

In vivo histological evaluation of the effect of the topical application of estrogen hormone on wounds healing in ovariectomized rabbits

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ABSTRACT

Background: Wound healing, as a normal biological process in the human body, is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. Growth factors released in the traumatized area promote cell migration into the wound area (chemotaxis), stimulate the growth of epithelial cells and fibroblasts (mitogenesis), initiate the formulation of new blood vessels (angiogenesis), and stimulate matrix formation and remodeling of the affected region. One of factors that effects on wound healing is a sex hormones and one of these hormones is an estrogen hormone. A wide range of cutaneous cell types (eg, fibroblast, endothelial, epithelial, and inflammatory) expressed estrogen receptors, indicating potential estrogen responsiveness.

Materials and methods: Thirty two female New Zealand rabbits were used in this study. All animals were ovariectomized, and incisional wounds were done on the right (experimental for estrogen hormone application) and left (control) sides of face for each animal, the control side was left to heal normally. Histological assessment regarding the count of inflammatory cells was performed for healing intervals (3, 7, 10, 14 days).

Results: Topical estrogen hormone application revealed enhancement of wound healing by reducing wound size and stimulating matrix deposition in comparison to control.

Conclusion: Topical estrogen cream application results in significant progress of cutaneous wound healing, leaving no scar or crust formation and can minimize the probable wound complications.

Key words: Estrogen hormone, wound healing. (J Bagh Coll Dentistry 2015; 27(1):100-104).

INTRODUCTION

Skin is the biggest external defense system. Skin covers the outside of the body but has other functions beside the defense mechanism. It serves as a mechanical barrier between the inner part of the body and the external world. It consists of three layers, the outer layer is called epidermis, the middle layer is dermis and the inner most layer is hypodermis ⁽¹⁾. A wound is defined as a defect or break in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physical condition ⁽²⁾.

Wound healing is a complex process consisting of four steps: haemostasis, inflammatory reaction, proliferation and remodeling, all of which are regulated by cytokines and growth factors released by cells in the wounded area. ⁽³⁾.

Estrogens are a class of hormones produced in the ovaries, or made from other hormones in fat cells. There are three estrogens: estradiol, estratriol and estrone. These natural hormones work more efficiently compared to synthetic estrogens or, even worse, animal estrogens. In the case of the skin the differential targeting of estrogen receptors to promote healing in aged subjects is a real therapeutic possibility ⁽⁴⁾.

Estrogen, stimulate proliferation of keratinocytes and suppresses apoptosis and thus prevents epidermal atrophy.

Estrogen also enhances collagen synthesis, and estrogen and progesterone suppress collagenolysis by reducing matrix metalloproteinase (MMP) activity in fibroblasts, thereby maintaining skin thickness ⁽⁵⁾

With a reduction of steroid hormones following ovariectomy, alternatively activated macrophage markers were reduced ⁽⁶⁾

MATERIALS AND METHODS

Materials:

- Estrogen cream (ALDO-union)
- Anesthetic solution: Ketamine Hydrochloride (Ketamin 50mg/ml){1 ml/kg body weight}; Xylocain (10%,){1 ml/kg body weight}.
- Zylazine (20mg/ml).
- Formalin 10%, Ethanol alcohol 96%, Xylol, Paraffin wax.
- Hematoxylen and eosin (H&E).

Method

Thirty two female New Zealand white rabbits age from 6-12 months were used as animal model in this study, their weights ranged between 1.5-2.5 kg .The experimental animals were divided into four groups, eight animals for each healing interval (3,7,10,14 days) all animals were subjected to ovariectomy operation, and all animals left about two weeks after doing

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ovariectomy operation. Then animals in each group were operated as follows:

- a- Right facial skin was operated as experimental side .
- b- Left facial skin was operated as control side.

