

THE EFFECT OF ESTROGEN ON WOUND HEALING IN RATS

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ABSTRACT

Objective: Cutaneous wound healing involves multiple cooperative molecular processes such as inflammation, angiogenesis, wound contraction, granulation tissue formation, reepithelialization, and matrix deposition. We studied the effects of topical estrogen on wound healing in male rats.

Methodology: This experimental study was done on 40 male rats. A circular wound with a diameter of 2cm was induced on each rats right flank. Twenty rats received topical estrogen (case group). And twenty other rats received placebo (control group). After the 5th, 10th, 15th, 30th, 35th, 40th and 45th days, healing process was compared between the two groups.

Results: On the 10th and 20th days the total healing surface in the case group was about 89.9% and 100% respectively and 75% and 98,4% (p<0.05 and P>0.05) in the control group.

Microscopic views revealed the formation of epithelial layer and hair follicles, progressive angiogenesis without scarring in case group. But neither hair follicles nor complete epithelial layer in the control group

Conclusion: Topical estrogen administration results in significant progress of cutaneous wound healing, leaving no scar or crust formation. Topical estrogen administration accelerates healing without changing plasma estrogen level and can minimize the probable wound complications.

KEY WORDS: Wound healing, Estrogen, Angiogenesis.

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INTRODUCTION

The healing process has been a matter of discussion for many years. Non healing or chronic wounds are a significant healthcare problem today; the quest for better wound-healing agent is perhaps one of the oldest challenges for medical practice, one such agent that has been tried in wound healing is estrogen.¹⁻³ Knowing the fact about the steps and mechanisms of wound healing, collagen metabolism and extra cellular matrix, has nowadays

dramatically increased our ability to treat acute and chronic wounds.² Wound healing improves both macroscopically and microscopically as the level of TGF- β 1 increases.⁴ In addition, topically applied estrogen can reverse the marked delay in acute incisional wound repair presented by ovariectomized young female rats, indicating that estrogen modulates both the rate and quality of wound healing.⁵ Metalloproteinases are involved in extracellular matrix and basement membrane remodeling and destruction during physiological and pathological processes, inducing wound healing.^{6,7} Elastase and metalloproteinase are mainly products of neutrophils. Estrogen has been shown to decrease neutrophils number and activity. Thereby decreasing the level of Elastase and metalloproteinase which leads to improve matrix formation, retain skin thickness and enhance wound healing.⁶⁻⁸ In this study the effect of local estrogen on wound

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healing was assessed. The main goal was to determine whether or not estrogen administration could accelerate wound healing and angiogenesis.

MATERIALS AND METHODS

All animal procedures were in accordance with the declaration of Helsinki and with the guide for the care and use of laboratory animals. This was an experimental study, which was carried out in the animal's lab of Isfahan University of Medical Sciences. The study was done on 40 Albino male rats (body weight 150±5 grams, age 3±0.5 months).

They were randomly allocated into two groups. Following a 0.5cc injection of 1% xylocain solution intra-dermal on right side flank and then we induced a circle shaped wound of 2cm in diameter in anesthetized skin so that the fascia was exposed. The wounds in the case group rats were treated with a daily topical dose of 0.5mg estrogen and topical gentamicin 0.1% while the control group rats had only topical gentamicin 0.1%.

The course of treatment and study lasted for 45 days. Blood sample were obtained from the nasal canthus of all the rats on the 1st and the 45th days of the course. The samples were sent to lab to have the levels of serum estrogen measured. Animals were housed one per cage

(temperature approximately 20-25C °) and the wounds were appropriately covered with moist saline dressing to prevent rats from grooming or licking, their diet provided with standard laboratory food and water. The wounds sizes were measured on the 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th and 45th day of the course and total wound healing surfaces in each group were calculated and results were compared with the control group. We used an information recording sheet for each rat. On the 20th day a 5mm biopsy was obtained from each wound. This biopsy included skin, healed wound tissue and normal margin. Slides were all filed and stored for comparison and scoring. A histological scoring system was previously validated through similar experimental models, and was based on the qualitative and quantitative aspects of healing, such as the degree of reepithelialization, granulation tissue formation, presence of inflammatory cells and angiogenesis.⁹⁻¹¹ The quantitative aspects of the score were evaluated by the percentage of the tissue presenting the specific qualitative features of wound healing. The criteria have been indicated in (Table-I).

RESULTS

During the 45-day period of receiving estrogen the 20 rats of the case group demonstrated

Table-I: Scoring of histological changes in wound healing

| Score | Reepithelialization | Granulation tissue | Inflammatory cells | Angiogenesis |
|-------|---|---|---|--|
| 0 | Absence of epithelial proliferation in ≥70% of the tissue | Immature and inflammatory tissue in ≥70% of the tissue | 13 - 15 inflammatory cells per histological field | Absence of angiogenesis, presence of congestion, edema hemorrhage |
| 1 | Poor epidermal organization in ≥60% of the tissue | Thin immature and inflammatory tissue in ≥60% of the tissue | 10 - 13 inflammatory cells per histological field | 1 - 2 vessels per site, edema hemorrhage congestion |
| 2 | Incomplete epidermal organization in ≥40% of the tissue | Moderate remodeling in ≥40% of the tissue | 7 - 10 inflammatory cells per histological field | 3 - 4 vessels per site, moderate edema and congestion |
| 3 | Moderate epithelial proliferation in ≥60% of the tissue | Thick granulation layer and well formed collagen matrix in ≥60% of the tissue | 4 - 7 inflammatory cells per histological field | 5 - 6 vessel per site, slight edema and congestion |
| 4 | Complete epidermal remodeling in ≥80% of the tissue | Complete tissue organization in ≥80% of the tissue | 1 - 4 inflammatory cells per histological field | More than 7 vessels per site vertically disposed toward the epithelial surface |

the following states; Wounds in nine of them were fully healed on 10th day. On the same day the area of wounds in four of them had decreased to one-fourth, i.e. 0.5cm, while in 7 rats the size of the wound area had reduced to half of the original size ($P < 0.05$).

