Morphometric study of the corpus cavernosum after anabolic androgenic steroid administration in pubertal and adult rats[1]

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ABSTRACT

PURPOSE: To evaluate the penile morphological modifications of pubertal and adult rats chronically treated with supra-physiological doses of anabolic androgenic steroids.

METHODS: Forty-eight male Wistar rats were distributed into four groups: two control groups, 105- and 65-day-old (C105 and C65, respectively) injected with peanut oil (vehicle); and two treated groups, 105- and 65-day-old (T105 and T65, respectively) injected with nandrolone decanoate at a dose of 10 mg Kg^{-1} of body weight. The rats were injected once a week for eight weeks. The rats were then killed and their penises were processed for histomorphometric analyses. The mean of each parameter was statistically compared.

RESULTS: A corpus cavernosum reduction of 12.5% and 10.9% was observed in the T105 and T65 groups, respectively, when compared with their respective control groups. The cavernosum smooth muscle surface density diminished by 5.6% and 12.9% in the T65 and T105 groups, respectively, when compared with their controls. In contrast, the sinusoidal space increased by 17% in the T105 group and decreased by 9.6% in the T65 group.

CONCLUSION: The use of supra-physiological doses of AAS promotes structural changes in the rat penis, by altering the proportions of corpus cavernosum tissues, in both pubertal and adult treated animals.

Key words: Penis. Anabolic Agents. Erectile Dysfunction. Rats.

Introduction

Anabolic androgenic steroids (AAS) are synthetic testosterone derivatives that can have major effects on the human body. It is known that both testosterone and AAS diminish body fat and increase muscle mass and, thus, improve athletic performance. This effect has prompted indiscriminate AAS use by professional and amateur athletes, both youth and adults, in an attempt to improve their sport performance[1].

One study showed that 1 to 3 million men and women in the United States have used AAS[2]. According to some authors, these drugs are commonly used in doses 10- to 100-fold greater than the physiological levels, involving a single drug or a mixture of various AAS[3-7].

Many side effects of this practice have been reported, including disorders of the urogenital system[8]. It is known that high doses of nandrolone decanoate reduce testicular volume and the length of the seminiferous tubules in rats[9]. AAS can also decrease the weight and volume of the prostate, and increase the prostatic epithelium width in rats[10].

Regarding the penis, it is well known that adequate levels of testosterone are necessary to maintain its normal morphology and the proper functioning of erectile bodies[11,12]. Therefore, it was hypothesized that AAS may affect penile morphology. To the best of our knowledge, there is no information on the effects of supra-physiological doses of AAS on the corpus cavernosum tissue. Thus, this study aimed to assess the penile morphological modifications of pubertal and adult rats chronically treated with supra-physiological doses of AAS.

Methods

All experiments were performed in accordance with the Brazilian laws for scientific use of animals, and the project was approved by the local ethics committee (protocol no. CEUA/036/2012).

We studied 48 male Wistar rats which were kept in a room with controlled temperature (22 ± 2°C) and an artificial dark-light cycle (lights on from 7:00 a.m. to 7:00 p.m.). They were fed standard rat food and water *ad libitum*. The rats were distributed into four groups: control rats 105-day-old (C105) (n = 10), control rats 65-day-old (C65) (n = 14) injected only with the carrier (peanut oil), treated rats 105-day-old (T105) (n = 10), and treated rats 65-day-old (T65) (n = 14). The treated rats (T65 and T105) were injected with nandrolone decanoate at a dose of 10 mg Kg^{-1} of body weight while the control groups (C65 and C105) were injected with 90% peanut oil (diluted in benzoic alcohol) as carrier[1,10]. Both the steroid hormone and carrier were administered by intramuscular injection once a week for eight weeks. This treatment protocol was established to simulate a commonly used protocol in humans during puberty or at early adulthood, as previously published elsewhere[1,10].

The rats were killed by anesthetic overdose (intraperitoneal thiopental injection) when they were 161- (C105 and T105) or 121-day-old (C65 and T65). The penises were dissected and fixed in 4% buffered formalin. The penile mid-shaft was processed for paraffin embedding to obtain 5-µm sections, which were stained with picrosirius red or Masson's trichrome.

