HEPARIN EFFECT ON VEGF AND EGFR AFTER DEEP SECOND AND THIRD DEGREE BURNS IN RATTUS NOVERGICUS STRAIN WISTAR

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ABSTRACT

The objective of this study was to study the effect of heparin on angiogenesis and epithelialization acceleration. The design of this study was experimental. This study used forty eight rats randomly divided into four groups. A group of deep second degree burns and a group of third degree burns were each divided into two groups, experimental and control groups. The experimental group was divided into 2, both treated with 1 ml heparin 100 IU/kg and saline solution injected beneath the burnt skin once daily, in which 1 group received it for 5 days while other up to 10 days. The control group was divided into 2, both treated with 1 ml saline solution injected beneath the burnt skin once daily, in which 1 group received it for 5 days while other up to 10 days. Observation was done at fifth and tenth day. Parameter of angiogenesis was VEGF count. Parameter of epithelialization acceleration was EGFR count. VEGF and EGFR counts were evaluated with immunohistochemistry. Results showed a significant difference was found between VEGF in control and treatment groups demonstrated by considerably increased VEGF count in the treatment groups. There was a significant difference in EGFR in control group compared to that in treatment group with evidence of considerably greater EGFR count in treatment group. A significant difference was found between VEGF and EGFR in deep second degree burns and those in third degree burns, demonstrated by considerably increased VEGF and EGFR count in the latter. There was no significant difference between VEGF and EGFR counts on day 5 and day 10. In conclusion, subcutaneous injection of heparin could promote VEGF and EGFR counts by means of promoting angiogenesis and epithelialization acceleration resulting in wound healing acceleration.

***Keywords**: Heparin, burn, angiogenesis, epithelialization*

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INTRODUCTION

Burn is destruction or loss of tissue caused by contact with source of heat such as fire, hot water, chemical, electricity and radiation which come into contact with skin, mucosa and deeper tissues (Marzoeki 1993; Moenadjat 2001). Cell damage due to burn can increase when zone of stasis deteriorates and became zone of coagulation due to secondary ischemic process (Noble, et al., 1977). Angiogenesis and epithelialization are important processes of wound healing when a wound is said to have healed if already covered with epithel. Growth factors (GF) required in angiogenesis are vascular endothelial growth factor (VEGF) and angiopoietin. VEGF emerges as the most important GF in vascular development during embriogenesis to the extent of angiogenesis on adult (Cotran et al. 1999).

GF playing a potential role in epithelialization are EGF (epithelial growth factor) and its receptor (EGFR), present in all epithelial and stromal cells. Increasing EGF in fact hastens the de novo synthesis rate of EGFR, resulting in increasing EGFR count (Sizemore et al. 1996). EGFR modulation holds tremendous promise in promoting wound repair and limiting scarring (Wells 1999). Increased EGFR will expedite signaling of proliferation, migration and accelerating wound healing. Activation of EGFR alone is not performed by EGF but also by transforming growth factor-α (TGF α), amphiregulin and heparin-binding EGF (HB-EGF), so that EGFR is more suitable as a parameter of epithelization (Wells 1999).

Besides being used as an anticoagulant, in higher dosage, heparin can be used as an anti inflammatory agent, for accelerating angiogenesis (Polykratis et al. 2004), cellular growth and development (Sasisekharan and Venkataraman, 2000) so that wound healing is accelerated (Galvan 1996; Saliba 2001). Heparin administration will strengthen the bond between growth factor and its receptor enabling it to emit signals into the cellular nucleus causing proliferation and differentiation of fibroblasts and vascular endothelial cells (angiogenesis) accelerating wound healing (Iozzo and Antonio

2001). This research was performed to evaluate the effect of heparin on the increase of VEGF and EGFR in Rattus novergicus strain Wistar with deep second degree and third degree burns on day 5 and day 10 using immunohistochemical examination. Day 5 was chosen as a time of initial proliferation and on day 10 angiogenesis process has started to decrease.

MATERIAL AND METHODS

The design of this research is experimental with post test control group design. Design of this study was experimental. This study used forty eight rats randomly divided into four groups. A group of deep second degree burns and a group of third degree burns were each divided into two groups, experimental and control groups. The experimental group was divided into 2, both treated with heparin 100 IU/kg diluted with normal saline up to 1 ml and injected beneath the burnt skin 1 hour after the burn injury, then afterward administered beneath the burnt skin once daily, in which 1 group received it for 5 days while other up to 10 days. The control group was divided into 2, both treated normal saline injected beneath the burnt skin 1 hour after the burn injury, then afterwards injected beneath the burnt skin once daily, in which 1 group received it for 5 days while other up to 10 days.

