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The Effect of Papain from Papaya Latex on the Levels of VEGF in Burn Wound Healing in Rat (*Rattus novergicus*)

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Abstract

Burn is a health concern in the community because burn injury will lead to physical disability and even death. Therefore, a proper treatment is needed in burn. Nowadays, natural ingredients such as honey bees, *aloe vera*, and *papain* have been used in burns as topical treatments. Papain is an enzyme found in papaya latex. This enzyme has a role in increasing the epidermal thickness and the vascular network due to burn. This study was aimed to know the effect of papain on the levels of VEGF which plays a role in angiogenesis in burn wound healing. An experimental study has been carried out on 15 young rats which were divided into three groups: control group, papain group and silver sulfadiazine group as a comparison group. A full thickness burns was made by placing a hot boiled metal for 20 seconds on their shaved dorsum which has been anesthetized before with 2% lidocaine. At day 5, levels of VEGF serum obtained from rat's eye was measured by ELISA. Results showed that average levels of VEGF in the control group is 68.511 pg/ml, in papain group is 96.077 pg/ml and in a comparison group is 77.823 pg/ml. these results showed that papain can increase the levels of VEGF which has an angiogenic character and suggested that papain plays a role in accelerating of granulation tissues formation. However, a further investigation on the expressions or on the levels of other growth factors and cytokines in burn wound healing needs to be developed.

Keywords Papain, Burn, VEGF, ELISA

Background

A burn is a serious problem for the community in developing countries because it can cause physical damage or even death and it requires longer treatment and high cost. Its complication such as disability will affect morbidity and psychological of burn victims (1). A burn is a type of injury caused by heat, chemicals, electricity and radiation. The damage and the severity of burns depend on the sites, depth and extent of injury (2).

Temperature and duration of contact have a synergistic effect in depth of burn. Burns are classified as first-degree (superficial), second-degree (partial - thickness) and third-degree (full-thickness) depending on how deep and severe they penetrate the skin's surface. A full-thickness burn destroy epidermis and dermis layer. Therefore, of all burn injuries, the third-degree of burns have higher morbidity and deformity, require high cost due to intensive treatment in a specialized unit as well (2-3). Moreover, a proper treatment is needed in order to minimize the complex effects caused by burns 1. The gold standard in topical burn treatment is silver sulfadiazine cream 1%-(SSD 1%), a wide broad spectrum antimicrobial agent for burn wound treatment. Though its effectiveness is good, it has side effect as well such as resistance and delay wound healing process (4). Previous study reported the effect of 1% SSD in comparison with propolis in burn wound healing: the average of healing duration of 1% SSD is longer and the amount of microbial colonization is more on SSD (5-6), the same results obtained in comparison with honey (7). Other natural ingredients that are empirically well known to have an effect on wound healing are papain contained in papaya latex.

Papain is a group of hydrolase enzyme which catalyzed a substrate with a help of water. There were many studies had been carried out on the role of papain in wound healing, but these studies focused on histological examination in which can be seen the amount of liquefying scab associated with a reduction of necrotic tissues (8). Previous study reported that Papain (from papaya) and Bromelain (from pineapple) were able to enhance IL-6 release which plays a role in inflammatory phase (9). The precedent research showed that papain can increase the epidermal thickness, blood vessels networks, and dermal collagen. A research to study the definite mechanism about the effect of papain in molecular level to the increase of blood vessels formation (angiogenesis) (10) and the destruction of necrotic tissues in burn has never been done. Recent study demonstrated the effect of topical papain on the levels of TGF-β in partial-thickness burn, there was a reduction on the levels of TGF-β compared with control and SSD 1% and this result indicated that papain is able to act as an anti-inflammatory (11). TGF-β is also known to play a role in the formation of granulation tissues and collagen type 1, and increased regulation of angiogenic growth factors (12). However, a research to study the effect of papain on *Vascular endothelial growth factor -A* (VEGF-A) which contributed in angiogenesis needs to be developed.

VEGF is a growth factor that plays a role in enhancing migration and proliferation of endothelial cells (13), increasing vascular permeability, and strongly controlling interaction between angiogenic and non-angiogenic mediators (14), initiating monocyte migration and acting as a survival factor for endothelial cells (15-16). The role of VEGF in stimulating endothelial migration and proliferation will lead to a formation of capillary tubule. This angiogenic capillary bud will penetrate the blood clot at the wound site and form microvascular networks in granulation tissues. These vascular networks provide oxygen and nutrient to accelerate wound healing (17). For that reason, a research on the effect of papain from papaya latex on the levels of *Vascular endothelial growth factor -A* (VEGF-A) in burn compared with 1% SSD-treated burn and control (with vaseline) was conducted.

Materials and Methods

Papain

The enzyme papain obtained from Nacalai Tesque Kyoto Japan factory in the liquid form containing 25 mg in 1.2 ml.

