation (Greene, 2009). There are dozens of growth factors that have been detected. Each growth factor is in a different place on the body and generally has the same function but works differently depending on its location. In specific α granules. There were several specific growth factors for platelets, namely PDGF, IGF-1, EGF, and TGF-β, but there are two main growth factors, namely PDGF and TGF-β (Puspita, 2014).

The benefit of PRP in burns is uncertain because of the limited clinical trials of PRP in burns. Preparation of PRP is usually done before surgery or other medical procedures, but it is difficult to do in burn patients, given the hemodynamic conditions that may be disturbed. PRP only increases the relative percentage of platelets in plasma, whereas the absolute number of platelets in the plasma of burn patients may be much lower so that the effectiveness of PRP in burn patients cannot be compared with other patients (Pallua, 2010). Despite this, there have been some reports regarding the effectiveness of the CCP for burns. Aracena et al. Reported that administering PRP to 10 patients with eye burns accelerated the reepithelialization of the eyelids and cornea. The study of Kazakos et al. Demonstrated the effectiveness of PRP in the management of acute wounds and burns (Kazakos, 2009). PRP is widely used in sports medicine such as ACL injuries, PRP is used to speed up rest time for athletes to return to the field more quickly (Figueroa, 2015). In the field of orthopedics, PRP is used to reduce joint pain, tendonitis symptoms, osteoarthritis, and low back pain (Sampson S, 2010). To reduce hair loss, PRP can also be applied directly to the scalp. In dermatology and aesthetics, PRP is used to reduce wrinkle lines, abdominal striae, scars, and skin rejuvenation (Puri N, 2015).

In theory, PRP could be useful for burns. However, PRP induces a severe inflammatory response in burns and is feared to stimulate the formation of excessive granulation tissue or hypertrophic scarring. Excess granulation tissue is not expected in burns with superficial or partial defects, but it may be necessary for burns with deep defects (full-thickness burns) (Pallua, 2010).

2.7 How PRP works in disposing collagen

PRP contains a mixture of bioactive agents extracted from platelets and plasma. Various growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) are secreted by α-granules from platelet concentrates that have been activated by aggregation. There are more than 30 bioactive substances in α-granules. Various growth factors and cytokines facilitate the accumulation of the extracellular matrix and aid in cell proliferation, angiogenesis, and cell migration. Matrix metalloproteinase (MMP) is involved in the aging process by degrading collagen and extracellular matrix proteins. PRP increases the expression of collagen type I, MMP-1, and mRNA in human fibroblasts. PRP induces the synthesis of new collagen by fibroblasts (Kim,). Adding PRP (Platelet Rich Plasma) or PPP (Platelet Poor Plasma) will significantly assist the proliferation of human dermal fat stemcells and fibroblasts in cell culture.

Collagen is the most abundant protein found in the extracellular matrix. The first amino acid component contained in collagen is glycine (~ 33%), L-proline (L-Pro), L-Hydocyproline (L-Hyp) (21%), and alanine (11%). L-Hyp is a collagen-specific amino acid. By measuring L-Hyp in the urine, it allows the metabolism of collagen in the body and determines the degree of collagen degradation. Therefore, L-Hyp is rarely detected without collagen, so L-Hyp is a specific biomarker for collagen degradation and is used to investigate collagen-related diseases (Sakamoto et al., 2015).

Collagen peptides are formed from the hydrolysis process of collagen and are commonly used for functional food. Prolyl-hydroxyproline (Pro-Hyp) is the main collagen peptide component that stays in the bloodstream after the digestion of collagen peptides. Pro-Hyp or peptides containing hydroxyproline are difficult for in vivo hydrolysis and can play an important role in the target tissue (Kimira et al., 2017).

Hydroxyproline is a non-essential amino acid that has 2 isomers, namely trans-4-hydroxy-L-proline and trans-3-hydroxy-proline. trans-4-hydroxy-L-proline differs from the amino acid proline in that there is a hydroxyl group attached to the γ carbon atom while the trans-3-hydroxy-proline hydroxyl group is attached to the β carbon atom. Hydroxyproline is an important component of the main structural protein, collagen, and plays an important role in collagen synthesis and stability (Vastava et al., 2016).

