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5α-Dihydrotestosterone enhances wound healing in diabetic rats

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ABSTRACT

Wound healing involves a complex interaction between the cells, extracellular matrix and oxidative response.
Aims: Analyze the effects of 5α-Dihydrotestosterone (5α-DTH) ointment in cutaneous wound healing by secondary intention in diabetic Wistar rats.
Main methods: Rats (302.23 ± 26.23 g, $n = 48$) were maintained in cages with food and water *ad libitum* in accordance with the Guiding Principles in the Use of Animal Ethics Committee. Diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg). Three skin wounds (12 mm diameter) were created on the animals' back, which were randomized into 6 groups according to the application received: VT group: Vehicle (lanolin), SA group: 0.9% saline solution, NC group: Non-diabetic, CP group: positive control (silver sulfadiazine 0001%), T1 group: Testosterone (10%), T2 group: Testosterone (20%) emulsified in lanolin. The applications were made daily within 21 days, and tissues from different wounds were removed every 7 days.
Key findings: Both groups treated with testosterone (T1 and T2) showed a significantly higher proportion of type I and type III collagen fibers. Superoxide dismutase levels were significantly higher on days 7 and 14 in testosterone treated groups. Protein carbonyls and MDA were lower in both groups.
Significance: We conclude that groups treated with 5α-DTH showed a better healing pattern with complete wound closure, and proved to have a positive effect on the morphology of the scar tissue as well as an antioxidant stimulating effect during secondhand intention skin wounds repair in diabetic rats.

1. Introduction

Impaired wound healing and other tissue abnormalities are known to be associated with diabetes [1]. The underlying biochemical mechanisms of the healing process involve mainly disturbances in collagen production, which result in delayed re-epithelialization in wounds, impaired migration and proliferation of keratinocytes and fibroblasts [2]. Different treatments have been tested to address this complex clinical problem, but only a few have been proven to be effective [3].

Wound healing under abnormal conditions can be due to impaired physiological processes and poor nutrition. Low testosterone concentration can result in thinner, more fragile skin and reduced insulin receptor expression. Testosterone and insulin interact in their actions on target tissues [4].

Testosterone is mostly produced by the testicles in men, as well as by the ovaries in women. A small amount is also produced by the adrenal glands in both genders [5,6]. Dehydroepiandrosterone (DHEA) and DHE-sulfate (DHEA-S) are among the intermediates in the biosynthesis of androgens — DHEA-S is converted to DHEA by sulfatase, which is expressed in several cell types including macrophages, keratinocytes and dermal fibroblasts [7]. Next, 17β-hydroxysteroid dehydrogenase (17β-HSD) converts DHEA in androstenedione (5-DIOL) and 3β-hidroxiesteroide dehydrogenase (3β -HSD) is responsible for converting 5 DIOL in testosterone. Finally, testosterone is converted in a more potent androgen, a 5α-dihydrotestosterone (5α-DTH) by the action of 5α-reductase (5α-R) [8]. Dermal cells express all the necessary enzymes to convert DHEA in 5α-DTH and assist in cutaneous distribution of these enzymes [7].

The decline in circulation of DHEA levels with aging and/or obesity has been associated with increased prevalence of Diabetes [9,10]. Physical activity was suggested as a DHEA stimulator, and 5α-DTH was reported to help decreasing blood glucose levels in insulin resistant patients [11]. Testosterone administrations in patients with hypogonadism have been known to lead to androgenic effects, such as increased protein synthesis, which facilitates a healing process [12]. Furthermore, there is evidence that the low serum testosterone concentration is related to the metabolic syndromes (obesity, hypertension, abnormal blood glucose, low HDL ratio, high LDL and triglycerides).

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Table 1
Area (mm^2) and rate of wound contraction (mm^2/day) in rats treated with 5α-dihydrotestosterone ointment.

Day	Area/contraction	VT	SA	NC	PC	T1	T2
0	A(mm^2)	120.67 ± 1.99	119.97 ± 2.99	121.46 ± 1.77	120.56 ± 1.77	121.77 ± 1.99	121.35 ± 1.11
	WCI(%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7	A(mm^2)	88.87 ± 5.43	89.45 ± 4.43	63.32 ± 3.23*	78.32 ± 3.23*	63.57 ± 5.43*	65.77 ± 4.43*
	WCI(%)	26.35 ± 4.20	26.35 ± 4.20	37.03 ± 4.31*	35.03 ± 4.31*	38.25 ± 4.20*	38.07 ± 4.50*
14	A(mm^2)	60.65 ± 4.65	63.75 ± 3.45	41.46 ± 4.55*	61.46 ± 4.55*	43.65 ± 4.65*#	42.68 ± 5.65*#
	WCI(%)	49.73 ± 5.21	48.53 ± 4.21	58.14 ± 4.98*	58.14 ± 4.98*	59.73 ± 5.21*	59.73 ± 5.21*
21	A(mm^2)	3.00 ± 0.00	3.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	0.00 ± 0.00*#	0.00 ± 0.00*#
	WCI(%)	90.00 ± 0.00	90.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Data are reported as mean ± S.D. A: wound area, WCI wound contraction index, VT: vehicle control, SA: saline solution, NC: Nondiabetic, CP: Silver sulfadiazine, T1: Testosterone 10%, T2: Testosterone 20%. Data are represented as mean ± SD.*, #, $p < 0.05$, and statistical differences between groups: * vs. SA and VT, # vs. CP (Kruskal–Wallis test).

