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Role of Topical Ritodrine Hydrochloride in Experimentally Induced Hypertrophic Scar in Rabbits

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Abstract

Hypertrophic scars are fibroproliferative illnesses caused by improper wound healing, during that, excessive inflammation, angiogenesis, and differentiated human dermal fibroblast (HDF) function contribute to scarring, whereas hyperpigmentation negatively affects scar quality. Over 100 million patients heal with a scar every year. To investigate the role of the beta 2 adrenergic receptor (β2AR); Ritodrine, in wound scarring, the ability of beta 2 adrenergic receptor agonist (β2ARag) to alter HDF differentiation and function, wound inflammation, angiogenesis, and wound scarring was explored in HDFs, zebrafish, chick chorioallantoic membrane assay (CAM), and a porcine skin wound model, respectively. A study identify a β2AR-mediated mechanism for scar reduction. β2ARag significantly reduced HDF differentiation, via multiple cAMP and/or fibroblast growth factor 2 or basic FGF (FGF2)-dependent mechanisms, in the presence of transforming growth factor betaβ1, reduced contractile function, and inhibited mRNA expression of a number of profibrotic markers. β2ARag also reduced inflammation and angiogenesis in zebrafish and CAMs in vivo, respectively. In Red Duroc pig full-thickness wounds, β2ARag reduced both scar area and hyperpigmentation by almost 50% and significantly improved scar quality. Indeed, mechanisms delineated in vitro and in other in vivo models were evident in the β2ARag-treated porcine scars in vivo. Both macrophage infiltration and angiogenesis were initially decreased, whereas DF function was impaired in the β2ARag-treated porcine wound bed. This data reveal the potential of β2ARag to improve skin scarring.

The purpose of this study was to assess the therapeutic effect of topical Ritodrine hydrochloride on hypertrophic scars in rabbits.

Thirty-two healthy male albino rabbits that divided in to 4 groups were included in the study (healthy; induced untreated hypertrophic scars; induced hypertrophic scars treated with 0.1% Triamcinolone acetonide (TAC) as a standard drug; and induced hypertrophic scars treated with 0.5% Ritodrine HCL gel twice daily for 21 days. Histopathology of skin sections, transforming growth factor beta1 TGFβ-1 level, and collagen III alpha1 in skin tissue were all used as outcome measures.

Compared to the induced hypertrophic scar group; treatment with Ritodrine significantly reduced means of TGF β1 and collagen III ($p \leq 0.01$); significantly reduce mean score of inflammation ($p \leq 0.001$), significantly lowered scar size ($P \leq 0.001$), and significantly lower mean scar height ($P \leq 0.001$), but no significant decrease in SEI ($P > 0.05$).

Therapy of induced hypertrophic scar with topical Ritodrine was successfully effective in rabbits. It reduced the immunological score (TGF-β1, collagen III), inflammation, and scar size in a substantial way. This effect was comparable (except in terms of SEI) to topical Triamcinolone acetonide efficacy.

Keywords: Hypertrophic scar, Ritodrine hydrochloride, Scar Elevation index, Collagen III.

دور الريتودرين هيدروكلوريد الموضعي في الندبة الضخامية المستحثة تجريبياً في الأرانب

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الخلاصة

الندبات المتضخمة هي أمراض تكاثرية ليفية ناتجة عن التئام الجروح غير السليم ، والذي يتميز بزيادة أو انخفاض في تنظيم بعض عمليات التئام الجروح. تؤثر الأنسجة المرتبطة بالندبات على ما يقرب من ١٠٠ مليون شخص في العالم المتقدم كل عام. يصيب التندب الضخامي ٣٢٪ إلى ٦٧٪ من الأشخاص في الدراسات ، وترتفع إلى ٧٥٪ عند الأطفال والشباب وذوي البشرة المصطبغة وتصل إلى ٩١٪ بعد إصابة الحروق ، اعتمادًا على عمق الجرح عامل النمو المحول بيتا ١ ، وهو سيتوكين بروتيفي ، مفرط في الخلايا الليفية الناشئة عن الندوب الضخامية ، بالإضافة إلى تعبير مطول عن مستقبلات عامل النمو المحول بيتا ١ -ذات الصلة. تفاعل مسارات تأشير عامل النمو المحول بيتا ١ ومستقبلات الأدرينالية بسبب نشاط الأرومة

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الليفية الذي تثبطه ناهضات مستقبلات بيتا ٢ الأدرينالية. تم تقييم دور نظام الإشارات الأدرينالية في إصلاح الجروح الجلدية مؤخرًا ووجد أن تنشيط مستقبل بيتا الأدرينالية يقلل بشكل ملحوظ انتقال الخلايا الكيراتينية ، وهي خطوة أساسية في إعادة الاندمال بتشكل النسيج الظهاري للجرح. بالإضافة إلى ذلك ، أفادت دراسة أن الانخفاض في تكوين الأوعية الدموية للجرح الطبيعي بوساطة مستقبلات بيتا ٢ الأدرينالية لديه القدرة على تقليل تندب الجرح وبالتالي قد يكون مفيدًا سريريًا ، لا سيما في الندبات الضخامية ، والمعروف أن لديها الأوعية الدموية المنظمة.

