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Stereological study of the effects of nandrolone decanoate on the rat prostate

Saied Karbalay-Doust *, Ali Noorafshan

Anatomy Department, School of Medicine, Shiraz University of Medical Sciences, Zand Avenue, Shiraz, Fars 71348-45794, Iran

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Abstract

It has been shown that nandrolone decanoate which is one of the anabolic-androgenic steroid compounds changes the testis structure and sperm quality but quantitative studies of the prostate have received less attention. Control rats received the peanut oil and experimental group received nandrolone decanoate for 14 weeks. Then the rats were left untreated for 14 weeks. After 14 weeks of withdrawal, the prostate was studied using stereological methods. The mean prostate weight decreased ~39% ($p < 0.009$) in nandrolone decanoate treated rats. The mean total prostate volume, glands, epithelia, fluids and collagen bundles reduced ~30% ($p < 0.03$), ~31% ($p < 0.03$), ~41% ($p < 0.02$), ~31% ($p < 0.05$) and ~59.5% ($p < 0.02$) in the experimental group. The mean total luminal surface of the glands and total length of the vessels decreased ~40% ($p < 0.02$) and ~46% ($p < 0.009$), respectively, in the nandrolone decanoate treated rats. The height of epithelium did show no difference. It can be concluded that nandrolone decanoate causes atrophic changes in the components of rat prostate.

Keywords: Nandrolone decanoate; Rat; Prostate; Stereology

1. Introduction

Anabolic-androgenic steroids (AAS) are synthetic derivatives of the male hormone testosterone. They can exert strong effects on human body that may be beneficial for athletic performance. AAS compounds were currently prescribed in the treatment of refractory anemia, hereditary angioedema, breast cancer and starvation states (Clark et al., 1997; Hartgens and Kuipers, 2004). The doses and combinations of these compounds used by athletes are typically in large excess (10–100-fold) of therapeutics doses. Many other adverse effects have been associated with AAS misuse, including disturbance of endocrine and immune function, alterations of sebaceous system and skin, changes of haemostatic system and urogenital tract. The evidence suggests that these compounds can produce changes in human and animal behavior and psychology (Clark et al., 1997; Hartgens and Kuipers, 2004; Feinberg et al., 1997; Pope and Katz, 1994), sperm quality (Holma, 1997; Nagata et al., 1999; Schurmeyer et al., 1984) and physiology (Torres-Calleja et al., 2001; Wroblewska, 1997; Koskinen et al., 1997; Ferry et al., 2000; Gayan-Ramirez et al., 2000; Joumaa and Leoty, 2001). Our previous study showed that nandrolone decanoate (ND) which is one of AAS compounds changed the testis structure (Noorafshan et al., 2005) and sperm quality (unpublished data) but quantitative study of the prostate has received less attention. Prostate is composed of tubulo-alveolar glands covered with a simple cuboidal or columnar epithelium. The glands produce a weak acidic secretion and are embedded in a connective tissue matrix containing vessels. Smooth muscles run between the separate glands as well as around the entire organ. The present study was conducted to evaluate the effects of nandrolone decanoate, which is one AAS on the prostate in rats by unbiased stereological methods. The evaluated parameters are total volume of the prostate, absolute volume of the tubulo-alveolar glands, epithelia and fluids of the glands, collagen bundles and smooth muscles, total luminal surface of the glands, height of epithelium and total length and diameter of the vessels.

2. Materials and methods

2.1. Ethics

All procedures were carried out under the ethical guidelines of the Shiraz University of Medical Sciences and the studies

* Corresponding author. Tel.: +98 711 2304372; fax: +98 711 2304372.
E-mail address: karbalas@sums.ac.ir (S. Karbalay-Doust).

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received prior approval of the Ethics Committee of Shiraz University of Medical Sciences.

2.2. Chemicals

Nandrolone decanoate (25 mg/1 ml) was purchased from Iran Hormone (Tehran, Iran).

2.3. Animals and treatments

Ten male Sprague–Dawley rats, with initial weight between 250 and 300 g, were kept in the animal house of Shiraz University of Medical Sciences while the environment temperature was maintained $22 \pm 2\ ^{\circ}C$ with 12-h light:12-h dark period. The animals had free access to diet during the experiment. The animals were randomly divided into two groups of control rats ($n = 5$) and experimental rats ($n = 5$). The control rats received the vehicle (peanut oil) 3 mg/kg each week for 14 weeks, and the experimental group received 3 mg/kg of ND dissolved in peanut oil each week for 14 weeks (Ferry et al., 2000; Gayan-Ramirez et al., 2000; Joumaa and Leoty, 2001). Then the rats were left untreated for 14 weeks withdrawn from the hormone injection. The selected dose was below other therapeutic uses of ND. According to Parfitt (1999), ND is usually given once every 3 or 4 weeks. Doses of 25–100 mg have been used after debilitating illness. Doses of 50 mg have been suggested for use in postmenopausal osteoporosis and doses of 25–100 mg postmenopausal breast carcinoma; therefore, the given doses here were lower than the therapeutic doses.