Such evidence supports the importance of this hormone for total homeostasis [13].

Although there are reports in the literature on the benefits of 5α-DTH in some diseases related to sexual function and metabolic syndrome [11], its action and applicability in wound healing under diabetes is not understood. Considering results showing a positive effect of this hormone in tissue vascularization in the genitals, we hypothesize that 5α-dihydrotestosterone will enhance skin repair in wounds of diabetic rats.

2. Materials and methods

2.1. Preparation of formulation and standard used

A 10% and 20% (*w*/w) of simple ointment containing 5α-DTH was prepared by mixing method in a ceramic and pestle emulsified in lanolin. For this, 5 g of testosterone was incorporated in 50 g of the base and 5 g of 5α-DTH was incorporated 100 g respectively. Silver sulfadiazine cream (0.01%) obtained from Rexin Pharmaceutical Pvt. Ltd. was used as standard drug for comparing the healing wound using of 5α-DTH in different animal models. Silver sulfadiazine is used to prevent and treat bacterial and fungi infections in burns and skin wounds [14,15].

2.2. Animals

Forty-eight Wistar rats (*Rattus norvegicus*), 5 weeks of age, weighing 198.25 ± 26.11 g, were obtained from the Central Animal House of the Health and Biological Sciences Center, Federal University of Viçosa, Brazil. Rats were fed commercial rat feed and provided water ad libitum. All animals were maintained in individual cages, cleaned daily, under controlled environmental conditions (temperature: 22 ± 2 °C, humidity: 60–70%, and light/dark cycle: 12/12 h). All procedures were approved by the University Animals Ethics Committee (registration No. 730/2014).

2.3. Experimental design

Prior to creating the wounds, the animals were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (20 mg/kg). Dorsolateral shaving of the animals was performed, and the area was degreased with ethyl ether (Merck®, Rio de Janeiro, Brazil), followed by using 70% ethanol and 10% povidone-iodine for

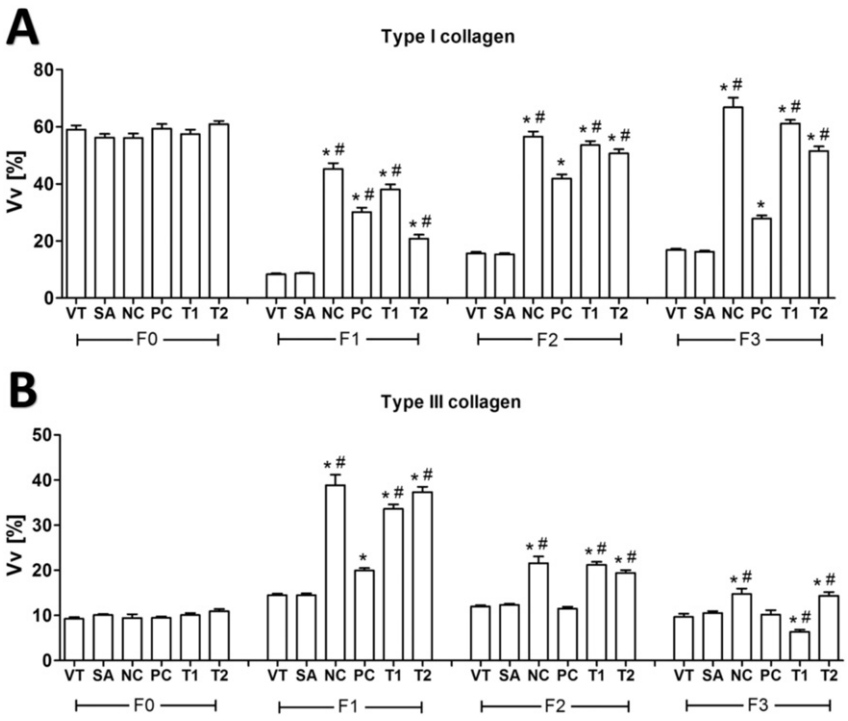

Fig. 1. A — Proportion of type I collagen fibers and B — Proportion of type III collagen fibers in the scar tissue of rats treated with 5α-dihydrotestosterone ointment. F0: normal untreated tissue, *F*1: treated tissue after 7 days, F2: treated tissue after 14 days, F3: treated tissue after 21 days. VT: vehicle, SA: saline solution, NC: No diabetic, PC: Silver sulfadiazine, T1: 5α-dihydrotestosterone 10%, T2: 5α-dihydrotestosterone 20%. Data are represented as mean ± SD.*, #, $p < 0.05$, and statistical differences between groups: * vs. Sa and VT, # vs. CP (Kruskal–Wallis test).