Histopathological study of Effects of continuous injection of dexamethasone on wound healing in rabbits

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Abstract

In this study 16 rabbit was divided in to two groups equally the first group was daily injected by dexamethasone(1 mg/Kg) for 21 days,the second group was left without any treatment as a control. An excision was made in the skin of rabbits and then sutured with silk band 2.5cm.The skin spicemen were taken for histopathological sections for wound healing evaluation at postoperative days 3,7and 14. The scars of the dexamethasone-treated group were less formation. The epithelization ,collagenization and the inflammatory cell infiltration was less intense in the dexamethasone- treated group in compare with the control animal.

Introduction

Corticosteroid markedly effect most aspect of wound healing. Systemic administration of corticosteroid which is frequently used as non –specific anti-inflammatory therapy in various diseases and traumas (1) may result in poor wound healing due to its catabolic effects in skin. The steroids reduce TGF- β and IGF-I production in wounds and that collagen deposition suffers by that mechanism (2). Dexamethasone is a potent glucocorticoid with a continuous action level ,due to its relative long biological half life (36-54hours) .it has pleomorphic effect on the immune system and may inhibit epidermal apoptosis by several mechanism :inhibition of T-cell activated apoptosis by

suppression of various cytokines such as TNF- α ;inhibition of interferon γ induced apoptosis; and inhibition of Fas-mediated keratinocyte apoptosis.(3). Dexamethasone reduce the number of cells in DNA synthesis and post wounding acanthosis of wound edge and regenerating epithelium (4). Dexamethasone has anti inflammatory effects and has been used during operation for decreasing edema formation and swelling and for preventing ischemia-reperfusion injury. Chronic or inappropriate use of dexamethasone can cause life threatening hormonal and metabolic changes(5).The aim of this study is to investigate the effect of chronic use of dexamethasone on skin wound healing.

Materials and methods

Experiments were conducted on 16 local rabbits weighing 1300–1700g, they were randomly assigned to two groups of eight animals, the first group was daily injected with dexamethasone (0.1 mg/Kg) for 21 days, intraperitoneally (IP) and the second group was left without any treatment as the control group. In a temperature-controlled and ventilated room with a 12-h light-dark cycle, animals were housed for at least 10 days before experiments and were given unlimited access to food and water. On the 22th day, after anesthesia was induced with ketamin (15ml/kg) and Xylazine (5ml/kg) intramuscularity (I.M), an excision was made in the dorsal midline of abdomen.Wound healing was evaluated at postoperative days 3, 7 and 14. On the

experiment day, The rabbit hair was closely shaved with an electrical razor, and the surgical field was disinfected with povidone-iodine and draped with sterile towels. All surgical procedures were performed under aseptic conditions . An incision, measuring approximately 5 cm, was made through the skin of each animal until the muscular fascia was exposed. Then the wound margins were opposed with a non absorbable interrupted suture. No postoperative antibiotics were given.On the 3,7 and 14th day, the wounded area was cut into a 5 x 1 cm strip under anesthesia. For histopathologic examinations,the biopsies were placed in 10% formaldehyde, embedded in paraffin, sectioned perpendicular to the wound, and stained with hematoxylin-eosin.

Histopathologic samples were examined

by using light microscope.

Result

During the observation period no animal died in control or dexamethasone groups. On the third day post operative in control animal there was thickness of wound epidermis, early inflammatory response characterized by abundance of neutrophils at the wound gap, a number of fibroblast can be seen in the dermis as in figure(1), while in dexamethasone group there is a dermal thickness, epidermal thickness was moderate with ulceration area with Carter like. The Carter has small diameter rich with inflammatory cells extending deep in the dermis like stream as in (Figure2). In the 7th day post operative control animal there was epithelization with new capillaries in the granulation

tissue with marked collagenization (figure3), dermal fibrosis, fibrosis between skeletal muscles with an area of ulceration in the dexamethasone group were noted. In control animal the blood vessels was less with great collagenization scab formation, and begin of epidermal epithelial regeneration (Figure4). On the 14th day post operative in dexamethasone group there is epidermal thickness, fibrosis, reduce diameter size of ulcer with decrease rate of inflammation (figure5), more fibrosis and keratosis (figure6). In control animal the blood vessels is less with great collagenization and epithelization.