Experimental models

White rats (Rattus novergicus) Wistar strain weighing 180-200 g were obtained from the laboratory of pharmacology, faculty of pharmacy Andalas University. Animals were kept in Anatomy laboratory of medical faculty of Andalas University for aklitimation purpose for 1 wk. The study protocol has got permission from the medical research ethics committee of Andalas University, Padang.

Research procedures

Rats were divided into three groups: control group (treated with vaseline), treatment group (treated with a dose 0.5 mg/kg BB of papain) and comparison group (treated with 1% silver sulfadiazine). Each of rats was anesthetized with 2% lidocaine, and then a full thickness burn was made by placed a copper plate weighing 150g that has been boiled in hot water with the temperature 100^{0}C on back of rats for 20 seconds (method Paramonov and Cheboterev cit Shuid et al., 2008). Then the treatments were given in accordance with the groups. At day 5, blood was taken from the orbital vein of rats which have been anesthetized with ether before. Serum obtained was used to measure the levels of VEGF-A. VEGF-A Kit used in this research is fabricated by R&D system. The levels of VEGF-A was measured by using ELISA method. Elisa is a detection technique based on antigen-antibody reaction. Antibodies are bounded with a specific enzyme as a marker. If there is a positive reaction, the enzymes will hydrolyte the substrate so that color changes occurred can be seen by using a spectrophotometer. Elisa Kit type used in this research was Elisa microplate.

Data analysis

To analyze the differences in the levels of the growth factor VEGF from each treatment, ANOVA test (analysis of variance) used when the data were normally distributed and if the data not normally distributed, the Kruskal-Wallis test was used.

Research ethics

In this research, before making burns on the rats, they were all anesthetized first with lidocaine plus decamidone (analgesic). Pre-research implementa-tion, an ethical clearance has been approved, but unfortunately the type of anesthetic used in this research was different from anesthetic proposed in the proposal due to the absent of anesthetic at the market. The analgesic used here was decamidon which advised by pharmacist.

Results, Discussion and Conclusion

The levels of VEGF serum of all groups of rats that having a full thickness burns measured by ELISA method, and the result can be seen in table 1.

Table 1. Levels of VEGF (pg/ml) serum of all groups that suffered from full thickness burn

Repeat number	Levels of VEGF (pg/ml) on each group		
	control	Papain	comparison
1	58.825	94.215	90.489
2	49.512	92.352	68.138
3	64.413	116.566	71.863
4	107.253	99.802	81.176
5	62.550	77.451	77.451
Mean	68.511	96.077	77.823
SD	22.406	14.124	8.674

Papain has been widely used to treat burns in experimental models and histologically demonstrated that papain may increase the epidermal thickness, vascular networks and number of dermal collagen compared with application of urea-base salve (10). Mechanism underlying that alteration may be caused by the release of growth factors, cytokines, chemokines, and expressions of adhesive molecules produced by keratinocytes. Growth factors produced by existing cells in the skin such as EGF, FGF, TGF-β, VEGF and PDGF, and those growth factor have a great role in wound healing like mitogen, migration of epithelial cells and formation of granulation tissues. The level of these growth factor increases when there is a wound (18).

The levels of VEGF of all group showed a variation in number on each repetition, it can be concluded that each animal has its own response on pathologic conditions. In table 1, it can be seen the levels of VEGF in rats treated with papain is higher (90.077 pg/ml) than the levels of VEGF in rats treated with SSD (77.823 pg/ml) and in control (68.511 pg/ml). The levels of VEGF was significantly different on each group (p=0.01). It suggested that papain has a better effect in wound healing than SSD 1%, especially in the formation of granulation tissues. It is well known that the formation of granulation tissues is affected by proliferation and migration of endothelial

cells and increased levels of VEGF after application of papain in burn may contribute to proliferation and migration of endothelial cells (19-20). Increased levels of VEGF may affect function of VEGF in forming other granulation tissues by increasing vascular permeability and MMP's activity. This circumstance contribute molecularly to a research conducted by Talgenhoff *et al.* that papain is able to upgrade vascular networks formation.

Increased levels of VEGF might also affect other functions of this growth factor that helping monocyte migration and thus affect the inflammatory process. A research had been carried out on the influence of papain on the levels of TGF-β (11) and on the levels of IL-6 (9), it was known that growth factors and cytokines play a role in inflammatory phase. TGF-β also helps in the formation of granulation tissues and is involved in the regulation of VEGF. This suggested that papain may be able to synergize the work of growth factors to accelerate wound healing (21).

At macroscopic examination, there was founded an elevated scab on burn wound in rats treated with papain, this condition helps to speed up wound healing process. This fact also supporting research conducted by Shuid et al who concluded that papaya latex is able to clean the wound, reduces scars on the skin, and relieves pain caused by burn in rat model (22). The reduction of scar tissues can be associated with the role of VEGF that helping migration of monocytes which play a role in phagocytizing damaged cells.

The levels of VEGF in burn treated with papain are significantly increased in comparison with control. This indicated that VEGF may play a role in angiogenesis and formation of granulation tissues. However, a further investigation on the role of papain in affecting levels or expressions of other growth factors which contributed in wound healing needs to be carried out.

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