Hydroxyproline, which is only present in collagen and elastin, is formed from the cotranslational hydroxylation of proline by the enzyme proline hydroxylase, which occurs before the synthesis of the polypeptide chain is complete. Carbon atoms in the proline residue at 4 positions from the proline residue that spearhead the glycine residue in the Pro-Gly-Xaa-Yaa sequence through the hydroxylation process (Ignat'eva et al, 2007).

Modification of the proline residue in the Xaa-Hyp-Gly sequence through the hydroxylation process increases the stability of triple helix collagen. Defect of collagen synthesis due to abnormal hydroxyproline which causes various disorders such as the destruction of connective tissue in tendons and ligaments and increases the risk of damage to blood vessels. Increased hydroxyproline secretion in the urine accompanied by the destruction of connective tissue is a symptom of vitamin C deficiency (Srivastava et al., 2016).

Two hydroxyproline isomers trans-4-hydroxy-proline and trans-3-hydroxy-proline have been identified in mammals, mostly in collagen and other extracellular proteins. trans-4-hydroxy-proline is present in type 4 collagen while trans-3-hydroxy-proline is found in type 1 collagen. These two amino acids are synthesized as a cotranslational and posttranslational modification through the hydroxylation process of proline residues by 2 different enzymes, namely prolyl-4 -hydroxylase and prolyl-3-hydroxylase. Prolyl-4-hydroxylase is present in vertebra, invertebrates, and plants. Besides prolyl-3-hydroxylase is not found in plants. Prolyl-4-hydroxylase plays an important role in the new synthesis mechanism of polypeptide chain collagen into triple helix collagen. The main role of 4-hydroxyproline in collagen causes prolyl-4-hydroxylase as a potential target in pharmacological modulation to increase collagen resistance in patients with various fibrosis disorders (Srivastava et al, 2016).

Hydroxyproline is synthesized in the lumen of the endoplasmic reticulum through the proline hydroxylation process by an enzyme-catalyzed reaction, namely prolyl hydroxylase. This hydroxylase reaction occurs in the addition of substrates that require molecular oxygen, Fe2+, vitamin C, and α-ketoglutarate. Collagen is stabilized by hydrogen bonds formed between the carbonyl oxygen and hydrogen from amino acids. Collagen contains a lot of proline, hydroxyproline, and hydroxylysine. Hydroxylase and lysine require vitamin C so that if there is a deficiency of vitamin C it causes scurvy. Complications in the chronic stage include bleeding gums, swelling of joints, and impaired wound healing due to impaired collagen stability. Researches also suspect that in the process of wound healing and inflammation, 4- prolyl hydroxylase is activated so that it hydroxylates proline (Srivastava et al., 2016).

Previous studies have suggested that hydroxyproline is important for the thermodynamic stability of triple-helical conformation and connective tissue where hydroxyproline plays a major part in collagen. Collagen-related diseases can cause serious pathological conditions and death (Srivastava et al., 2016).