Figure1: Section of skin in control group after 3 days, note thickness of epidermis rich with inflammatory cells. X10 (H&E)

Figure 2: Show the skin in dexamethasone group after 3days, note ulceration and Carter -like .X4 (H&E).

Figure3: Skin of control group after 7days, note scab formation (1), fibrosis (3), regeneration epidermal epithelial (2) .x10 (H&E).

Figure4: skin of dexamethasone group after 7days, note scab formation(1), fibrosis(2), regeneration of epidermal epithelial (3).x10 (H&E).

Figure5: Skin of dexamethasone group after 14days,note thick epidermis(1), fibrosis(2), reduce diameter size of ulcer(3) &decrease rate of inflammation.X10(H&E).

Figure6: Skin of dexamethasone group after 14days,note thick epidermis(1) ,more fibrosis(2), hyper keratosis(3).X10(H&E).

Discussion

The result of this study reveals thickening with epidermis with inflammatory cells in 3 days, developed to angiogenesis and epithelization with in the 7th days in control animal decrease the blood vessels number with great collagenization and scab formation in 14th day post operative. The wound healing process has been divided in to three phases: inflammation, proliferation and remodeling, however, the process is continuous and phases overlap (6). The fibrin clot is replaced by blood vessels, rich granulation tissue replaced by scar formation (7, 8). In dexamethasone group there is decrease in inflammation and this is agree with previously published studies (9,10) and contrast with (11) who found that corticosteroids-induced increased number of macrophage and impaired process of re-epithelization as glucocorticoid-inhibited keratinocyte growth process (12). Cortisone and other anti inflammatory steroid increase the integrity of the lysosome, lysosomal enzymes are known to take prominent parts in the inflammation. In 7 days post operative group treated with dexamethasone there is clear negative effect of dexamethasone on granulation tissue due to decrease process of neoangiogenesis and this is agree with

study of (13) who found that daily injection of dexamethasone inhibited a basal spong-induced angiogenesis. (14) found that corticosteroid decreased expression of transforming growth factor and B(TGF_β) and exogenous administration of this factor improved wound healing (15). Following damage to skin, the enhanced synthesis and secretion of extracellular matrix proteins by smooth muscle α-expressing myofibroblasts play a fundamental role in scar formation and healing (16,17,18). Corticosteroid therapy during the acute phase following coronary artery ligation lead to impaired scar formation (19,20). Glucocorticoids are potent anti-inflammatory agents that inhibit leukocyte adhesion and aggregation (21) and decrease the expression of several cytokines like monocytes (22,23,24), interleukin-1(IL-1) (25) and interleukin-6(IL-6) (26). They have a strong anti-proliferative effect and are able to inhibit SMC proliferation (27,28). In 14 day postoperative system the corticosteroids decrease extracellular matrix deposition and remodeling because its ability to decrease collagen synthesis (29).

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دراسة التغيرات المرضية النسيجية لتأثيرالحقن المستمر للديكساميثازون على عملية التئام الجروح في الارانب

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الخلاصة

في هذه الدراسة تم تقسيم 16 أرنب إلى مجموعتين تم حقن المجموعة الأولى بـ 1ملغ/كغم من عقار الديكساميثازون يوميا عن طريق الخلب لمدة 21 يوما وتركزت المجموعة الثانية دون اي معالجة واستعملت كمجموعة سيطرة.تم اجراء قطع جراحي في الجلد وتم خياطته بواسطة خيوط سلك قياس 2.5سم اخذت عينات الجلد لاجراء التقطيع النسيجي وذلك لتقييم عملية التئام الجروح بعد مرور 3،14،7 يوم على اجراء العملية الجراحية .ان عملية تكوين الندبة تكون اقل في المجموعة المعاملة بالديكساميثازون ،وكذلك عملية تكوين الخلايا الطلائية والياف الكولاجين وترشيح الخلايا الالتهابية تكون اقل في مجموعة المعالجة مقارنة مع مجموعة السيطرة.