Five days later (on the 15th day), wounds showed complete healing in thirteen rats, but the wound size in seven rats in this group was still about 0.5cm. During the last 25 days (from the 20th to the 45th day) there were no wounds in the estrogen-receiving group.

In the control group complete healing occurred by the 10th day in 15 rats, meanwhile wound size in 5 rats did not differ from the 1st day and the area of wounds in these rats decreased to 1cm on the 15th day and 0.5cm on the 25th day. All wounds in the control group completely healed by the 30th day.

Fig-1 shows the wound state according to time in both groups. In the case group, hair grew on the healed wounds of 13 rats by the 15th day and the remaining 7 rats had no hair on the healed wounds ($P = 0/0001$). On the 20th day, wounds in all 20 rats in the case group were covered with hair ($P = 0$). In the control group no hair grew on any of the wounds until the 25th day ($P = 0$). Between the 30th and 45th days, wounds in 16 rats were covered with hair and finally on the last day of the course in 4 rats, wounds still did not have any hair. Histological evaluation of the wounds revealed a

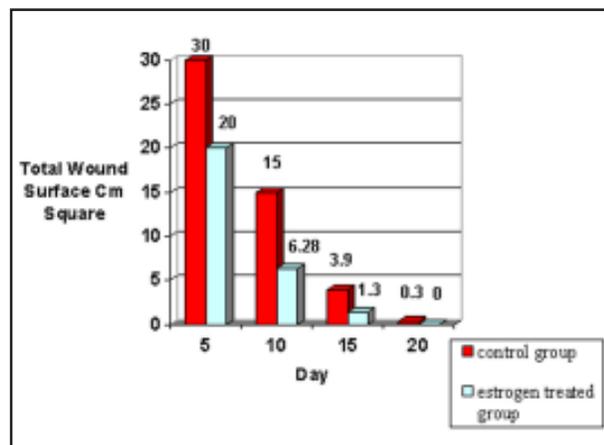


Fig-1: Wound surface in estrogen treated and control group

remarkable difference between control and estrogen treated mice (Fig-2).

Wounds in estrogen treated mice exhibited good reepithelialization and granulation tissue organization. In particular epidermal regeneration was characterized by well-structured epithelial layers with no evidence of crusting or intra-epithelial inflammatory cells.

DISCUSSION

In this study data implicate topical estrogen in increasing the extent of wound healing by reducing wound size and stimulating matrix deposition. In microscopic examination there was a marked increase in wound collagen deposition in estrogen treated rats.¹

Cutaneous wound healing is initially characterized by inflammation followed by the formation of granulation tissue, subsequent re-epithelialization, and finally tissue remodeling. Review of literature demonstrates improved wound healing in men and women treated with topical estrogen.^{8,12,13} An excessive inflammatory response with resultant proteolysis has been implicated in delayed wound healing.³⁻¹⁴ Elevated levels of proteolytic enzyme, such as Elastase, in association with high neutrophils count have been demonstrated in the

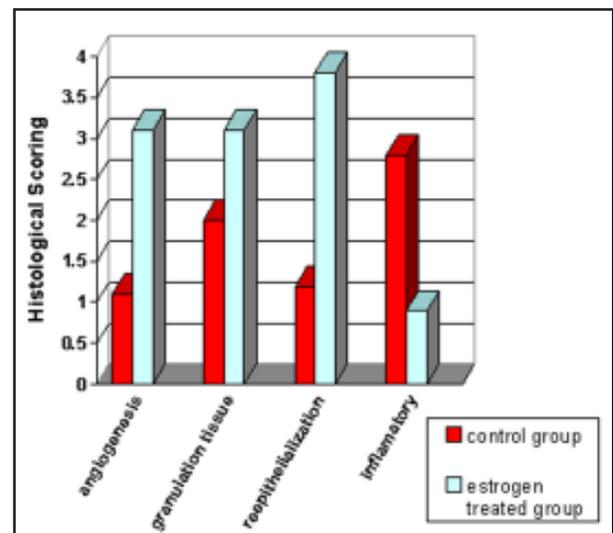


Fig-2: Angiogenesis, granulation tissue, reepithelialization and inflammatory cell in estrogen treated and control group by using histological scoring of Table-I on the 20th day.

wounds.¹¹ Initially, it was thought that estrogen increased the level of TGF- β 1 a cytokine involved in cell proliferation, differentiation, and matrix production.^{6,7} Estrogen has been shown to decrease neutrophils chemotaxis and adhesion, thereby decreasing the levels of Elastase in the wounds and allowing for improved cellular matrix formation.⁸ Estrogen regulates multiple signaling pathways involved in the interplays between growth factors, integrins, and proteases, key participants in new vessel formation.^{15,16}

The role of estrogen in wound healing is complex and not fully understood however, sufficient evidence supports its role in accelerating wound healing.

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