Images of the cross-sections stained with picrosirius red were captured under x12 magnification with a digital camera (AxionCan ERC5S, Karl Zeiss, Gottingen, Germany) coupled to a stereomicroscope (SteREO Discovery V8, Karl Zeiss, Gottingen, Germany). In these images, the cross-sectional areas of the penis, corpus cavernosum (with and without tunica albuginea), and tunica albuginea alone were measured in mm^2 using the ImageJ software (version 1.45s, National Institutes of Health, Bethesda, USA) using the "Free Hand" tool after calibration. The area of the tunica albuginea was estimated by the difference in the areas of the corpus cavernosum with and without the tunica albuginea[13].

In these sections stained with picrosirius red, by observation under x400 magnification and polarized light, the collagen types of corpus cavernosum were differentiated as types III (in green) and I (red/orange)[14]. These images were captured with a digital camera (DP70, Olympus, Tokyo, Japan) coupled to a microscope (BX51, Olympus, Tokyo, Japan).

The surface densities (Sv) of the corpus cavernosum connective tissue, sinusoidal space, and smooth muscle were measured by examining cross-sections stained with Masson's trichrome. For each animal, 25 corpus cavernosum photomicrographs were obtained under x400 magnification. The density of each of these structures was expressed as a percentage obtained by the point-counting method[11]. Briefly, a 100 points grid was superimposed over the images using the "grid" tool of the ImageJ software, and each structure touched by one point was counted as a connective tissue, sinusoidal space, smooth muscle, or other structure. The "cell counter" tool of the ImageJ program was used to count each structure separately.

For each parameter, the mean of each control group was compared to its age-matched treated group. Results were first analyzed using the D'Agostino & Pearson omnibus normality test. Parametric data were then compared using the Student's *t*-test, while nonparametric data were compared using the Mann-Whitney test. For

all analyses, two-tailed tests were used. All analyses were performed using the GraphPad Prism 5.0 software (GraphPad Software, San Diego, USA). Mean differences were considered significant at $p<0.05$. All results are presented as means ± standard deviation.

Results

Penile cross-sectional area

There was no difference on the penis cross-sectional area between the adult groups (C105 vs T105). When comparing the results of pubertal animals, a 5.6% reduction in the penile cross-sectional area was observed in T65 animals compared to C65 animals.

Corpus cavernosum cross-sectional area with and without the tunica albuginea

The cross-sectional area of the corpus cavernosum with the tunica albuginea was similar between C105 and T105 animals, but a 12.5% reduction in the corpus cavernosum without the tunica albuginea of the treated animals was observed compared to their age-matched controls.

Regarding the pubertal treated animals (T65), the area of the corpus cavernosum with and without the tunica albuginea was 6.9% and 10.9% smaller, respectively, than in C65 animals.

Tunica albuginea cross-sectional area

The cross-sectional area of the tunica albuginea was similar among the pubertal animals. When comparing the C105 and T105 groups, a 20% area increase was recorded in C105 animals.

Corpus cavernosum connective tissue density

When analyzing the connective tissue by point-counting planimetry, we observed a 6.8% increase in connective tissue density in the T65 group compared to the C65 group. T105 animals showed connective tissue density values similar to those of C105 animals (Figure 1).

Corpus cavernosum sinusoidal space density

Sinusoidal space density analysis showed a 9.6% reduction in group T65 compared to the C65 group. The density of the adult treated group (T105) was 17% higher than that of the C105 group (Figure 1).

