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Comparison of Deep Partial Thickness Burns Wound Healing with Resveratrol Gel and Paraffin Gauze in Wistar Rats

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Abstract

BACKGROUND: Various modalities of wound care in burn cases are offered, but there is no standard algorithm used. Paraffin gauze dressing, as one of the commonly used dressings, is considered to have many shortcomings. Resveratrol, a stilbenoid derived from plants, is reported to have a role in wound healing process; it has the potential to be one of the modalities for treating burns. This study aims to strengthen studies on the role of resveratrol in wound healing process, especially in burns compared with paraffin gauze.

AIM: This study conducted to prove that resveratrol gel more superior than paraffin gauze in deep partial thickness burns healing process.

METHODS: This research is a laboratory experimental study with a post-test only design, simple randomized, and single blind. A sample of 32 Wistar rats with deep partial thickness burns was divided into four control groups and four treatment groups. Control groups were treated using paraffin gauze. Treatment groups were treated using resveratrol gel. Group 1 of each group was assessed for epithelialization. Angiogenesis, fibroblasts, and collagen deposition was assessed on day 5 in group 2, day 14 in group 3, and day 21 in group 4.

RESULTS: Angiogenesis significantly higher in the treatment group than the control group on day 5 and 14 ($p = 0.047$ and 0.032) but not significantly different on day 21 ($p = 0.107$). The number of fibroblasts in the treatment group was significantly higher on day 5, 14, and 21 ($p = 0.004$; 0.038; and 0.005). Collagen deposition not significantly different on day 5 ($p = 0.342$) but significantly higher on day 14 and 21 ($p = 0.048$ and 0.002). The epithelialization process occurred earlier in the treatment group than in the control group ($p = 0.001$).

CONCLUSION: Topical administration of resveratrol gel accelerates the epithelialization process, which increases the number of angiogenesis, fibroblasts, and collagen deposition in deep partial thickness burns compared to paraffin gauze.

Introduction

Wound management in burns has its own challenges, because wound healing phases in burns has its own characteristics. Since the beginning, burn healing process faces complex problems that affect the subsequent phases and cause burn healing to take longer. Therefore, since the initial phase, wound management plays an important role.

The principle of moist wound treatment is known in burns wound management and moist environment is an important component in burn healing process. Various modalities are offered to achieve these conditions. Some of the modalities are dressings application and topical materials such as creams, gels, or liquids. In the use of these topical materials, several substances are added to stimulate wound healing. The selection of appropriate dressings and topical materials depends on several factors, including the depth of the burn, the condition of the wound bed, the location of the wound, the desired moist and drainage conditions, the frequency of dressing changes required, and the cost of treatment.

Although there are various modalities, there is no standard algorithm used in burns wound care. Especially in Indonesia itself, burns wound care in each burn centers is varied.

Paraffin gauze is one of the most used dressings for burns wound care. Paraffin acts as a soothing agent that is useful in stimulating epithelialization thus accelerate the wound healing process. Paraffin gauze dressings are well accepted by patients and less painful; besides that, it is widely available and more affordable.

Paraffin gauze dressing is considered to have many shortcomings, one of those is tends to stick on the wound surface when it starts to dry. This frail triggers the emergence of other modalities in treatments.

Resveratrol, a stilbenoid derived from plants, has been reported to have potential in wound healing, scarring, and anti-aging [1], [2]. Many studies have

shown that resveratrol has strong anti-inflammatory, immunomodulatory, chemopreventive, cardioprotective, hepatoprotective and antioxidant properties, as well as stimulates vascular endothelial function and enhances lipid metabolism. Resveratrol has been reported to stimulate endothelial nitric oxide synthase (eNOS) activity and facilitate the expression of vascular endothelial growth factor (VEGF), thereby providing vascular protection, increasing angiogenesis and blood supply in various experimental models [3].

However, studies of resveratrol as topical materials in wound healing, especially burns, have not been widely carried out. Lin *et al*., in 2016, and Zheng *et al*., in 2020, have reported the effect of topical application of resveratrol on superficial partial thickness burns in experimental mice. In these studies, resveratrol significantly increased healing activity. Seeing the potential for resveratrol to be one of the modalities for treating burns, this research aims to strengthen the previous studies and compare them with other modalities that have been widely applied.