كان الغرض من هذه الدراسة هو معرفة كيفية تأثير الريتودرين هيدروكلوريد الموضعي على الأرانب ذات الندوب الضخامية.

تم تضمين اثنين وثلاثين من ذكور الأرانب البيضاء التي تم تقسيمها إلى ٤ مجموعات في الدراسة (صحية ؛ ندوب تضخمية مستحثة غير معالجة ؛ ندوب تضخمية مستحثة تم علاجها بـ ٠,١٪ تريامسينولون أسيتونيد كدواء قياسي ؛ والندوب الضخامية المستحثة التي تم علاجها باستخدام ٠,٥٪ ريتودرين هايدروكلوريد جل مرتين يومياً لمدة ٢١ يوماً. تم قياس عامل النمو المحول بيتا ١ و والكولاجين ٣ الفا ١, وتم قياس أنسجة الجلد المستحثة.

مقارنة بمجموعة الندبات الضخامية المستحثة غير المعالجة , وجد ان العلاج بالريتودرين يقلل بشكل كبير من مركبات عامل النمو المحول بيتا ١ والكولاجين ٣ (P<0.001) ؛ تقليل متوسط درجة الالتهاب بشكل ملحوظ (p <0.001) ، انخفاض متوسط حجم الندبة بشكل ملحوظ (P< 0.001)، وانخفاض متوسط ارتفاع الندبة بشكل ملحوظ (P<0.001) ، ولكن لم يحدث انخفاض كبير في مؤشر تقييم الندبة (p>0.05) .

علاج الندبة الضخامية المستحثة بالريتودرين الموضعي كان فعالاً بنجاح في الأرانب. لقد قلل من درجة عامل النمو المحول بيتا ١, والكولاجين ٣ ، والالتهاب ، وحجم الندبة بطريقة كبيرة. كان هذا التأثير مشابهًا الى فعالية علاج التريامسينولون أسيتونيدالموضعية بأستثناء مؤشر تقييم الندبة.

الكلمات المفتاحية: ندبة تضخمية ، ريتودرين هيدروكلوريد ، مؤشر ارتفاع الندبة ، الكولاجين الثالث.

Introduction

Hypertrophic scars are fibroproliferative illnesses caused by improper wound healing, which is characterized as an increase or reduction in the regulation of certain wound healing processes [(1)]. During wound healing, excessive inflammation, angiogenesis, and differentiated human dermal fibroblast (HDF) function contribute to scarring, whereas hyperpigmentation negatively affects scar quality. Over 100 million patients heal with a scar every year. To investigate the role of the beta 2 adrenergic receptor (β2AR) in wound scarring, the ability of beta 2 adrenergic receptor agonist (β2ARag) to alter HDF differentiation and function, wound inflammation, angiogenesis, and wound scarring was explored in HDFs, zebrafish, chick chorioallantoic membrane assay (CAM), and a porcine skin wound model, respectively. A study identifies a β2AR-mediated mechanism for scar reduction. β2ARag significantly reduced HDF differentiation, via multiple cAMP and/or fibroblast growth factor 2 or basic FGF (FGF2)-dependent mechanisms, in the presence of transforming growth factor betaβ1, reduced contractile function, and inhibited mRNA expression of a number of profibrotic markers. β2ARag also reduced inflammation and angiogenesis in zebrafish and CAMs in vivo, respectively. In Red Duroc pig full-thickness wounds, β2ARag reduced both scar area and hyperpigmentation by almost 50% and significantly improved scar quality. Indeed, mechanisms delineated in vitro and in other in vivo models were evident in the β2ARag-treated porcine scars in vivo. Both macrophage infiltration and angiogenesis were initially decreased, whereas DF function was impaired in the β2ARag-treated porcine wound bed. These data collectively reveal the potential of β2ARag to improve skin scarring. [(2)] It is elevated, red, inflexible, and causes serious functional and esthetic issues. Collagen type III aligned parallel to the epidermal surface with many collagen nodules is the main component. Hypertrophic scars are also characterized by nodular formations including alpha smooth muscle actin-expressing myofibroblasts and smaller vessels [(3)]. Pathological scarring is a difficult to predict and prevent post-operative consequence [(4)].

Scar-related tissues affect roughly 100 million persons in the developed world each year [(5)]. Hypertrophic scarring affects 32 percent to 67 percent of people in studies, rising to 75 percent in children, young adults, and those with pigmented skin [(6)] and up to 91 percent after a burn injury, depending on the depth of the wound [(2)]. Scar formation's underlying mechanisms are complex, and they can be influenced by a variety of circumstances [(7)]. In adult tissue, the physiologic reaction to wounding is the creation of a scar, which can be divided into three separate phases: inflammation, proliferation, and remodeling [(8)].