After 14 weeks of withdrawal the rats were sacrificed and the prostate (ventral and dorsal lobes) was removed and weighed. Then the tissue was immersion fixed in solution of 4% buffered formaldehyde. The time period of one spermatogenesis is about ~49 days in rats (Kolasa et al., 2004); therefore, 14 weeks ND injection (98 days, i.e. twice the spermatogenesis period) and then another 14 weeks discontinuation of the injection seem to be reasonable periods for recovery of the sperm quality.

2.4. Stereological study

Estimation of prostate volume and volume density of the prostate components does not require isotropic uniform random (IUR) sections but IUR sections are necessary for the estimation of luminal surface of the prostate glands and vessels length. These sections were obtained by the orientator method (Mattfeldt et al., 1990; Gundersen et al., 1988a,b). Briefly, for generating the isotropic uniform random sections, warm paraffin was filled into a plastic syringe the tip of which had been cut off. Then each prostate was embedded in it. After cooling of paraffin, the paraffin cylinder was pulled out from the syringe. Then the cylinder was located in the center of a circle with 36 equidistant divisions along the perimeter. A random number between 0 and 36 was selected using a random table and the cylinder was cut in the selected direction. The first cut was performed in the tissue free space of the cylinder. The first cut edge of the cylinder was placed parallel to the 0–0 direction of a second circle with sine-weighted non-equidistant 97 divisions along its perimeter. A new random number between 0 and 97 was chosen and the specimen was cut in the new direction. The second cut was also performed in the tissue free space of the cylinder. This new cut surface was the isotropic face of the cylinder. Complete serial sections (5 μm thickness) were then cut from the isotropic face of the embedded tissue.

Cavalieri estimator was used to estimate the total volume of the prostate. After staining with Heidenhain's azan, 10–12 sections were selected in a systematic random manner and examined using a projection microscope at final magnification of 21.5. It has been proved statistically that 10–12 sections are sufficient for stereological study (Gundersen et al., 1988a,b; Mouton, 2002). The volume of the prostate was obtained by point-counting method and the following formula (Gundersen et al., 1988a,b; Mouton, 2002):

$$V_{(\text{prostate})} = T \cdot \sum P \cdot \left(\frac{a}{p}\right)$$

where "$\sum P$" is the number of points landing within the object transect on each section, "a/p" the area associated with each point and "T" is the distance between the selected sections.

For estimating volume density of the glands, the epithelia and fluids of the glands, collagen fibers, smooth muscle, luminal surface of the glands, length and diameter of the vessels, the sampled sections were viewed using a Nikon E-200 microscope. A personal computer and a monitor were connected to a color video camera mounted on top of the microscope. Each section was subsampled by systematic quadrants of 8556.25 μm^2 and composed of 25 points and, with a gap between quadrants of 1 mm in the horizontal direction and 1 mm in the vertical direction, using the microscope's stage vernier (Fig. 1). The volume density of the glands, epithelia and fluids of the glands, collagen bundles and smooth muscle were estimated by means of stereological software designed at Shiraz University of Medical Sciences. The stereological probes composed of 25 points were superimposed on the images of the tissue sections viewed on the monitor at final magnification of 1835. The volume density (Vv) was obtained using the following formula (Gundersen et al., 1988a,b; Mouton, 2002):

$$\text{Vv} = \frac{P_{(\text{structure})}}{P_{(\text{ref})}}$$

where "$P_{(\text{structure})}$" indicates the number of points hitting the parameters and $P_{(\text{ref})}$ is the number of points hitting the reference space. The absolute total volume of each structure was estimated by the volume density multiplied by the total volume of the prostate (Fig. 2).

The luminal surface of the glands was another estimated parameter. It should be noted that luminal microvilli could not be seen under light microscope, so we estimated the luminal surface excluding the microvilli. For estimating the luminal surface of the glands, a probe of 25 lines was superimposed on the images of the tissue sections viewed on the monitor at final magnification of 454 and the following formula (Mouton, 2002):

$$\text{Total surface} = \frac{2 \cdot \sum I}{l/p \cdot \sum P} \cdot V_{(\text{prostate})}$$