Analysis of number of inflammatory cells

It was performed by counting inflammatory cells, in histological sections (H&E stained), for each animal and in four microscopic fields at x40 magnification. Scores for intensity of inflammatory reaction:

- 1 Absent or very few inflammatory cells.
- 2 Mild: average number less than 10 inflammatory cells
- 3 Moderate: average number 10-25 inflammatory cells
- 4 Severe: average number greater than 25 inflammatory cells ⁽⁷⁾

Histological preparation

All tissue specimens, samples and controls, were fixed in 10% neutral formalin and processed in a routine paraffin blocks. Each formalin-fixed paraffin-embedded specimen had serial sections were prepared as follows: 5µm thickness sections were mounted on clean glass slides for routine Haematoxylin and Eosin staining (H&E), from each block of the studied sample (experimental and the control groups) for histo- pathological re-examination.

RESULTS

Three days duration

Control group

After three days of skin incision, the histological view of wound site of control group shows the defect area, no epithelium is formed yet and necrotic tissue is seen (figure1).



Figure 1: View of wound site of control group after 3days shows defect area with necrotic tissue. H&EX10.

Experimental group

Microphotograph view of skin of 3days duration at wound site of experimental group shows area of acute inflammation and epithelial cells migration also shows granulation tissue formation and hair follicles are formed (Figure 2).

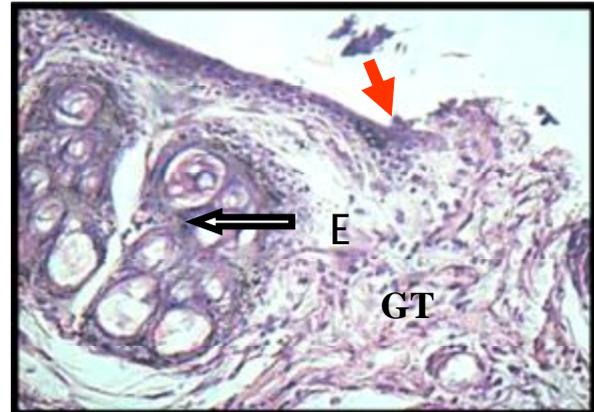


Figure 2:View at wound site of experimental group after 3days shows area of acute inflammation and epithelial cells migration (red arrow), cross section of epithelia(E) and granulation tissue (GT). H&EX20.

Seven days duration

Control group

Histological findings of wound site of control group of 7days duration, shows complete epithelialization, the subepidermal layer is congested with numerous blood cells and there is decreased number of inflammatory cells, together with deposition of loose collagen fibers (figure3).

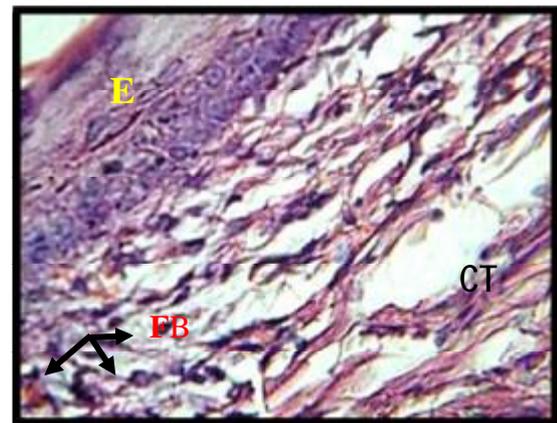


Figure 3: Microphotograph of control group, after 7days showing complete epithelialization (E), loose fibrous connective tissue and fibroblasts (FB) H&EX40.

Experimental group

Histological findings of experimental group of 7 days duration showed reduction in

inflammatory cells and replacement of granulation tissue by fibrous connective tissue with scattered fibroblasts and complete epithelialization is seen too (Figure4).