According to recent study, combination therapies of steroids (especially Triamcinolone) should be recommended for the treatment of pathological scars. These therapies have the advantage of good curative effects and fewer side effects. But not useful for patients who cannot tolerate the side effects. [(9)]

There are multiple interactions between fibrotic and anti-fibrotic growth factors, cells, extracellular matrix (ECM) components, and other enzymes within these stages, which often overlap [(10)]. Transforming growth factor beta 1 (TGF-β1) is a family of growth factors thought to be the master regulator of fibrosis, and its effects on collagen deposition, cell proliferation, immunological regulation, apoptosis, differentiation, and several other processes have been well documented in hypertrophic scar [(11)].

TGF-β is released in three isoforms (TGF-β1, 2, and 3) as inactive latent precursors that must be activated before binding to TGF β receptors [(12)]. TGF-β signaling appears to be altered in hypertrophic generated fibroblasts (due to increased phosphorylation of the receptor SMAD proteins) and lower expression of the inhibitory SMAD 7 in hypertrophic scar derived fibroblasts [(13)]. The majority of wound-healing cells produce TGF-β in

an inactive state that actively stimulates fibroblast chemotaxis to the site of injury [3].

TGF-β1, a profibrotic cytokine, was found to be overexpressed in fibroblasts originating from hypertrophic scars, as well as a prolonged expression of the related TGF-β receptors [14].

The interaction of the TGF-β1 and adrenergic receptor signaling pathways due to fibroblast activity inhibited by beta 2 adrenergic receptor agonists (β2-ARag).[15]

β-ARags are G protein-coupled receptors (GPCRs) for the endogenous catecholamines, adrenaline and noradrenaline. There are three β-AR subtypes: β1-AR, β2-AR, and β3-AR, which differ in their protein sequences and respond differently to their catecholamine ligands. [16] β-ARags can all couple to Gαs activating the membrane effector enzyme adenylate cyclase (AC) which generates the secondary messenger molecule cyclic adenosine monophosphate (cAMP) by catalysing the conversion of adenosine triphosphate to cAMP. [17]

In dermis, β2-ARag promote fibroblast migration and proliferation via Rous Sarcoma Oncogene-mediated transactivation of the epidermal growth factor receptor and the cAMP-mediated activation of protein kinase A (PKA), respectively, in two-dimensional assays in vitro [18]. The authors are evaluating the role of the adrenergic signaling system in cutaneous wound repair and recently found that β2-adrenergic receptor (β2-AR) activation markedly decreases keratinocyte migration, an essential step in wound reepithelialization. [19] In addition, a study reported that the reduction in normal wound angiogenesis mediated by β-ARag have the potential to reduce wound scarring and may thus be useful clinically, particularly in hypertrophic scarring and keloids, known to have upregulated vasculature. [20]

Similarly; Isoxsuprine (β-ARag) is a drug with the ability of direct relaxation of uterine and vascular smooth muscle fibers, stimulation of beta adrenoceptors, production of positive chronotropic and inotropic effects, and dilatation of blood vessels and in particular those supplying skeletal muscles. There are three principal mechanisms that induce the pharmacodynamics of this drug. The first is the stimulation of beta adrenoceptors, the second is the inhibition of α-adrenoceptors, and the third one is the direct papaverine-like spasmolytic of smooth muscles. [21] The observation that beta blockers induce the formation of skin pathology through the enhancement of angiogenesis,[22, 23] we suggest that the use of beta agonist, such as Isoxsuprine, may counter act this mechanism, resulting in reduction of scar size and resultant disfigurements.

Excessive wound inflammation contributes to scarring. A zebra fishtail wound model was used to visualize neutrophil guidance to wounds in real time. [24] β2-ARag reduced neutrophil recruitment by 60% after 6 hours. Although angiogenesis is essential for wound repair, reduced angiogenesis is linked with improved healing [25] and less angiogenesis occurs in non-scarring oral wounds [26] and scarless fetal wounds. [27] β2-ARag significantly reduced angiogenesis in the chick chorioallantoic membrane assay (CAM) by 29%. [2, 28]

The goal of this study was to investigate the activity of β2-ARag (Ritodrine hydrochloride) in the treatment of hypertrophic scar in rabbits.

Materials and Methods

The present study included 32 healthy male albino rabbits between the ages of 6 and 12 months. The animals were given 48 hours to acclimate to the animal room conditions of controlled temperature (28–30°C) and free access to water and food before beginning the work. Al Nahrain University College of Medicine's Institute Review Board accepted the current study's protocol. Ketamine (45 mg/kg) and xylazine (5 mg/kg) injections were used to anesthetize rabbits in the hypertrophic scar model. On the first day, surgical wounds were created using an 8 mm biopsy punch. On the ventral surface of one ear, four injuries were precisely made down to cartilage. After achieving homeostasis with manual pressure, the perichondrial layer was removed, and the wounds were bandaged with sterile gauze for 1 day. On the 30th day, the scars were detected [29].

