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Original Article

Testosterone replacement maintains smooth muscle content in the corpus cavernosum of orchiectomized rats

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Abstract *Objective:* To evaluate the effects of testosterone on the maintenance of corpus cavernosum (CC) structure and apoptosis.
Methods: Animals were divided into three groups: sham operation group ($n = 8$) underwent sham operation; Orchiectomized (Orchiec)+ oily vehicle group ($n = 8$)underwent bilateral orchiectomy and received a single dose of oily vehicle by intramuscular injection (i.m.) 30 days after orchiectomy; and Orchiec + T group ($n = 8$) underwent bilateral orchiectomy and received a single dose of testosterone undecanoate 100 mg/kg i.m. 30 days after the surgery. Animals were euthanized 60 days after the beginning of the experiment with an anesthetic overdose of ketamine and xylazine. Blood samples and penile tissue were collected on

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euthanasia. Azan's trichrome staining was used to evaluate smooth muscle, Weigert's Fucsin-Resorcin staining was used to evaluate elastic fibers and Picrosirius red staining was used to evaluate collagen. Apoptosis was evaluated using TUNEL technique. Statistical significance was set at $P \leq 0.05$.

Results: Testosterone levels decreased in Orchiec + oily vehicle when compared to *sham operation* and Orchiec + T groups ($P < 0.001$). Testosterone deprivation reduced trabecular smooth muscle content and penile diameter and testosterone replacement maintained both parameters ($P = 0.005$ and $P = 0.001$, respectively). No difference was observed in the content of sinusoidal space ($P = 0.207$), elastic fibers ($P = 0.849$), collagen ($P = 0.216$) and in apoptosis ($P = 0.095$).

Conclusion: Normal testosterone levels maintain CC smooth muscle content and do not influence elastic fibers, collagen content and apoptotic index. Further studies should be performed in order to investigate the mechanisms by which androgen mediates its effects on CC structure.

1. Introduction

Testosterone (T) is known to be an essential hormone involved in normal sexual male response, including erectile function [1,2]. Besides that, it is well established that normal erectile function requires the correct balance of hormonal factors, such as T, and corpus cavernosum (CC) histological structure [1,2]. Although there are clinical evidences demonstrating that low levels of T are associated to erectile dysfunction (ED) [3], this subject is still controversial, since some authors have not observed any association between T levels and ED [4]. In fact, the exact role of androgens in erectile function and dysfunction remains unclear [1,5,6].

Traish et al. [5,6] observed that androgen deprivation by surgical castration damages the histological structure of CC, which leads to veno-occlusive dysfunction, an important cause of organic ED [1,5,6]. Recently, Miranda et al. [7] evidenced that T deprivation decreases smooth muscle and sinusoidal space content, an effect reversed by testosterone replacement. The decrease of smooth muscle content in response to androgen deprivation is believed to be due to increased cellular apoptosis [1,8]. In fact, there are several data demonstrating increased apoptotic cells following androgen deprivation [1,8]. Besides that, T replacement prevents CC structures apoptosis, suggesting that androgen may have a role in apoptotic cascade [8].

Therefore, the aim of the study is to evaluate the effects of T on the maintenance of CC histological structures (smooth muscle, collagen, elastic fibers and sinusoidal space) and on apoptosis in order to better elucidate the interplay among androgens and CC structures.

2. Materials and methods

2.1. Animals and study design

The experimental protocol was approved by Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) Ethical Committee for Research and all efforts were made to minimize discomfort, distress and animals' suffering. All experimental procedures were carried out according to the International Guiding Principles for Biomedical Research Involving Animals of the Council for International Organization of Medical Sciences and The International Council for Laboratory Animal Science.

Ninety-day old male Wistar rats (~250 g), obtained from the animal facility of UFCSPA, were used. The animals were maintained under standard conditions of temperature (22 ± 2)°C with a 12 h light/dark cycle (lights off at 5 p.m). The animals were fed a standard laboratory rat chow and had water available *ad libitum*.

Animals were divided into three groups: *sham operation* group ($n = 8$) underwent *sham operation*; Orchiectomized (Orchiec) + oily vehicle group ($n = 8$) underwent bilateral orchiectomy and received a single dose of oily vehicle by intramuscular injection (i.m.) 30 days after orchiectomy; and Orchiec + T group ($n = 8$) underwent bilateral orchiectomy and received a single dose of testosterone undecanoate (Nebido®;Bayer Schering Pharma, Berlin, Germany) 100 mg/kg i.m. 30 days after the surgical procedure [9]. Both oily vehicle and testosterone undecanoate were injected into the animals' muscle biceps femoris in the right hind leg.

All surgical procedure were performed under sterile condition and ketamine and xylazine (10 mg/kg and 80 mg/kg i.p, respectively) anesthesia. Animals from Orchiec + oily vehicle and Orhiec + T groups were submitted to bilateral orchiectomy. The surgical procedure was performed with a 2-cm scrotal midline incision and both testes were removed. *Sham operation* group underwent the same surgical procedure and manipulation; however, testes were not removed. Ibuprofen (Buprovil®;Multilab, São Jerônimo, Brasil) 20 mg/kg, 8-8 h, was given by gavage during two consecutive days. Animals' body weight was monitored for Tundecanoate dose adjustment.

All animals were euthanized 60 days after the beginning of the experiment with an anesthetic overdose of ketamine

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