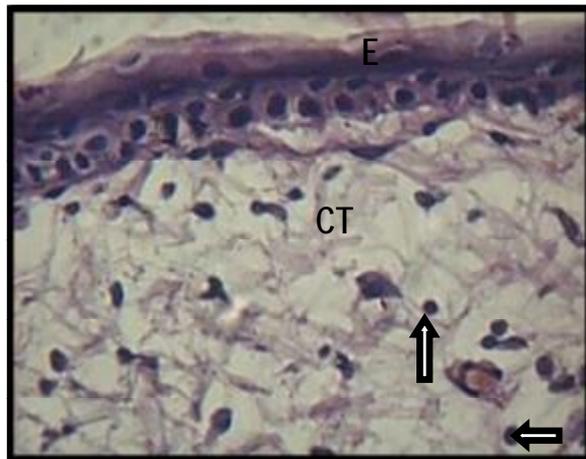


Figure 4: View of wound site of experimental group after 7days shows loose fibrous connective tissue (CT), scarce number of inflammatory cells (IC) (arrows) and thin newly formed epithelium. H & Ex40.

Ten days duration

Control group

Histological section at wound site of ten days duration of control group shows reepithelialization with no rete-ridges, newly formed hair follicles and remodeling of collagen fibers can be detected (Figure5)



Figure 5: View at control wound site after 10days illustrating thin newly formed epithelium (E), new hair follicles (HF) and remodeling fibrous connective tissue (CT). H&EX20

Experimental group

Histological section at wound site of experimental groups at 10 days duration shows

that the new epithelium is completely formed with no rete-ridges and number of fibroblasts with remodeling fibrous connective tissue (Figure 6).

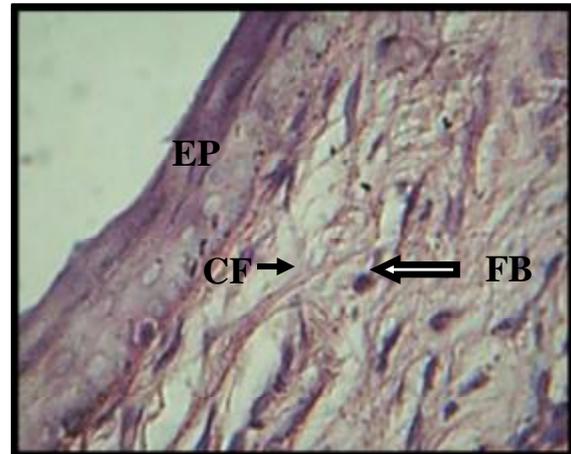


Figure 6: Micophotograph at experimental wound site of 10 days duration shows numerous fibroblasts (FB) with maturing collagen fibers (CF) and the epidermis is fully formed. H&EX40.

Fourteen days duration

Control group

Histological section at wound site of 14days duration of control group shows fibrous connective tissue with few blood capillaries also remodeling of collagen fibers which seem to be still maturing can be detected (Figure 7)

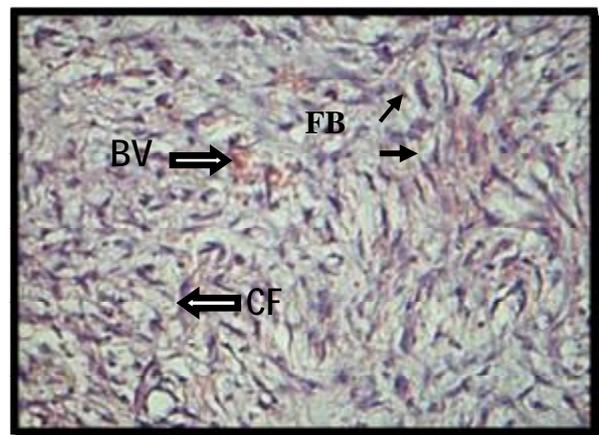


Figure 7: View at control wound site of 14 days shows mature collagen fibers (CF), fibroblasts (FB) and blood capillaries (BV). H&EX20.