Preparation of gels formulations

Gels formulations of chemicals were prepared as following: First, in order to prepare base gel from hydroxypropyl methyl cellulose (HPMC) approximately 3 g of gelling agent HPMC was weighted and added to75ml of warm distilled water (70 ºC) then stirred with magnetic stirrer for 2hours to obtain homogeneous gel (solution A). Second, Solution B of chemicals was prepared as following: 1. One hundred milligram (0.1g) of triamcinolone acetonide was weighted then dissolved in 10 ml of absolute ethanol alcohol to prepare (solution B) as slandered drug. 2. Five hundred milligram (0.5g) of Ritodrine hydrochloride was weighted then added to 10 ml absolute ethanol with the purpose of make (solution B) as suspected active drug.

Solution A and B were mixed thoroughly and the final weight was made up to 100 ml [30]. All the samples were allowed to equilibrate for at least 24 h at room temperature [31].

Treatment groups

The treatment groups are as follows (each with eight animals):

Group I: healthy animals;

Group II: hypertrophic scars were induced and the animals were left untreated (only base gel);

Group III rabbits with induced hypertrophic scars were given 0.1 percent Triamcinolone acetonide (TAC) as a standard drug;

Group IV rabbits with induced hypertrophic scars were given 0.5 percent Ritodrine HCL. Drugs were

given as a formulated topical gel twice a day for 21 days.

Collection and preparation of samples

After anesthetizing the animals at the end of the experiment (51 days), samples were taken using an 11 mm punch biopsy with a margin of more than 3 mm of adjacent skin [32] and submitted for histological and immunohistochemical investigation.

Each wound sample was preserved in a 10% formaldehyde solution processed in section for histopathological and immunohistochemistry examinations.

Preparation of formalin-fixed paraffin-embedded tissues

The fixative volume was 20 times that of the tissue on a weight-per-volume basis, and the tissue was fixed for at least 48 hours at room temperature before being treated with gentle agitation [33] Tissues were subsequently embedded in paraffin blocks.

Tissue sectioning and slide preparation

Using a microtome, serial sections of 3–5 µm thickness were produced, and 105 slides were made from each wound paraffin block. To prevent tissue sections from folding during the mounting method, sections were mounted on ordinary slides (for Hematoxylin and Eosin (H&E) staining) and positively charged slides (for immunohistochemistry) using a water bath at 45°C. Each slide was labeled with a pencil to carry the same number on its paraffin block. [34]

Assessment of histopathological changes in skin sections (Height of the scar, scar elevation index, and scar size)

The scar elevation index (SEI) is calculated as the ratio of the highest vertical height of the scar region between the perichondrium and the skin surface to the highest vertical height of the normal area around the scar between the perichondrium and the skin surface. A blinded examiner used a calibrated ocular reticule to measure each wound; histopathological scores reflecting **scar size** [35].

Inflammation was assessed by an expert pathologist and graded as mild, moderate, and severe. Mild inflammation was given a score of 1, moderate inflammation was given a score of 2, and severe inflammation was given a score of 3; while a score of 0 was given for no inflammation[36].

Immunohistochemistry IHC detection and procedure of collagen III, TGF-β1

(I) Anti-collagen III antibody: Rabbit polyclonal antibody to collagen III (Code number: MBS822102) (MyBioSource, USA). (II) Anti- TGF-β1antibody: Rabbit polyclonal antibody to TGF-β1 (Code number: ab190503) (Abcam, UK). On positively charged slides, five-micrometer thick sections were cut, and the staining treatment was carried out according to the manufacturer's instructions with the (ab80436 staining kit). Collagen III alpha1 and TGF-β1 immunohistochemistry kits are employed for detection.

Evaluation of IHC results

Under X20 light microscopy, the expression of TGF-β1 and collagen protein was measured. The extent of the immunohistochemical reactivity of ECM proteins like collagen was determined by ranking signal intensities on a scale of – (absence), + (mild), ++ (moderate), and +++ (marked) [37]. TGF-β1 immunoreactivity was determined by examining stained slides. A scoring system was established, with the average intensity of the expression being recorded as the score: Absence of immunoreactivity received a value of zero, mild immunoreactivity received a score of one, moderate immunoreactivity received a score of two, and strong immunoreactivity received a score of three [38].

Statistical analysis

Two statistical software packages were used to gather, summarize, analyze, and present data: the statistical package for the social sciences (SPSS version 22) and Microsoft Office Excel 2013. All data are presented as means ± standard deviation. The Mann–Whitney U test and the unpaired t-test were used to compare mean values between the two groups. Kruskal–Wallis test was used to analyze data for multiple comparisons. $P \leq 0.05$ was considered significant.

Results

Healing rate

As illustrated in Figure 1, the normal healing process of the untreated induced hypertrophic scar involves three overlapping phases: inflammation (0–3 days), cellular proliferation (3–12 days), and remodeling (3–6 months). As a result, inflammation could be seen in group II on the 1st day in all animals, with partial wound closure b on the 4th day and severe fibrosis formation (100 percent induction) starting on the 30th day.

In the Triamcinolone-treated group, healing signs appeared immediately after treatment, with disappearance of inflammatory signs.

Figure 2a shows complete wound closure and scar thickness reduction after 21 days of treatment.

After administration of Ritodrine gel (Group IV), signs of wound healing gradually appeared. At the end of the 21-day period, there was a decrease in inflammatory signs, wound edges converging and closing, and a partial reduction in scar thickness (Figure -3).