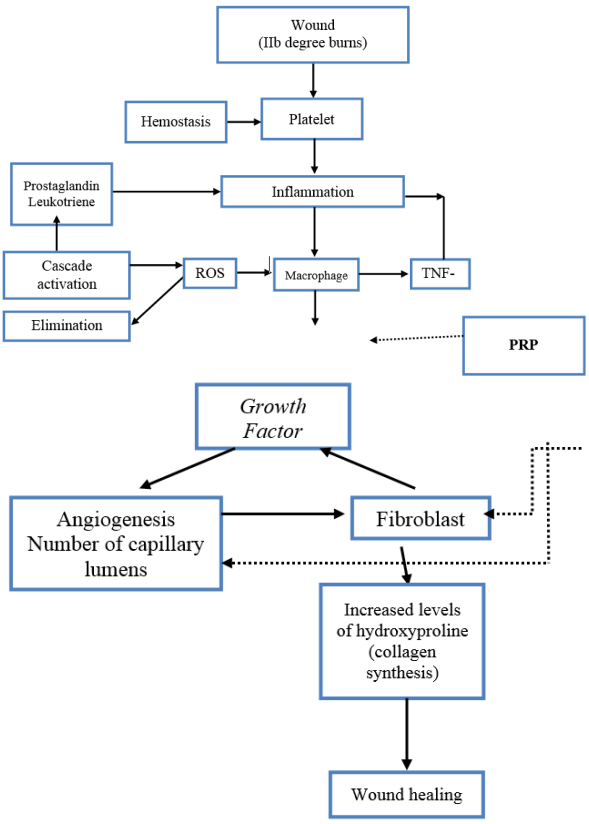
The wound is a disruption of the cellular structure and anatomical integration of the epithelium on the skin or tissue. Injuries can arise from pathological mechanisms both internally and externally in the organs involved. Based on the process and healing, wounds can be classified into 2 categories, namely acute and chronic wounds. Acute wounds indicate damage to external tissue that can be caused by burns, chemicals, or mechanical injuries and usually heals in 6 to 14 days. Chronic wounds are a continuation phase of acute wounds when the acute wound cannot heal within the time duration specified above or originates from deeper wound tissue involving the surrounding tissue. Apart from the cause and type of injury, any wound impairs normal cell structure and tissue function causing unwanted bleeding, pain, and inflammation (Srivastava et al, 2016).

Protein deficiency during the wound healing process can reduce the formation of new capillary tissue, fibroblast proliferation, collagen and proteogenesis synthesis, and remodeling and wound contraction so that sometimes it can lead to suppression of the immune system. A normal and stable extracellular matrix structure is a sign that the wound healing process has been completed. Collagen degradation causes the release of free hydroxyproline and other peptides. Hydroxyproline is a biochemical marker during the wound healing process is commonly used to evaluate the amount of tissue collagen and an indicator of collagen turnover after the wound healing process. The increase in hydroxyproline in granulation tissue is an indicator of increased collagen turnover which indicates good collagen maturation and proliferation during the wound healing process (Srivastava et al., 2016).

Several types of collagen as a specific structural protein can be directly examined by immunochemical analysis. The drawback of this technique is the production of antibodies to the applied collagen and the use of special preparations. Another test is to determine individual amino acids. In this study, the examination was focused on examining hydroxyproline, which is an amino acid that forms collagen by using the chromatographic method of amino acid separation by spectrophotometric detection of hydroxyproline. The disadvantage of this examination is that the concentrations of hydroxyproline and proline cannot be determined separately (Ignat'eva et al., 2007).

Various analysis techniques can be used to determine L-Hyp in serum and urine. The calorimetric method is used to measure the L-Hyp which is involved in the oxidation process of the hydrolyzed L-Hyp. However, this process requires time, control of the oxidation process, and the color formation reaction (Sakamoto et al, 2015).

2.8 Theoretical frameworks



METHODS

3.1 Research Type

This study is an experimental study to assess the effect of PRP on wound healing in IIb degree burns in Wistar rats. The research design used in this study was a simple experimental design (*Post Test Only Control Group Design*).

3.2 Research Time and Place

This research was conducted at the Pharmacy Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. The research was conducted from February to March 2019.

3.3 Populasi dan ISampel

The approximate sample size is determined according to the Federer formula:

$$(n-1)(t-1) \geq 15$$

n = samples per group
t = number of treatment groups

$$(n-1)(t-1) \geq 15$$
$$(n-1)(2-1) \geq 15$$
$$n-1 \geq 15$$
$$n \geq 16$$

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion criteria

Wistar rats weighing 250-300 grams.

3.4.2 Exclusion criteria

Rats that have abnormalities in the skin either due to trauma.

3.5 Variable

3.5.1 Dependent variable

Burn evaluation was assessed on day 14, the Iwistar rat dorsum was excised and then the hydroxyproline level was assessed.

3.5.2 Independent variable

Giving PRP in the topical form which was then covered with an OpSite drape and the edges were sewn after the hot iron was dipped in boiling water (100^0 C) for 10 seconds measuring 2x2 cm for 35 seconds on the dorsum of rats whose hair had been shaved until IIb degree burns were obtained.

