Influence of hypoandrogenism in skin wound healing resistance in rats

Influência do hipoandrogenismo na resistência cicatricial cutânea em ratos

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ABSTRACT

Objective: The objective of the present study is to verify the effect of testosterone depletion on healing of surgical skin wounds at different ages and postoperative times. **Methods:** Forty-four Wistar male rats were divided into four groups: Group 1y (n = 11) – young control, sham-operated rats (30 days-old); Group 1A (n = 10) – adult control, sham-operated rats (three to four months old); Group 2Y (n = 10) – young rats after bilateral orchiectomy; and Group 2A (n = 11) – adult rats after bilateral orchiectomy. After six months, a linear incision was performed on the dorsal region of the animals. The resistance of the wound healing was measured in a skin fragment with a tensiometer, on the 7th and 21st postoperative days. **Results:** The wound healing resistance was higher in Group 1Y than in Group 2Y after seven days ($p < 0.05$). Wound healing resistance at 21 days was higher than at seven days in all groups ($p < 0.05$). Late wound healing resistance was not different between young and adult rats. **Conclusions:** Bilateral orchiectomy decreased the wound healing resistance only in young animals at the seventh postoperative day.

Keywords: Orchiectomy; Hypogonadism; Testosterone; Androgen; Wound healing; Rats

RESUMO

Objetivo: Verificar o efeito do hipoandrogenismo na cicatrização de lesão cirúrgica cutânea em diferentes períodos pós-operatórios. **Métodos:** Quarenta e quatro ratos Wistar machos foram distribuídos nos seguintes grupos: Grupo 1Y – controle, ratos jovens (30 dias de vida); Grupo 1A – Controle, ratos adultos (três a quatro meses de idade); Grupo 2Y – Orquiectomia, ratos jovens; Grupo 2A – Orquiectomia, ratos adultos. O Grupo 2 foi submetido a orquiepididimectomia total bilateral, já o Controle recebeu incisão e sutura escrotal. Após seis meses, realizou-se incisão linear no dorso dos animais. A resistência das cicatrizes foi medida com um tensiômetro, utilizando um fragmento de pele. Esse procedimento foi feito no 7º e no 21º dia após a realização das incisões. **Resultados:** A resistência da cicatriz no sétimo dia foi maior no Grupo 1Y do que no grupo 2Y ($p < 0,05$). A resistência cicatricial após 21 dias foi maior do que a encontrada após sete dias em todos os grupos ($p < 0,05$). Não se verificou diferença entre as resistências cicatriciais tardias entre os ratos jovens e adultos. **Conclusões:** A orquiectomia reduziu a resistência cicatricial nos animais jovens no sétimo dia pós-operatório.

Descritores: Orquiectomia; Hipogonadismo; Testosterona; Androgênio; Cicatrização de feridas; Ratos

INTRODUCTION

Wound healing may be impaired by several conditions, such as hyperglycemia, hypoproteinemia, infections and impaired collagen synthesis. Androgens cause positive nitrogen balance and increase protein synthesis. Due to the anabolic effect of these hormones, their use was proposed for accelerating wound healing. Several studies showed that wounds are sensitive to androgen stimulation and that its deficiency causes anomalous healing[1-3]. The pro-mitotic activity of these mediators, in physiologic concentrations, seems to increase the production of granulation tissue in wounds. On the other hand, high doses of testosterone have an inhibitory effect on granulation tissue[4].

About 95% of serum testosterone is secreted by the interstitial cells of the testicles, a process under control of interstitial cell stimulating hormone (luteinizing hormone), produced in the anterior pituitary. The remaining is synthesized by the adrenal. Therefore, bilateral orchiectomy causes near total testosterone depletion, which allows assessing the effects of the absence of this hormone in different organic reactions[5].

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Due to the need of more investigations comparing the effects of testosterone in skin regeneration, the present study has the objective of comparing skin healing resistance in different age groups and different postoperative periods.

METHODS

This study was performed in accordance to the recommendations of the International Guidelines of Animal Protection[6] and approved by the Ethics Committee in Animal Experimentation of Universidade Federal de Minas Gerais (UFMG).