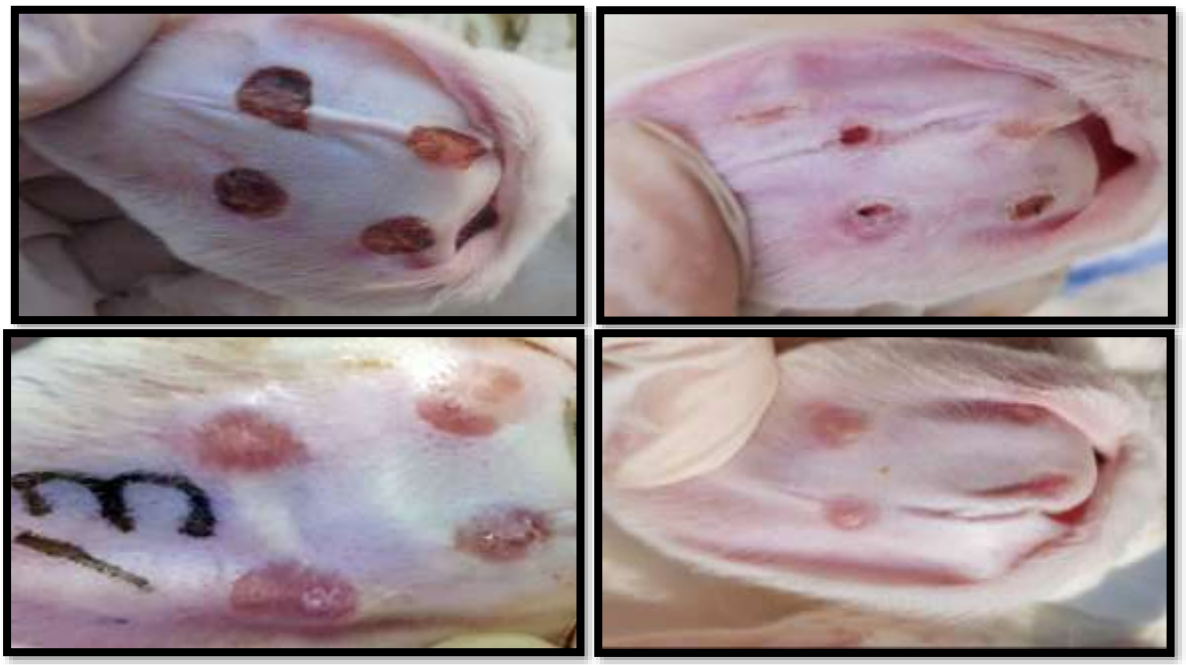

Figure 1. Gross morphological features of healing rate in the induced hypertrophic scar of rabbits during 30 days

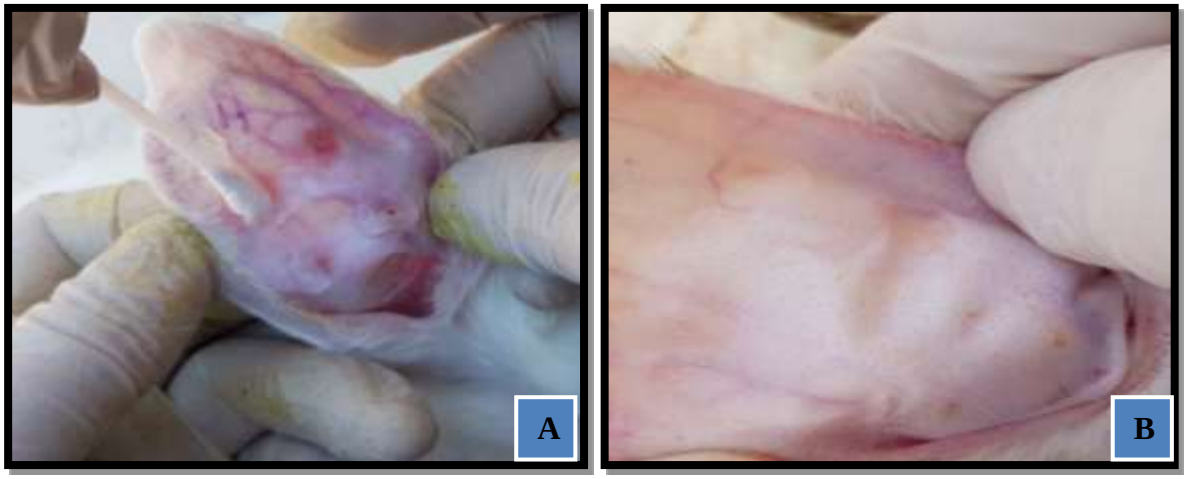

Figure 2.Treatment with triamcinolone acetonide (G3).