After anesthesia was performed with ether, deep second degree burns were produced by holding fast the head of a nail, which was previously immersed in boiling water 100^{o}C for 5 minutes, onto the backs of the rats for 3–4 seconds. Third degree burns were made by holding fast the head of a nail, which was previously immersed in boiling water 100^{o} C for 5 minutes, onto the backs of the rats for 10 seconds. On days 5 and 10, biopsies of burnt tissue were performed using standard operative procedure excising the whole burnt tissues which were then fixed on filter paper and placed in bottles already filled with formalin 10% and buffer. After fixation, specimen were sent to Pathology Laboratory in the Faculty of Medicine at Airlangga University Surabaya for routinely embedded in paraffin and sections were cut onto subbed glass slides. Immunohistochemistry was carried out on polyclonal antibodies (ABs) against VEGF and EGFR at Biomedical Laboratory Brawijaya University Malang to obtain VEGF and EGFR counts. Prior to statistical analysis, normality test was performed on VEGF and EGFR counts associated with treatment, degree of burn and observation time yielding a normal distribution and correlation test between VEGF and EGFR was performed yielding a positif correlation result. Then multivariat analysis of varians (MANOVA) was performed.

RESULT

Table 1. Mean and standard deviation of VEGF associated with treatment, degree of burn and observation time

Observation day	Treatment		Control	
	IIB	III	IIB	III
5	16.00±1.10	19.67±2.42	5.83±1.17	5.67±1.86
10	14.50±0.55	19.33±3.01	5.00±1.55	7.67±1.21

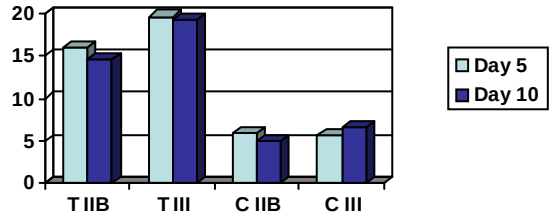

Figure 1. Mean and standard deviation VEGF associated with treatment, degree of burn and observation time.

Table 2. Mean and standard deviation of EGFR associated with treatment, degree of burn and observation time

Observation day	Treatment		Control	
	IIB	III	IIB	III
5	13.83±1.72	19.50±2.88	3.83±0.98	5.50±0.84
10	14.67±1.03	17.50±1.38	4.83±1.33	6.00±0.63

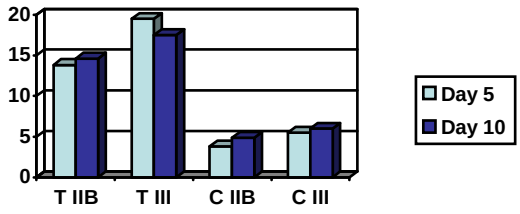

Figure 2. Mean and standard deviation EGFR associated with treatment, degree of burn and observation time.

DISCUSSION

There was no significant difference in VEGF and EGFR counts day 5 compared to those on day 10. This may due to the short interval between observation time and

the fact that the increase was not linear as the proliferation phase proceeded to maturation/ contraction phase. A significant difference was found between VEGF and EGFR in deep second degree burns and those in third degree burns, demonstrated by considerably increased VEGF and EGFR count in the latter. This finding could be caused by tissue destruction in third degree burn which was much more severe than in deep second degree burn, so the inflammation mediator activation was much greater, and activating the release of more GF.

Table 3. Multivariat test VEGF and EGFR associated with treatment, degree of burn and observation time

Effect	p Wilks' Lambda value	Description
Treatment	0.0001	S
Degree	0.0001	S
Observation time	0.923	NS
Treatment * Degree	0.0001	S
Treatment * Observation time	0.161	NS
Degree * Observation time	0.021	S
Treatment * Degree * Observation time	0.353	NS

* interaction S : significant NS : not significant

Table 4. Multivariat Analysis of Varians VEGF and EGFR associated with treatment and degree of burn

Variation	MANOVA	
	F value	P value
Treatment		
• VEGF	461.875	0.0001
• EGFR	626.983	0.0001
Degree		
• VEGF	90.750	0.0001
• EGFR	96.333	0.0001
Treatment * Degree		
• VEGF	8.091	0.0007
• EGFR	9.797	0.0003

This research demonstrated a signicant difference between VEGF counts in control and in treatment groups demonstrated by considerably increased VEGF counts in these treatment groups. There was a significant difference in EGFR in the control group compared to that in the treatment group receiving daily subcutaneous injection of heparin for 5 and 10 days with evidence of considerably greater EGFR count in the treatment groups. This finding supported previous researches on the role of heparin in angiogenesis. Iozzo (2001) found that the signaling pathways activated by FGFs and VEGFs are essentially heparan sulfate–dependent, as evidenced by the failure of cells deficient in heparan sulfate biosynthesis to activate these pathways and the ability of exogenous heparin/HSPG to restore their function (Iozzo & Antonio 2001). Saliba (2000) found that full thickness 1.5 cm burns in rats [9] treated with heparin intramuscularly for 7 days starting 4 h postburn, healed in 24 days, 7 days faster then rats not given heparin. When heparin was administered in rats 24 h after injury, healing time was prolonged by 4 days to 28 days. Heparin-rats had more blood vessels and improved blood flow in and around the burn bases compared to control rats (Saliba 2001).

CONCLUSION

Daily subcutaneous injection of heparin for 5 and 10 days could promote VEGF and EGFR counts by means of promoting angiogenesis and epithelialization acceleration resulting in wound healing acceleration.

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