A total of 44 male Wistar rats (*Rattus norvegicus albinus*) were used. They originated from Central Animal Laboratory of Instituto de Ciências Biológicas of UFMG and were transferred to the Central Animal Laboratory of the Medical School. The animals were allocated to appropriate cages with up to four rats per cage and kept at room temperature with natural light, with 12 hours of light and 12 hours of darkness according to the murine circadian cycle described in the literature[5,7]. They were followed daily and received water and food *ad libitum*, with no addition of protein, caloric or lipid supplement of any kind during the entire study period. For the weight assessment of the animals, an electronic precision scale (Marte®, model AS500) with 500 g maximum capacity and 0.01 g sensitivity was used.

According to their age, the rats were labeled with the letter Y for young animals (30 days old with weights between 55 and 80 g) and with the letter A for the sexually mature adult rats (three to four months old and with weights between 250 and 350 g). They were randomly distributed in groups according to the operative procedure performed:

- Group 1 Y (n = 11): young control rats;
- Group 1 A (n = 11): adult control rats;
- Group 2 Y (n = 11): orchiectomy, young rats;
- Group 2 A (n = 11): orchiectomy, adult rats.

All surgical procedures were performed on the Research Laboratory of the Surgery Department of the Medical School, in accordance to technical guidelines of asepsis and antisepsis.

All rats were anesthetized with the association of ketamine hydrochloride (Ketalar®, Pfizer, São Paulo), 50 mg/kg body weight and xylazine hydrochloride (Rompum®, Bayer, São Paulo) 5 mg/kg body weight intramuscularly in the right gluteus region[8]. Throughout the entire anesthesia period, heart and respiratory rates were assessed, as well as the animals' voluntary movements, in order to determine the level of anesthesia and possible anesthetic complications.

The rats were immobilized with surgical tape over surgical boards in supine position. Surgeries were performed through an anterior middle scrotal incision, opening of the vaginal tunic and testicles exposure. The procedure thereafter, depended on the group of the animals. In Group 1, the scrotal skin was sutured with chromic catgut 3-0 (Catgut chromic Polysuture®). In Group 2, the spermatic funicles were ligated and cut. The testes and the epididymes were removed and the scrotum sutured with chromic catgut 3-0 (Catgut chromic Polysuture®).

Six months after the surgeries, all animals were weighed again and under anesthesia, as previously described, they underwent a 3.0 cm long mid dorsal incision, involving all skin layers and subcutaneous tissue, up to the muscular fascia. After this, the wound borders were sutured with four simple stitches, with nylon 4-0 (Nylon®, Ethicon, São Paulo).

Skin healing resistance was assessed on the 7th and the 21st postoperative days, in a 4 x 1 cm skin fragment transversal to the scar, with the scar in its mid portion. The suture was carefully removed and the fragment was subjected to resistance and tension testing by means of a tensiometer.

Histology was done in preparations stained with hematoxillin-eosin and Gomori trichrome. The scar fibrous neoformation thickness was measured in three different regions (close to the cranial end, in the mid portion and close to the caudal border).

Statistical analysis was done using the SPSS for Windows 10.0 package. The results of healing tension were compared using the Mann-Whitney test. The significance level adopted was $p < 0.05$.

RESULTS

All animals were killed at the end of the study. Skin scars did not show macroscopic abnormalities. In the seventh post-operative day it could be seen that the healing area was delicate, while in the 21st day it became firm. There was no infection or necrosis.

The results of healing resistance are depicted in Table 1. Healing resistance in young orchiectomized rats (Group 2Y) was lower than in the Control Group (Group 1Y) seven days after the skin incision ($p = 0.038$). However, there was no difference between the two groups after 21 days. No difference was seen between Groups 1A and 2A. Healing resistance after 21 days was higher than that found after seven days in the two groups ($p = 0.013$).

Histology in the seventh post-operative day showed a slight increase in collagen and the inflammatory infiltrate was composed of polymorphonuclear cells, plasma cells, lymphocytes and macrophages, with

Table 1. Healing resistance, mean ± standard deviation (g/cm²), on skin of rats at 7 and 21st days after skin incision

Groups	Days after incision on the dorsum	
	7th	21st [b]
1Y (n = 11)	503.6 ± 167.8	1215.0 ± 428.1
1A (n = 10)	495.9 ± 259.5	1126.7 ± 297.8
2Y (n = 10)	419.6 ± 135.6[a]	1216.4 ± 287.3
2A (n = 11)	532.6 ± 278.1	1264.6 ± 394.4

Group 1Y: n = 10, control, young rats; Group 1A: n = 10, control, adult rats; Group 2Y: n = 10, orchiectomy, young rats; Group 2A: n = 10, orchiectomy, adult rats; [a]ss different from mean as compared to control (p = 0.038); [b] healing resistance greater on the seventh day

evidence of vascular congestion. On the other hand, on the 21st day, a homogenous morphologic pattern was observed, with better arranged fibroblasts and collagen fibers, which were thicker and with parallel orientation. There was no morphological difference between the groups of the same postoperative period (Figures 1 and 2).