A. Application of topical gel on induced model B. After 21 days of treatment

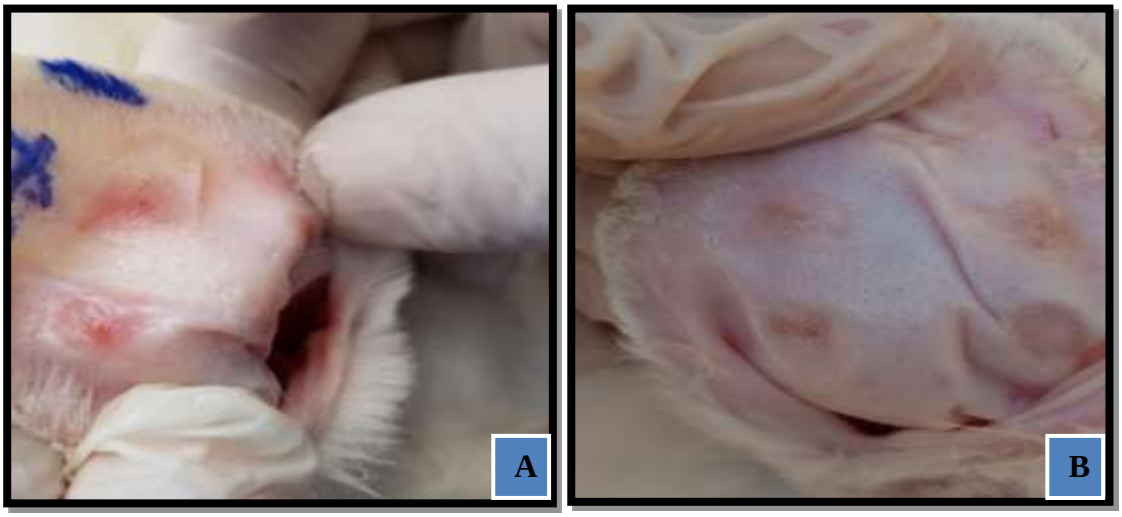

Figure 3. Treatment with Ritodrine (G4).A. Application of topical gel on induced model B. After 21 days of treatment

Immunohistochemical results

Tables 1 & 2 demonstrates immunohistochemical results for TGF-β1 and collagen III. According to the healthy control and the induced hypertrophic scar group recruited in the current investigation, there was a highly significant increase in mean immunohistochemistry scores of TGF-β1 and collagen III among induced hypertrophic scar group (p≤ 0.001).

Compared to the induced hypertrophic scar group, treatment with Triamcinolone acetonide and Ritodrine significantly reduced IHC expression scores for TGF-β1 and collagen III (p ≤0.01). Table 1 & 2.

Table1. Mean TGF-β1 scores in control and study groups:

GROUPS	Mean ± SD	P-value
Healthy control (G1)	1.13±0.35	<0.001*
Induced hypertrophic scar (G2)	3.0±0.0	
0.1%TAC Steroid (G3)	2.0±0.54	0.002*
0.5%Ritodrine (G4)	1.75±0.46	<0.001*

Kruskal-Wallis test. SD standard deviation; P indicate the level of significance at (P≤0.05); * indicate a significant difference between induced hypertrophic scar and the other groups

Table2. Mean collagen III scores in control and study groups

GROUPS	Mean ± SD	P-value
Healthy control (G1)	1.0 ±0.0	<0.001*
Induced hypertrophic scar (G2)	3.0 ±0.0	
0.1%TAC Steroid (G3)	2.13 ±0.64	0.01*
0.5%Ritodrine (G4)	2.0 ±0.54	<0.002*

Kruskal-Wallis test.SD: Standard deviation; P indicate the level of significance at (P≤0.05); ***** indicate a significant difference between induced hypertrophic scar and the other groups

Histological results

Inflammation

As shown in Table 3, the histopathological score reflecting the scar in the experimentally generated hypertrophic scar was very high and significantly increased in the untreated induced hypertrophic group compared to the healthy control (P<0001). In comparison to the induced hypertrophic scar group, treatment with Triamcinolone acetonide and Ritodrine resulted in a significant reduction in mean score of inflammation (p ≤0.001).

Table3. Mean inflammation score among study groups

GROUPS	Mean ± SD	P-value
Healthy control (G1)	**0±0**	**<0.001***
Induced hypertrophic scar (G2)	**2.75 ±0.46**	
0.1%TAC Steroid (G3)	**0.75±0.46**	**<0.001***
0.5%Ritodrine (G4)	**1.13±0.35**	**<0.001***

Kruskal-Wallis test.SD: Standard deviation; P indicate the level of significance at (P≤0.05); ***** indicate a significant difference between induced hypertrophic scar and the other groups

Scar size

In the untreated induced hypertrophic scar group, histopathological scores reflecting scar size were significantly higher (P <0.001) than in the healthy group, with mean (3.0±0.0) compared to (0.0±0.0) in the healthy group.