Experimental group

View of skin section at wound site of experimental group at 14 days revealing complete epithelialization, the new epithelium is thin with no rete-ridges and healed wound site with fibrous

connective tissue with reduced cellular component can be detected (Figure 8).

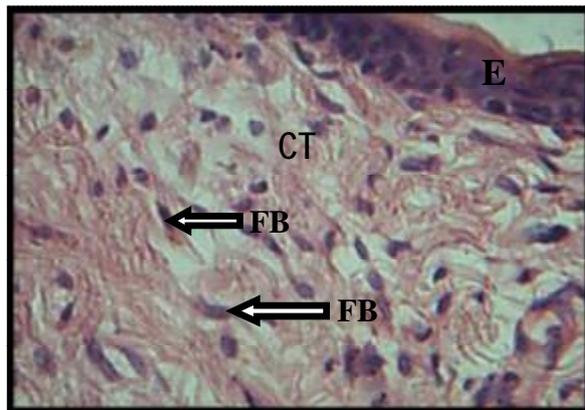


Figure 8: Microphotograph at skin section of experimental group after 14 days shows complete epithelialization, dense fibrous connective tissue (CT) with reduced number of fibroblasts (FB) (arrows). H&Ex40.

The results of the present study have shown a higher count numbers of all estimated inflammatory cells for experimental group in 3 and 7 days than in control group, while a higher count was detected at 10 and 14 days for control group than experimental group, and the highest mean values were recorded at the 3 and 7 days for both experimental and control groups.

Table 1: Distribution of the observed frequencies and percentages of the inflammatory cells in different periods

Periods	Score	Control		MS	SD	Exp.		MS	SD	P value
		No	%			No	%			
3 Days	1	0	0	2.87	0.64	0	0	3.87	0.35	0.022
	2	2	25			0	0			
	3	5	62.5			1	12.5			
	4	1	12.5			7	87.5			
7 Days	1	0	0	2.75	0.46	0	0	3	0.53	0.332
	2	2	25			1	12.5			
	3	6	75			6	75			
	4	0	0			1	12.5			
10 Days	1	0	0	2.37	0.51	0	0	2.12	0.35	0.264
	2	5	62.5			7	87.5			
	3	3	37.5			1	12.5			
	4	0	0			0	0			
14 Days	1	0	0	2.12	0.35	6	75	1.25	0.46	0.022
	2	7	87.5			2	25			
	3	1	12.5			0	0			
	4	0	0			0	0			

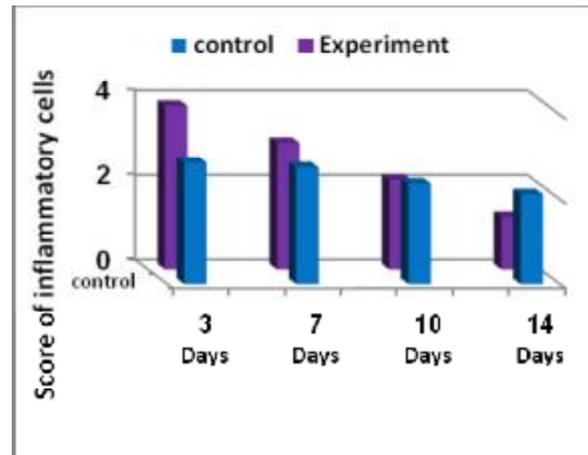


Figure 9: Cluster bar chart of inflammatory cells parameter's means of score values in different periods.

DISCUSSION

Skin healing involves cross-reactions between cells from the epidermis and dermis, with the participation of cytokines, growth factors and modulation of the extracellular matrix. This occurs in three stages: 1) inflammatory reaction; b) formation of granulation tissue; and c) remodeling of the granulation tissue⁽⁸⁾.

The result of present study showed the defect area, no epithelium is formed and necrotic tissue at three days of skin incision in control group⁽⁹⁾.