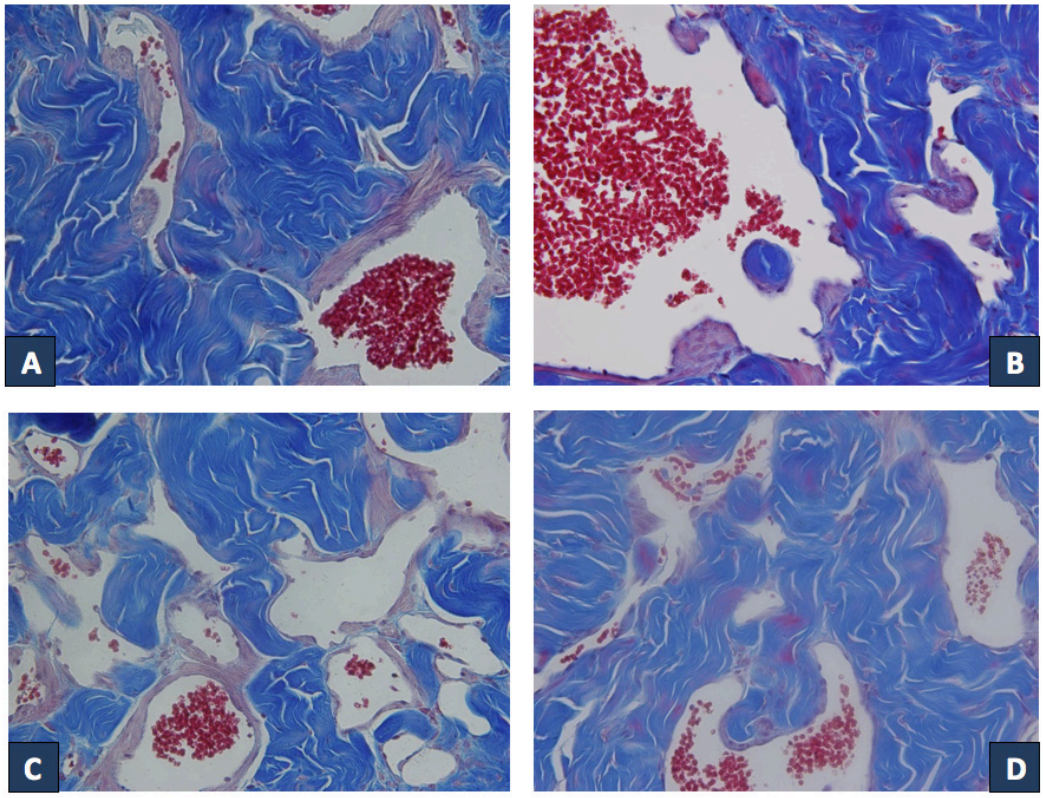

FIGURE 1 - Photomicrograph of penile corpus cavernosum from rats showing the differences among control animals and animals treated with anabolic androgenic steroids. A) Group C105; B) Group T105; C) Group C65; D) Group T65. Masson´s Trichrome, x400.

Corpus cavernosum smooth muscle density

Corpus cavernosum smooth muscle density was 5.6% lower in the pubertal treated group than in its control group. In the adult groups, the T105 group showed a 12.9% reduction when compared with the C105 group (Figure 1). All the morphometric data of pubertal and adult animals are shown in Tables 1 and 2, respectively.

TABLE 1 - Penile morphometrical data from pubertal control animals (C65) and animals treated with supra-physiological doses of androgenic anabolic steroids (T65).

	C65	T65	*p* value
Penile cross-sectional area (mm^2)	7.48 ± 0.52	7.06 ± 0.66	< 0.01
Corpora cavernosa area with tunica (mm^2)	5.45 ± 0.39	5.07 ± 0.49	< 0.01
Corpora cavernosa area without tunica (mm^2)	2.98 ± 0.39	2.65 ± 0.29	< 0.001
Area of the tunica albuginea (mm^2)	2.47 ± 0.27	2.55 ± 0.48	0.70
Sv of the sinusoidal space (%)	28.7 ± 11.6	26.0 ±10.2	< 0.05
Sv of connective tissue (%)	54.1 ± 13.4	57.8 ± 11.9	< 0.01
Sv of smooth muscle tissue (%)	16.0 ± 5.4	15.1 ± 5.0	< 0.05

Data are presented as means ± standard deviation. Data was compared by Student's *t*-test considering significant at p<0.05. Sv, surface density.

TABLE 2 - Penile morphometrical data from adult control animals (C105) and animals treated with supra-physiological doses of androgenic anabolic steroids (T105).

	C105	T105	*p* value
Penile cross-sectional area (mm^2)	7.22 ± 0.92	7.35 ± 1.16	0.57
Corpora cavernosa area with tunica (mm^2)	5.32 ± 0.71	5.43 ± 1.03	0.66
Corpora cavernosa area without tunica (mm^2)	2.92 ± 0.34	2.55 ± 0.40	< 0.01
Area of the tunica albuginea (mm^2)	2.40 ± 0.45	2.88 ± 0.70	< 0.05
Sv of the sinusoidal space (%)	24.5 ± 8.2	28.7 ± 13.8	< 0.05
Sv of connective tissue (%)	59.5 ± 11.1	57.2 ± 15.5	0.40
Sv of smooth muscle tissue (%)	15.1 ± 6.3	13.1 ± 4.6	< 0.01

Data are presented as means ± standard deviation. Data was compared by Student's *t*-test considering significant at p<0.05. Sv, surface density.

Collagen types

Picrosirius red-stained cross sections of corpus cavernosum observed under polarized light of both T65 and T105 animals showed a higher predominance of green collagen (collagen type III), compared to the control animals. The latter showed a greater amount of red/orange collagen.