Materials and Methods

This research was an experimental study using a post-test only design, simple randomized, and single blind, to prove that topical resveratrol gel administration accelerated the healing process of deep partial thickness burns in Wistar rats compared to paraffin gauze. Subjects based on predetermined groups were observed at different times.

This study used 32 Wistar rats as research subjects. The research subjects were divided into 8 groups, namely, four control groups and four treatment groups. Deep partial thickness burns were created on the backs of male Wistar rats following anesthetization with an intramuscular injection of ketamine 10% (20 mg/kg body weight) and xylazine 2% (10 mg/kg body weight). Briefly, the hair on the backs of the rats was removed using a hair remover under anesthesia, and the back was subsequently disinfected with Chlorhexidine gluconate 4%. The back skin was subjected with a 100°C soldering iron tip for 10 s. The treatment group was intervened with the application of topical resveratrol gel 0.5%, while the control group was treated using paraffin gauze. Wounds are treated every 3 days.

On day 5, 14, and 21, a skin biopsy sample was taken 1 cm × 1 cm from the wound in the center of the wound in the control and treatment groups; then, wound was stitched and treated until it heals. The biopsy samples were fixed with 10% formalin buffer; sections were fixed in 5 μm paraffin wax and stained with hematoxylin eosin.

Neovascularization (number of blood vessel lumens), number of fibroblasts, and collagen deposition were observed at 400 times magnification under an Olympus CX33 Digital Biological Microscope trinocular microscope with PC Imaging Software Windows OS EP50 in 5 fields of view. The epithelialization rate was observed clinically where the wound was declared healed if a pink lesion was uniform and thorough on the wound surface, dry, and not attached to the dressing.

The data are expressed as the estimated mean standard deviation. The results were analyzed using one-way analysis of variance and Dunnett's multiple comparison tests. Statistical analyses were performed using SPSS software version 23.0. $p < 0.05$ was considered to indicate a statistically significant.

Results

Wound healing rates were measured on different days. In all groups, wound healing rates increased in line with duration. Significant increases in wound healing activity were observed in Wistar rats treated with topical resveratrol gel, compared with those that received control treatments. The effects of the treatments on the wound healing activity levels in Wistar rats with deep partial thickness burns are presented in Tables 1 and 2. In this model, the resveratrol-treated Wistar rats exhibited a significant increase in wound healing rate and a decreased time to epithelialization, compared with the control Wistar rats [4].

Table 1: Descriptive analysis and normality test

Day	Group	Variable	Mean ± SD	Shapiro-Wilk test
5	Treatment (resveratrol)	Angiogenesis	8.50 ± 1.73	0.19*
		Fibroblast	31.25 ± 3.86	0.71*
		Collagen deposition	54.85 ± 7.00	0.98*
	Control	Angiogenesis	6.00 ± 1.41	0.16*
		Fibroblast	20.00 ± 2.94	0.73*
		Collagen deposition	50.73 ± 3.85	0.06*
14	Treatment (resveratrol)	Angiogenesis	6.00 ± 0.81	0.68*
		Fibroblast	35.00 ± 5.59	0.34*
		Collagen deposition	71.62 ± 4.42	0.11*
	Control	Angiogenesis	4.25 ± 0.95	0.27*
		Fibroblast	29.50 ± 7.32	0.67*
		Collagen deposition	59.71 ± 8.57	0.75*
21	Treatment (resveratrol)	Angiogenesis	2.25 ± 1.25	0.40*
		Fibroblast	15.75 ± 4.27	0.40*
		Collagen deposition	84.79 ± 4.89	0.51*
	Control	Angiogenesis	0.75 ± 0.95	0.27*
		Fibroblast	6.00 ± 1.41	0.16*
		Collagen deposition	66.43 ± 4.59	0.97*
	Treatment (resveratrol)	Epithelialization	13.50 ± 1.73	0.23*
	Control		20.25 ± 0.75	0.12*

*Normal distribution if $p > 0.05$. SD: Standard deviation.