3.6 Research Mechanism

As many as 30 Wistar rats that had met the inclusion and exclusion criteria were then kept in similar conditions, namely kept in cages with a temperature of 22 ° C and maintained humidity. Randomly, the rats were then grouped into 3 different treatment groups. Group 1 was the experimental group who was given PRP after experiencing burns, group 2 was the control group who had burns given normal saline afterward, group 3 was the donor group whose blood was used for PRP preparation. All experimental animals were sedated through intraperitoneal injection of ketamine hydrochloride. In sterile conditions, using an iron that has been dipped in boiling water for 10 seconds, applied for 35 seconds on the dorsum of rats that have been shaved to induce burns. With the Meeh formula (A = 10 x W2 / 3, where A = area in cm2, 10 is a constant and W = weight in grams) the surface area of Wistar rats is calculated and burns are made 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 covered with OpSite and sutured around the edges after topical PRP application. In group 2, the burn was closed with Opsite and sutured at the ends as well after saline administration. After the action, the mice were placed in the same conditions and given the same food. The wound was excised on day 14 and sent to the histopathology laboratory. The specimens were fixed with 10% formaldehyde solution then made paraffin blocks. The specimen was examined for the content of the hydroxyproline examination procedure, which is the basis of collagen constituents. The skin tissue was then dried at 600C for 12 hours and hydrolyzed with HCl6N for 24 hours at 1100C. After being neutralized by adding 1 mL of buffer solution, 1 mL of NaOH and 1 mL of aquabidest, and a total of 3 mL (3000 L), then the sample is pipette 300 L plus aquabidest up to 1000 L, mixed with 1 mL CuSO40.01 M, 1 mL 2.5 N NaOH, and 1m L H2O26%. The solution was then stirred and incubated at 800C for 5 minutes. After the incubation process is complete, the solution is cooled and 4 ml of H2SO4 3 N and 2 mL4-dimethyl-amino-benzaldehyde are added. The total amount of solution becomes 10 mL. Samples were incubated again at 700C for 16 minutes, cooled at 200C, and measured the absorption at a wavelength of 559 nm using a UV-Vis spectrophotometer. The amount of hydroxyproline in the sample was calculated against the standard curve of hydroxyproline.

PRP preparation

PRP was taken by 2-stage centrifugation. Blood was taken from the donor group mice and then rotated at a speed of 1500 rpm, 20°C for 10 minutes then the plasma fraction was separated from the red blood cells. Then the plasma fraction was again centrifuged at 2000 rpm, 20°C for 15 minutes to become PRP and plasma-poor platelets.

3.7 Data Analysis

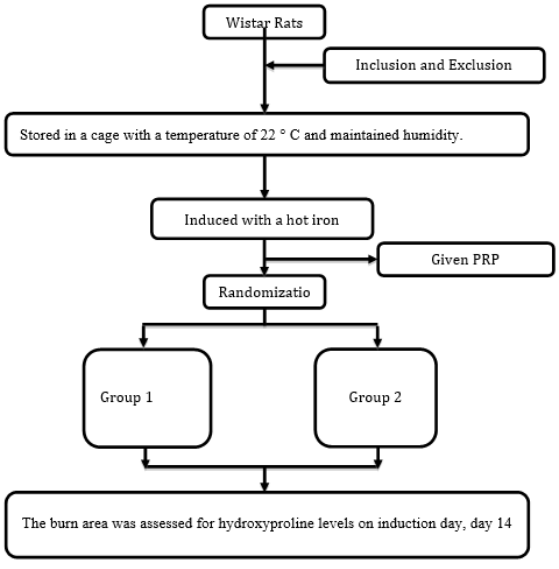
The data obtained are then presented in mean ± SD for numeric data that are normally distributed and median (min-max) for data that are not normally distributed. Then an unpaired T-test was performed on data with normal distribution or Mann Whitney/Wilcoxon for data with the abnormal distribution. The p-value <0.05 was considered significant.