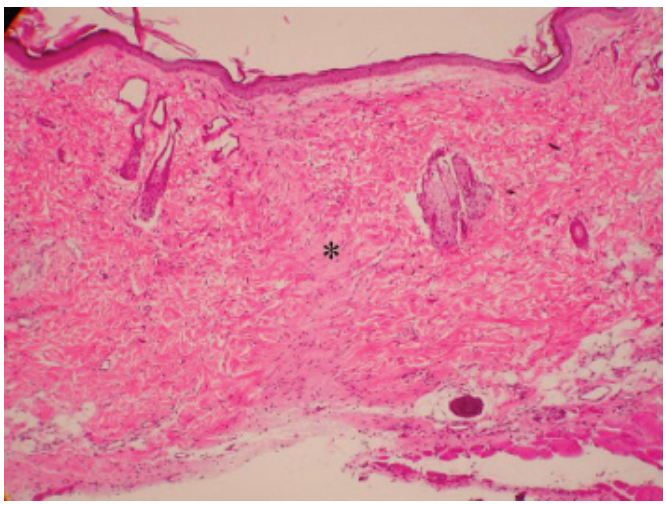

Figure 1. Histological section of skin scar (*) of control rat (animal 4 of Group 1A) on the 21st postoperative day (HE, 40X)

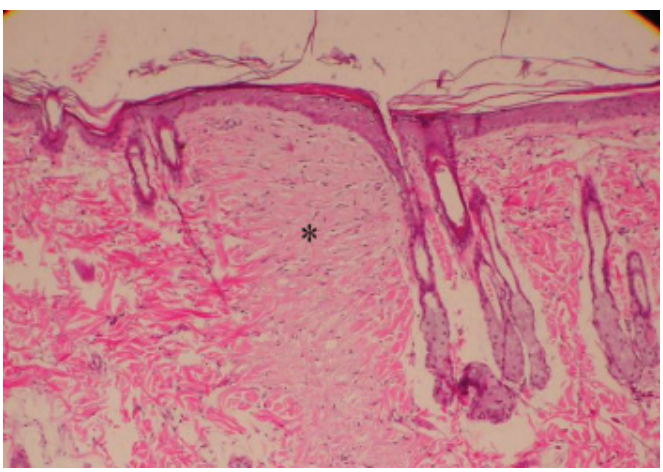

Figure 2. Histological section of skin scar (*) of orchiectomized rat (animal 7 of Group 2A) on the 21st postoperative day (HE, 40X)

DISCUSSION

Sexual maturation in rats is completed around 50 days after birth and their highest fertility occurs between 100 and 300 days of life[9]. Comparisons between the young and adult animals were made because the influence of endocrine changes in the initial phase of life is different from that in adults[10].

One of the functions of testosterone is to stimulate protein synthesis in different tissues, such as skeletal muscle, kidneys and male reproductive organs. Skin fibroblasts may convert testosterone to dihydrotestosterone, which may change the synthesis of collagen and of mucopolysaccharides. It should be noticed that the testosterone levels fall after trauma and surgical stress and this may cause healing complications[11]. Although the androgens have no direct anabolic effect on the epidermis, they may modulate keratinocyte maturation[12]. Several authors reported increased velocity of epidermis proliferation after testosterone treatments[13-16].

In the present study, the increasing healing resistance with time was expected and agrees with the literature[11]. Shamberger et al. found no testosterone influence on wound healing. It is likely that this hormone acts only in early healing stages of sexually immature rats[11].

Indications for orchiectomy are well established and the results do not change such indications. However, new studies should be carried out to fill the knowledge gap still remaining as to the effects of hypogonadism. They should observe the undesirable effects of orchiectomy considering that the elderly are a group in whom this type of surgery may be indicated[17]. With this in mind, it is important to emphasize that hypogonadism is not only a consequence of orchiectomy, as it may be part of the natural aging process, besides being one of the possible complications of severe orchitis or more rarely, of the congenital absence of testicles[18].

CONCLUSIONS

It was concluded that the skin healing resistance was lower in the early healing stages of castrated sexually immature rats, which may indicate the influence of testosterone in the skin healing process.

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