Comparative analysis using independent t-test for variables of angiogenesis, number of fibroblasts, and collagen deposition on days 5, 14, and 21 is shown in Tables 3-5. Angiogenesis was significantly higher in the treatment group than the control group on day 5 and 14 ($p = 0.047$ and 0.032) but not significantly different on day 21 ($p = 0.107$). The number of fibroblasts in the treatment group was significantly higher on day 5, 14, and 21 ($p = 0.004$; 0.038 and 0.005). Collagen deposition was not

Table 2: Homogeneity test

Day	Variable	Levene test of homogeneity
5	Angiogenesis	0.70*
	Fibroblast	0.61*
	Collagen deposition	0.23*
14	Angiogenesis	0.50*
	Fibroblast	0.35*
	Collagen deposition	0.12*
21	Angiogenesis	0.78*
	Fibroblast	0.08*
	Collagen deposition	0.60*
	Epithelialization	0.35*

*Homogen (p ≥ 0.05)

significantly different on day 5 (p = 0.342) but significantly higher on day 14 and 21 (p = 0.048 and 0.002). The epithelialization process occurred earlier in the treatment group than in the control group (p = 0.001).

Table 3: Independent *t*-test of angiogenesis, fibroblast, and collagen deposition on days 5, 14, and 21 between treatment and control groups

Variable	Group	Day	Mean ± SD	Mean difference	CI 95%	p
Angiogenesis	Treatment (resveratrol)	5	8.50 ± 1.73	2.50	1.23–5.23	0.047*
	Control		6.00 ± 1.14			
	Treatment (resveratrol)	14	6.00 ± 0.81	1.75	0.21–3.28	0.032*
	Control		4.25 ± 0.95			
	Treatment (resveratrol)	21	2.25 ± 1.25	1.50	−0.43–3.43	0.107*
	Control		0.75 ± 0.95			
Fibroblast	Treatment (resveratrol)	5	31.25 ± 3.86	11.25	2.42–17.19	0.004
	Control		20.00 ± 2.94			
	Treatment (resveratrol)	14	35.00 ± 5.59	5.50	5.38–16.78	0.038*
	Control		29.50 ± 7.32			
	Treatment (resveratrol)	21	15.75 ± 4.27	9.75	4.24–15.25	0.005*
	Control		6.00 ± 1.41			
Collagen deposition	Treatment (resveratrol)	5	54.85 ± 7.00	4.12	−5.65–14.63	0.342
	Control		50.73 ± 3.85			
	Treatment (resveratrol)	14	71.62 ± 4.42	11.91	0.11–23.7	0.048*
	Control		59.71 ± 8.57			
	Treatment (resveratrol)	21	84.79 ± 4.89	18.36	10.14–26.57	0.002*
	Control		66.43 ± 4.59			

*Significant (p ≥ 0.05). SD: Standard deviation, CI: Confidence interval.

Angiogenesis in the treatment group was indeed higher, but overall wound healing activity decreased (higher on day 5, and decreased on day 14 and 21), as well as the number of fibroblasts, whereas collagen deposition in the treatment group higher than control and overall wound healing activity increased (increasingly on day 14 and 21). The wound healing process in the treatment group was faster, because there was an increase in activity in each phase (Figures 1 and 2).

Table 4: Independent t-test of epithelialization rate between the treatment and control groups

Variable	Group	Mean ± SD	Mean difference	p
Epithelialization	Treatment (resveratrol)	13.50 ± 1.73	6.75	0.001*
	Control	20.25 ± 1.50		

*Significant (p ≥ 0.05). SD: Standard deviation.

Discussion

Wound healing is a response to an injury aimed at reconstructing damaged tissue, this process requires precise coordination of connective tissue repair, re-epithelialization, angiogenesis, fibroblast proliferation, production of several extracellular matrix proteins, and growth factors to accommodate generation of new tissue and healing of the wound. Angiogenesis is required during wound healing to supply oxygen and metabolites to new tissue hence disposes of metabolic waste products during wound repair. The role of angiogenesis may also be a key regulatory process in wound healing, as impaired angiogenesis leads to delayed or unsuccessful wound healing.