3.8 Operational Definitions

Table 3. 1. Operational definitions

Variable	Definitions
IIb degree burns	Burns that reach the dermis, but there are still healthy epithelial elements remaining. Symptoms that arise are pain, bubbles, or bullae filled with fluid exudate that comes out of the blood vessels due to increased wall permeability.
PRP	PRP was obtained from the results of the centrifugation of blood taken through a puncture into the heart of the donor group rats.
Treatment group	After attaching 2x2 cm of hot iron that has been dipped in boiling water for 10 seconds for 35 seconds on the dorsum of the rats whose hair has been shaved until second-degree burns are obtained, the wound area is smeared with PRP gel once, which is 10 minutes after sticking the hot iron. then closed with a transparent cover.
Control group	After attaching a hot iron measuring 1x1cm for 35 seconds on the dorsum of the rats whose hair has been shaved until IIb degree burns are obtained, the wound area is given 0.9% NaCl then covered with a transparent cover.
Hydroxyproline (μg)	Amino acids are the result of proline modification which is catalyzed by the enzyme prolyl-4-hydroxylase (P4H) during the post-translates protein process. The amino acid hydroxyproline can be found in the protein domains of collagen, elastin, conotoxin, and argonaute.
Wound evaluation method	Wound evaluation Burn evaluation was assessed on day 14, the wound on the dorsum of the Wistar rat was excised to then assess the hydroxyproline level in the wound.

3.9 Research pathways



4.1. Research Sample Characteristics

In this study, 40 samples were analyzed for data. On the 14th day, hydroxyproline levels were measured using a spectrophotometer. The bodyweight of the study sample was measured on the first day when the sample was to be subjected to burn induction. This weight data can be seen in table 4.1.

Table 4.1. Research Sample Characteristics

Sample	Weights (gram)	Hydroxyproline levels(μg/g)
PRP	255	0,95
PRP	270	0,88

PRP	285	0,9
PRP	292	0,99
PRP	263	1,02
PRP	248	1,3
PRP	287	1,31
PRP	298	1,19
PRP	265	0,9
PRP	262	0,77
PRP	277	0,92
PRP	284	0,95
PRP	270	1,21
PRP	286	0,66
PRP	267	0,95
PRP	300	0,92
PRP	276	1,22
PRP	288	1,05
PRP	277	1,14
PRP	258	0,9
Control	255	0,6
Control	280	0,68
Control	285	0,42
Control	295	0,77
Control	281	0,36
Control	278	0,6
Control	285	0,7
Control	279	0,85
Control	270	0,42
Control	267	0,33
Control	279	0,29
Control	289	0,81
Control	275	0,75
Control	291	0,39
Control	271	0,88
Control	277	0,99
Control	281	0,92
Control	293	0,7
Control	282	1,02
Control	270	0,99

	PRP group	Control group	p-value
Samples number	20(50)	20(50)	0,336*
Weight (gram)	275,40 ± 14,53	279,20 ± 9,56	

* Data analysis was performed using an independent T-test. A p-value of <0.05 indicated statistically significant results.

Based on table 4.1. Out of a total of 40 study samples, 20 (50%) samples were assigned to the PRP-treated group, and 20 (50%) samples were assigned to the control group. The table also shows that the group given PRP had a mean initial body weight of 275.40 ± 14.5 grams while the control group had a bodyweight of 279.20 ± 9.56 grams. This result did not show a statistically significant difference with a p-value of 0.336.

4.1. The difference in Hydroxyproline Levels

Furthermore, this study looked at differences in hydroxyproline levels in the group given PRP and the control group. The PRP group had hydroxyproline levels of 1.01 ± 0.17 μg/g while in the control group, the hydroxyproline levels were 0.67 ± 0.24 μg/g. These results have a statistically significant difference with a p-value of <0.001.

Table 4.2. Hydroxyproline levels

	PRP group	Control group	p-value
Hydroxyproline levels (μg/g)	1,01 ± 0,17	0,67 ± 0,24	<0,001*

* Data analysis was performed using an independent T-test. The p-value <0.05 indicated statistically significant results.