In comparison with the induced non-treated groups, both Triamcinolone acetonide and Ritodrine treatment resulted in a significant reduction in scar size (P <0.001) (Table 4)

Table 4.Mean scar size score in control and study groups

GROUPS	Mean ± SD	P-value
Healthy control (G1)	0±0	<0.001*
Induced hypertrophic scar (G2)	3.0 ±0.0	
0.1%TAC Steroid (G3)	0±0	<0.001*
0.5%Ritodrine (G4)	0.13±0.0	<0.001*

Kruskal-Wallis test.SD: Standard deviation; P indicate the level of significance at (P≤0.05); * indicate a significant difference between induced hypertrophic scar and the other groups

Height and scar elevation index (SEI)

According to the healthy control and induced untreated groups, there was a highly significant difference in mean height and Scar elevation index (P≤0.001).

The mean scar height and scar elevation index in the Triamcinolone acetonide group were significantly lower than that of induced untreated group (P≤0.001).

In addition to; Ritodrine treated group was compared to the induced untreated group and there was a significant reduction in scar height (P=0.047) but no significant decrease in SEI (P>0.05). Table 5 and Figure 4

Table 5. Scores evaluation and scores among study groups

Parameters		**G1 N=8**	**G2 N=8**	**G3 N=8**	**G4 N=8**
Height of scar	Mean	0±0	756.25	285.0	687.5
	SD	0±0	40.7	27.91	76.5
	P-value	<0.001*		<0.001*	0.047*
Scar elevation index	Mean	0±0	8.03	3.03	7.3
	SD	0±0	0.87	0.42	1.07
	P-value	<0.001*		<0.001*	0.156*

Kruskal-Wallis test.SD: Standard deviation; P indicate the level of significance at (P≤0.05); * indicate a significant difference between induced hypertrophic scar and the other groups

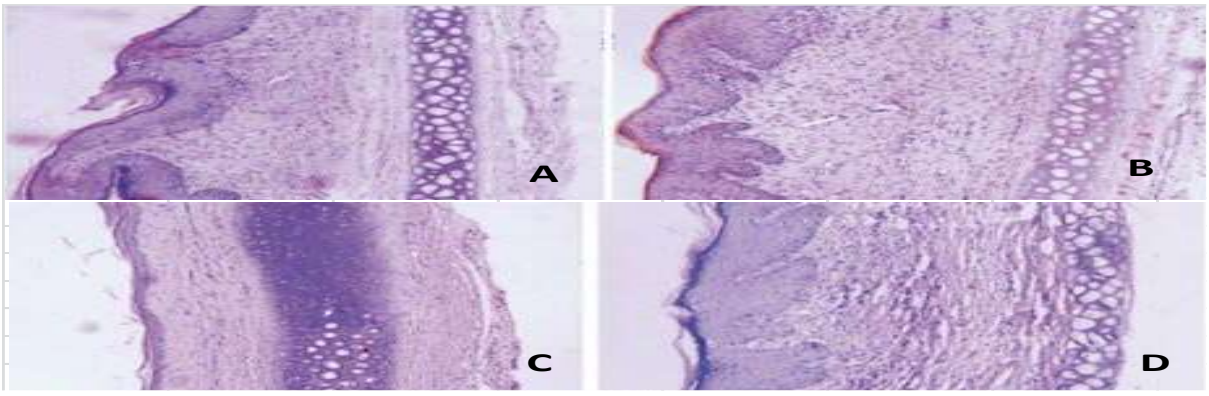

Figure4. Represent height of scar from perichondrium to skin surface(x4)

A) Normal skin (110µm) B) induced hypertrophic scar tissue (700 µm), (C) treated hypertrophic scar of 0.1% TAC steroid gel (320µm), (D) treated induced hypertrophic scar of 0.5% Ritodrine gel (700 µm)

*(10x, 4x): ordinary Hematoxylin and eosin stain.

Discussion

Scarring following surgery or injury is difficult to predict, and both physicians and their patients are highly concerned with minimizing scar appearance and value as clinically meaningful even small improvements in scarring. Despite a plethora of various in vivo and in vitro studies, to date only limited information is available on the exact cause of hypertrophic scar and keloid formation. Knowledge of the cellular and molecular mechanisms implicated in the development of these fibroproliferative disorders remains relatively poor because of the lack of representative and well-recognized animal models of human hypertrophic scar formation.[39]

Some herbs have also been found to be helpful in the treatment of hypertrophic scars. In a rabbit ear model, Phytosterol 0.3 percent extract of Chenopodium murale reduced scarring and was nearly as effective as Triamcinolone acetonide. [40]

In a rabbit ear model of hypertrophic scar, a phytosterol fraction derived from Fumaria Officinalis significantly reduced Transforming growth factor beta 1 (TGF-β1) on HTS. [41]

TGF-β1 controls the expression of fibrosis-related proteins such as Type I and III collagens [10]. It can also stimulate the transformation of fibroblasts into myofibroblasts, which are important cells in the formation of hypertrophic scars (HTS) and are characterized by enhanced collagen synthesis and cytokine up regulation [42].

In the current work, HTS in the rabbit's ear model was successful since there were substantial changes in cellular response to growth factor (TGF-β1) between induced HTS and normal skin, which is consistent with a previous study. [43] After 21 days of treatment, topical Triamcinolone acetonide significantly reduced TGF-β1 compared to the untreated group ($P<0.001$), which is consistent with (44), which demonstrated substantial variations in pro-inflammatory cytokines TGF-β1 and collagen III in a rabbit ear model after treatment with topical Triamcinolone acetonide.