Table 5: Analysis of variance test between treatment groups on the parameters of angiogenesis, number of fibroblasts, and collagen deposition

Variable	Sum of squares	df	Mean square	F	p
Angiogenesis					
Between groups	158.37	5	31.67	20.92	<0.001*
Within groups	27.25	18	1.51		
Total	185.62	23			
Fibroblast					
Between groups	2.419,33	5	483.86	22.53	<0.001*
Within groups	386.50	18	21.47		
Total	2.805,83	23			
Collagen deposition					
Between groups	3.086,74	5	617.34	18.32	<0.001*
Within groups	606.39	18	33.68		
Total	3.693,13	23			

*Significant (p ≥ 0.05).

The result of this study shows that the administration of topical resveratrol gel increased the amount of angiogenesis in deep partial thickness burns of Wistar rats compared to paraffin gauze. This is similar with other studies which state that resveratrol induces angiogenesis in FOXO1-Dependent in mice with Hind Limb Ischemia. Administration of resveratrol in human umbilical vein endothelial cells can increase FOXO1 phosphorylation and cytoplasmic localization to induce angiogenesis [5]. Resveratrol has a positive effect on VEGF expression and granulation in healing wounds. Administration of resveratrol increased VEGF expression depending on the concentration given. Higher granulation in wound tissue due to high VEGF expression accelerates wound healing. The mean granulation in the resveratrol-treated group was significantly higher than in the control group [6].

Several previous studies have suggested that resveratrol has a contrast effect on angiogenesis which may exert pro-angiogenetic or anti-angiogenetic effects depending on several factors. Resveratrol dose and cell type have been shown to influence its effect on angiogenesis [7]. Pro-angiogenetic effects were detected in tissues affected by ischemia reperfusion injury, such as peri-infarction in the myocardium [8], while anti-angiogenic effects were observed during tumor growth [9]. This strengthens the reason that resveratrol can accelerate the tissue healing process in ischemic burns.

Administration of resveratrol reduces oxidative stress levels and improves the healing process by increasing cell proliferation and cell migration quality [10]. The study by Celen *et al*. (2018) showed that the proliferative and migratory activity of fibroblast

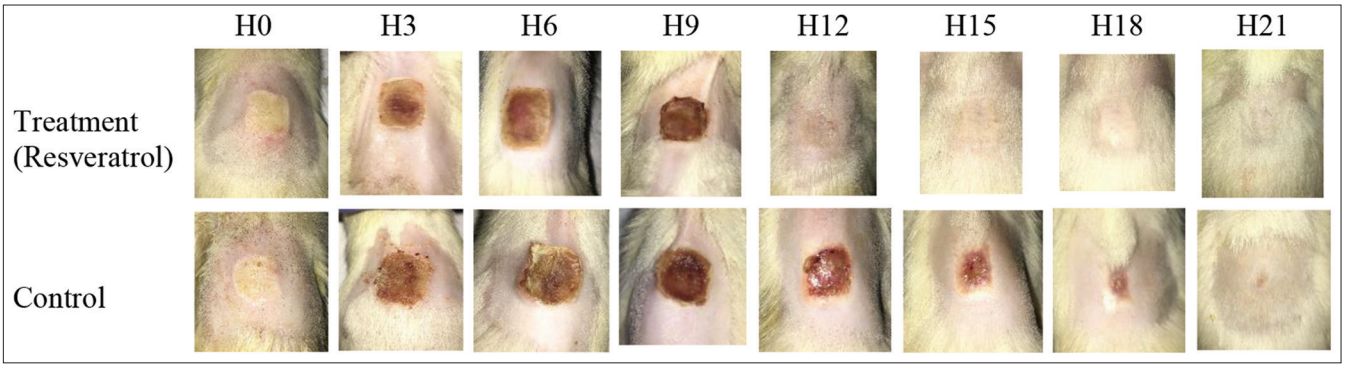

Figure 1: Images of wound healing. Representative images of Wistar rats in control group and the groups treated with resveratrol

cells was significantly increased by resveratrol-containing organogels [11].