Discussion

It is well known that androgenic hormones are important in sexual differentiation and male genital organ formation[15]. Furthermore, testosterone acts on other tissues that are not directly related to the genital system such as the adipose and muscle tissues[16,17]. Because of these effects, many athletes, both professionals and amateurs, youths and adults, use synthetic testosterone derivatives irresponsibly. Anabolic androgenic steroids are more powerful than testosterone and are often used at much higher concentrations than the physiological levels. Their use results in muscle mass increase and adipose mass decrease, enabling users to achieve aesthetic results or athletic performances that could not be obtained without the use of these hormones. Although AAS are Class III drugs, with their use being subject to strict rules, illegal access to these substances without medical guidance is easy[18].

However, besides the desired esthetic and/or athletic effects, high serum concentrations of these hormones cause important negative physiological changes that jeopardize the health of users. In addition to changes in vital organs such as the heart, kidneys, and liver, studies have shown that urogenital system organs are also affected. For example, the prostate and testes of animals injected with these hormones show pronounced

morphological changes, compatible with functional alterations[8-10]. Several studies have considered the effects of AAS; however, to the best of our knowledge, this is the first study to characterize the penis of rats after AAS use.

We observed important alterations both in the cross-sectional area of erectile structures and in corpus cavernosum composition after the chronic use of nandrolone decanoate in rats. These changes could be explained because the penis is an organ that responds strongly to testosterone.

In a study in which rats were subjected to androgen deprivation, a 21% reduction in the penis cross-sectional area was observed. These animals also showed reduced smooth muscle and sinusoidal space in the corpus cavernosum[12]. Changes in the penile erectile tissue were also noted in another study where the serum level of testosterone was 57% reduced. This reduction resulted in smooth muscle reduction and cavernosum connective tissue increase[11].

Interestingly, in the present study, animals treated with synthetic androgen showed changes comparable to those seen on animals subjected to androgen deprivation (i.e., smooth muscle reduction, connective tissue increase, and penis and/or corpus cavernosum area reduction). Since AAS are known to depress the production of endogenous testosterone, the changes observed might be due to this negative feedback mechanism. The measurement of serum testosterone levels in animals receiving AAS would be of interest for further explaining this theory.

Correct proportions of the elements that compose the corpus cavernosum (connective tissue, smooth muscle, endothelium, and sinusoidal space) are required for proper erectile function. It has been demonstrated that in individuals with erectile dysfunction, the proportions of these elements are altered[19]. Therefore, it is possible that the changes observed in animals treated with high AAS doses might be associated with impaired erectile function. Thus, in future studies, it would be interesting to check erectile function by cavernosometry in animals treated with high AAS doses.

Adolescence is a highly sensitive period for organic and sexual development. Often at this period, an interest for experimenting with drugs and sex is developed. In addition, the desire to improve their athletic appearance prompts some adolescents to abuse AAS without considering the negative side effects. Several articles have also shown that the use of anabolic steroids by teenagers is sometimes motivated purely by a desire to experiment with restricted drugs and not by a desire to enhance their appearance and/or sport performance[20]. AAS are also commonly used along with other legal drugs (alcohol and tobacco) as well as with illegal substances[21]. The profile of young steroid users appears to be distinct from that of adult users, whose steroid use is usually associated with sport practice in order to gain muscle mass and lose body fat. In addition, it is known that many adults also use steroids for purely esthetic purposes[22].

The knowledge that AAS use promotes pronounced changes in the penile tissue of rats might discourage the abusive use of these drugs. The risk of penile damage might outweigh the desire to improve their appearance and sport performance in many adolescents and young adults.

In the present study, we observed changes in the penises of pubertal and adult animals immediately after AAS treatment. However, we cannot determine whether the changes caused by the use of these drugs are permanent or whether the changes could be restored after their use is discontinued. Also, studying the effects of other dosages and schemes of AAS administration on penile morphology would be of interest since different drugs, doses and schemes are used by athletes. Finally, although the penile tissue of rats differs from that of humans, the two species possess the same penile structural components and respond very similarly when exposed to many experimental situations [13,23]. Thus, the results obtained in our study are likely to be relevant to humans.

Conclusion

The use of supra-physiological doses of anabolic androgenic steroids promotes structural changes in the rat penis, by altering the proportions of corpus cavernosum tissues, in both pubertal and adult treated animals.

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