In the current study, Ritodrine administration for 21 days in the induced hypertrophic scar rabbit model resulted in a significant reduction in immunohistochemical expression of the proinflammatory cytokine TGF-β1, which is consistent with previous study, which found that salbutamol and Formoterol; which are beta2 adrenergic receptor agonists (β2-ARag), reduce TGF-β1 gene expression (in vitro). [28]

In addition to a drop in TGF-β1 after treatment with Triamcinolone acetonide, one possible mechanism for collagen distribution in the ECM is the influence on plasma protease inhibitors, allowing collagenase to breakdown collagen [45].

In addition, 7 days of topical Olodaterol (β2-ARag) treatment reduced TGF-β mediator by 50 to 70% in bleomycin-treated mice, which is consistent with our findings [46].

Ritodrine's exact mechanism, as well as how cAMP interferes with the TGF-β1 signaling cascade, are unknown. The interference of cAMP with TGF-β1 specific Smad3/4-dependent gene expression is one of the proposed reasons. Additionally, cAMP may suppress fibrotic responses by inhibiting TGF-β1 stimulated ERK1/2 and JNK activation via the PKA or EPAC pathways [47].

Collagen type III is primarily found parallel to the epidermal surface in hypertrophic scars [1], and the current study findings showed that collagen III expression is elevated in the induced HTS group, which is consistent with Oliveira et al [48]. This study also discovered a considerable reduction in collagen III in Triamcinolone acetonide, which is consistent with other literature [49].

Ritodrine, a (β2-ARag) medicine, reduced collagen III in mice wounds, which is analogous to previous research that described the effect of Salbutamol a (β2-ARag) in mice wounds and reported a significant decrease in collagen III after 5 and 10 days of follow-up. [50]

Furthermore, Salbutamol and Formoterol (β2-ARag) during wound healing resulted in a significant reduction in collagen synthesis, which is consistent with the findings of this study. [28]

In terms of inflammation, the current study found that Triamcinolone acetonide considerably reduced the inflammation and had anti-inflammatory activity after 14 days of therapy in a rabbit wound model, which was essentially identical to previous findings [51].

Ritodrine also had an effect on the inflammatory process after 21 days of treatment, resulting in a significant drop in inflammation, which is consistent with a study reported that a β2-ARag reduced neutrophil recruitment in zebrafish wounds within hours of wounding. There was also a decrease in macrophage in the induced scar of the porcine model after 7 days, with a minor increase after 14 days, and no difference after 21 days. [28]

β2-ARag has been reported to have anti-inflammatory effects in addition to their effects on smooth muscle relaxation in the airways. They have been shown to inhibit the expression of inflammatory mediators and to reduce capillary permeability and formation of plasma exudate and tissue edema [52, 53]. Also; it was reported that β2-ARag reduced carrageenan-induced paw edema in rats and that effect was attenuated when the β2-receptors were blocked by a non-selective β-receptor antagonist [54]. Another study found that β2-ARag inhibited the production of TNF in macrophages and carrageenan-induced paw edema was reduced by β-receptor antagonist in rats [54]. Showing that β2-ARag have anti-inflammatory effects in vitro and in vivo. [55]

Topical Triamcinolone acetonide reduced scar size significantly, which is consistent with another study reported that a reduction in scar size of 82.3 percent in the steroid group after 4 weeks.[56]

In comparison to the generated hypertrophic scar, the Ritodrine group showed a significant reduction in scar size.

Salbutamol (β2-ARag) reduces scar area in Red Durocs by 50% in previous study, which consistent the findings of the current study. [28]

The standard medicine Triamcinolone acetonide caused a significant decrease in height and SEI, which agrees with a previous study. [27]

After 21 days of therapy with Ritodrine, there was a significant reduction in height and no change in SEI in the rabbit ear model.

Salbutamol causes a 34 percent reduction in height in Red Durocs, which is consistent with our findings. [28]

The lack of a significant decrease in SEI following Ritodrine treatment may be due to other factors affecting ECM proliferation and disposition with no net reduction in scar index, or that the 3-weeks treatment time was too short to detect a considerable reduction in scar hypertrophy.

β2-ARag as a regulator of wound healing/scarring. There are currently no clinically tested or licensed interventions/pharmaceuticals available to reduce wound scarring/fibrosis or to improve scar hyperpigmentation. Topical Salbutamol significantly improved acute skin scarring in vivo and could have significant potential as a treatment. Future work will address the potential to improve hypertrophic scarring, keloid formation, and organ fibrosis.[28] Therefore, there are still shortcomings in Ritodrine, and its conclusions need to be further confirmed by well-designed and rigorous RCTs.

Conclusion

Induced hypertrophic scar therapy with topical Ritodrine proved successful in rabbits. It reduced the immunological score (TGF-β1, collagen III), inflammation, and scar size in a substantial way. This effect was comparable (except in terms of SEI) to topical Triamcinolone acetonide efficacy.

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