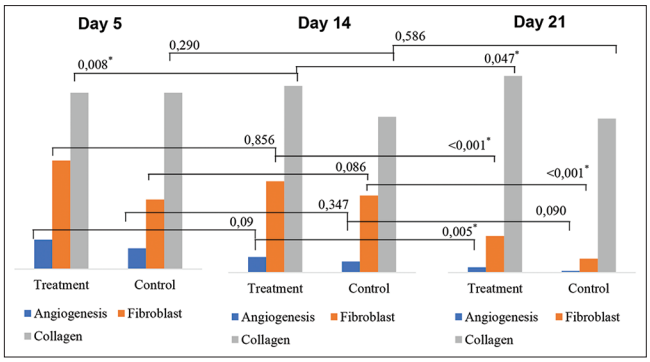

Figure 2: Post Hoc Test on angiogenesis, fibroblast variables, and collagen deposition on days 5, 14, and 21

However, the effect of resveratrol on tissues and cells was found to change depending on the dose as a therapy in the wound healing process. The results of research by Bosutti and Degens (2015) showed that low doses of resveratrol can stimulate cell migration, while high doses inhibit cell migration [12].

Other studies have shown that resveratrol can inhibit fibrogenesis and induce apoptosis in keloid fibroblasts, but not uniquely in normal skin. The mechanism is not yet clearly understood and further investigation needs to be carried out. Resveratrol has a strong antifibrogenic effect by inhibiting the activity of nuclear factor-κB (NF-B) and the production of transforming growth factor-b1 (TGF-β1) in rats with carbon tetrachloride (CCL4)-induced liver cirrhosis [13]. These findings explain the potential of resveratrol in increasing fibroblast activity while preventing keloid formation.

Dose-dependent characteristic of resveratrol also affects the collagen deposition that occurs. Low doses of resveratrol have been shown to inhibit oxidation and inflammation, so can induce cell proliferation, and accelerates wound healing process. Otherwise, high doses of resveratrol can reduce collagen synthesis in several cell types or animal models. Research by Zeng *et al*. (2013), resveratrol was found to significantly inhibit cell growth, stop the cell cycle in G1 phase, induce apoptosis in fibroblasts, decrease hydroxyproline or collagen levels, and decrease type I and III procollagen mRNA expression levels [14]. This is also similar to the research conducted by Wei *et al*. (2016) who performed a red-stained examination of picrosirius collagen fibers and showed that resveratrol can decrease collagen deposition during adhesion formation in a mouse model.

This study also showed that the administration of topical resveratrol gel accelerated the epithelialization process in deep partial thickness burns in Wistar rats compared to paraffin gauze. These findings are similar with the previous studies by Wang *et al*. using a hydrogel composite containing resveratrol and the vascular EGF plasmid which significantly inhibits the inflammatory response, promotes the formation of micro blood vessels, and accelerates burn wound healing through its effect in accelerating the re-epithelialization process *in situ* [15].

The role of resveratrol on epithelial tissue is known to be mediated by its effect in increasing the rate of cell proliferation. Resveratrol was reported to be able to increase the secretion of EGF, HGF, PDGF, and TGF-β1 from cultured mesenchymal stem cells (MSCs) which showed a response to resveratrol stimulation in the process of cell proliferation and growth factor secretion, which also reported that the effect was dependent on topical doses [16].

Conclusion

Topical administration of resveratrol gel accelerates the epithelialization process, which increases the number of angiogenesis, fibroblasts, and collagen deposition in deep partial thickness burns compared to paraffin gauze. Weaknesses have been found in this study, including variations in the appearance of burns on the specimens because the usage of non-flat electric solder tip.

For the next study, we suggest using more observation parameters to prove significant effect of topical resveratrol gel administration and using several different concentrations to prove the precise gel concentration level for the deep partial thickness

burns healing. We also suggest creating deep partial thickness burns using modified flat surface electric solder tip. Wider burns area is needed to observe all variables in the same specimen.

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