DISCUSSION

Burns have an increasing prevalence, especially in modern life. In the United States, the number of patients receiving medical treatment for burns reaches 450,000 each year, this includes about 3,500 deaths (Latif, 1992; American Burn Association, 2013). In this study, the benefits of topical administration of platelet-rich plasma (PRP) for the healing process of mid-dermal burns in Wistar rats were assessed objectively in the form of measuring hydroxyproline levels using a spectrophotometer.

Marx in 2001 stated that PRP can be used to accelerate the wound healing process, where PRP is an autologous platelet that is concentrated in a small volume of newly drawn blood. Normally, the platelet count in PRP reaches 4 to 5 times the platelet count in routine blood (Marx, 2001). In 2003, Henderson stated that autologous topical PRP gel can function to accelerate the wound healing process by stimulating an inflammatory response which then increases the extracellular matrix and tissue granulation, tissue vascularity, fibroblast proliferation, and collagen production (Henderson, 2003).

The beginning of the emergence of opinion about PRP in the process of repairing wound conditions was obtained from an increase in the levels of growth factors found in wounds (Marx, 2001; Marx, 2004). And based on the previous statement which states that the platelet count in PRP ranges from 4 to 5 times, it is better if the platelet level in PRP is measured first to get rid of bias. PRP increases growth factor by about 3 to 30 times compared to whole blood (Arora, 2009).

This study used inclusion criteria for the bodyweight of Wistar rats as experimental animals ranging from 250 to 300 grams. As a result, in the PRP group, the average body weight of rats was 275.40 (± 14.53) grams compared to the control group, namely 279.20 (± 9.56) grams. The difference in body weight of rats in the two groups showed no statistically significant difference with a p-value of 0.336 or p> 0.05, so it is expected that the conditions of the two groups are homogeneous and the results obtained in this study are not biased due to differences in body weight conditions of the two groups. Previous studies have also used body weight in Wistar rats with a range of 250 to 300 grams, but there are also those with a narrower range of 275 to 300 grams (Ozcelik, 2016; Venter, 2016).

Furthermore, an assessment of hydroxyproline levels was carried out, were in the group with topical PRP administration, the results were 1.01 ± 0.17 μg/g, while the control group had lower results, namely 0.67 ± 0.24 μg/g. These results indicate a statistically significant difference with a p-value of <0.001. Ozcelik's previous research in 2016 also obtained similar results. Where the control group obtained a value of 0.69 ± 0.08 μg/g while higher results were obtained in the PRP group with a value of 0.95 ± 0.35 μg/g and the results showed a statistically significant difference (p=0,03).

Venter also said PRP can accelerate the healing of soft tissue wounds. Research conducted by Venter also found that the hydroxyproline levels in the group with topical PRP were higher than in the control group. The hydroxyproline content of the group with topical PRP was 0.94 ± 0.12 μg/g, while lower results were obtained in the control group with a value of 0.89 ± 0.10 μg/g. The statistical results of the two hydroxyproline levels showed a significant difference (p, 0.05) (Venter, 2016).

Klosova also said the use of PRP on burns can speed up wound healing time compared to the control group. PRP has a wide variety of growth factors and fibrin protein in high concentrations. This is why giving PRP to burns can accelerate wound healing. Klosova conducted research on burns that

were treated with PRP and those that were not given PRP. In the group given topical PRP, TGF 1 and EGF were higher than the control group. TGF 1 in the group given PRP had a result of 30.8 ± 9.8, while in the control group it was 23.7 ± 7.6. Likewise, the EGF in the PRP group had a value of 18.2 ± 6.3 and the control group was 14.1 ± 5.4. There was a significant difference between the groups that were given the PRP topic and those that were not (Klosova, 2013).

CONCLUSION

6.1. Conclusion

6.1.1. Hydroxyproline levels were shown to be higher in the group given PRP topically and showed statistically significant results.

6.1.2. The topical application of PRP can provide benefits in the healing process of mid-dermal burns.

6.1.3. PRP administration can accelerate the healing of mid-dermal burns.

6.2. Suggestion

6.2.1. Future studies should measure the platelet levels in PRP before it is applied topically to burns.

6.2.2. This study can be applied in daily clinical practice in patients with mid-dermal degree burns.

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