In experimental group at 3 days, histological observation showed area of granulation tissue formation, epithelial cells migration, new hair follicles were formed, numerous blood capillaries and numerous inflammatory cells⁽¹⁰⁾.

In control group at 7 days of incisional wound histological evaluation revealed complete epithelialization⁽¹¹⁾.

In experimental group at 7 days of incisional wound, the histological results showed reduction in inflammatory cell count and replacement of granulation tissue by fibrous connective tissue⁽¹¹⁾.

At 10 days of incisional wound, there is similar characteristic feature between experimental and control group and this period detected by newly formed epithelium, active fibroblast with remodeling of collagen fibers and granulation tissue⁽¹²⁾.

In the present study, the histological observation of skin wound at 14 days for control and experimental groups showed complete epithelialization, complete healing and reduce cellular inflammatory response⁽¹³⁾.

The result of the present study show that there was a marked increase in the number of inflammatory cells that infiltrated into the wound

sites in the experimental group during period of 3 and 7 days as a compare with control group, while a higher count was detected at 10 and 14 days for control group than experimental group⁽¹⁴⁾. The highest mean values were recorded at 3 and 7 days for both experimental and control groups⁽¹⁵⁾.

In conclusion; topical estrogen is a step in the direction of increasing the extent of wound healing by reducing wound size and stimulating matrix deposition, and estrogen administration results in significant progress of cutaneous wound healing, leaving no scar or crust formation. Histological findings revealed that estrogen treated wounds exhibited good reepithelialization and granulation tissue organization. Finally estrogen is the most important hormone to regulate skin hemostasis, increase collagen content and dermal thickness.

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الخلاصة

المقدمة: التئام الجروح يعتبر عملية بيولوجية طبيعية في جسم الانسان ويتحقق من خلال اربع مراحل مبرمجة بدقة عالية : وقف النزف والالتهاب والتكاثر واعادة البناء. هناك عوامل نمو تتحرر في المنطقة المصابة لتعزز وتشجع انتقال الخلايا الى منطقة الجرح (الانجذاب الكيميائي) وتحفز نمو الخلايا الظهارية والخلايا الليفية (تكون أو نمو الانقسام الخيطي) وكذلك تبدأ بتكوين اوعية دموية جديدة (تكون الاوعية والعروق) وتحفز تكوين القالب الأم واعدة بناء المنطقة المتضررة. وأحد العوامل التي تؤثر على شفاء الجروح هو الهرمونات الجنسية و واحد من هذه الهرمونات هو هرمون الإستروجين . معدل واسع من انواع الخلايا الجلديه مثل الخلايا الليفية والبطانية والظهارية والالتهابية تعتبر كمستقبلات للإستروجين . مما يدل على استجابة هرمون الإستروجين المحتملة.

المواد والعملي: استخدمت اثنين وثلاثين للأنثى ارانب نيوزيلندا في هذه الدراسة كل الحيوانات كانت مستأصلة المبيض . تم عمل الجروح الجراحية على الجانب الأيمن(الجانب التجريبي للتطبيق الموضوعي لهرمون الاستروجين والجانب الأيسر(جانب التحكم) من وجه كل حيوان , وترك جانب التحكم للشفاء بشكل طبيعي. التقييم النسيجي نسبه الى معدل الخلايا الالتهابية كان أنجز للفرات الشفاء الفاصل (3,7,10,14يوم).

النتائج: اظهر التطبيق الموضوعي لكريم الاستروجين تعزيز شفاء الجروح عن طريق تقليل حجم الجرح وتحفيز ترسب القالب الام مقارنة مع مجموعته التحكم.

الاستنتاج: اظهر الاستعمال الموضوعي لكريم الاستروجين تقدم ملحوظ على شفاء الجروح حيث لا يترك اثر او تكوين ندبه ويمكن ان يقلل مشاكل مضاعفات الجروح

الكلمات المفتاحية:هرمون الاستروجين , شفاء الجروح , علامات الجلد الكيميائي.