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Pathological Study of Androgen Effect on the Musculoskeletal System on Male Rat

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Abstract: Androgen have important effects on bone development and homestasis. Increasing recognition of morbidity and mortality attributable to osteoprosis in man stimulated considerable interest in recent years in the mechanisms by which androgen act on bone. The purpose of the present study was to investigate the effect of androgen deficiency on the musculoskeletal system of the male rat. Cyproteron acetate is an antiadnrogenic drug. It suppresses the actions of testosterone by blocking its receptors Its main indications are, prostate cancer and other conditions in which androgen action maintains the disease process. Retardation of muscle and bone growth in male rats that were exposed to cyproterone acetate were studied. 36 male rats were divided into 4 groups each. Group 1 served as the control whereas group II, III and IV received 2, 5 and 7mg/kg of the drug, respectively. After 10 weeks, animals were euthanized. Their different tissues of, Gastronemius, bicep, tricep, tibialis and soleus muscles were cut and weighed. The absolute muscle weighs showed significant difference among the different groups. Blood was collected to measure the testosterone level. The lowest level of testosterone was recorded in group IV. The histopathological studies revealed osteoprosis in group IV. The bone lesions included enlarged marrow cavity with thin bone trabeculae, wide seams of pale staining osteoid on bone. Hypertrophic zone of growth cartilage narrowed and distorted. It was concluded that cyproteron as an antiandrogenic hormonal drug caused osteopenia and significantly reduced bone and muscle mass to a varying extent in male rat.

Key words: Androgen · Rats · Cyproteron · Bone · Necrosis · Osteoprosis

INTRODUCTION

Androgen is necessary for male sexual differentiation before birth and sexual maturation during puberty and for the proper development and function of numerous tissues, including the central nervous system [1]. However, the role of androgens in other target organs including muscular tissue; the cardiovascular, central nervous and immune systems; and bone is less well-established. In addition, accumulating evidence indicated that age-related androgen loss can contribute to degenerative disorders disease in bone [2]. Cyproterone acetate is an antiandrogen, i.e. it suppresses the actions of testosterone and its metabolite dihydrotestosterone on tissues. It acts by blocking androgen receptors which prevent androgens from binding to them. Its main indications are prostate cancer, benign prostatic hyperplasia, piriapism, hypersexuality and other conditions in which androgen action maintains the disease process.

In the early 1940s, Albright and Reifenstein [2] were among the first to refer to the antiosteoporotic and anabolic properties of androgens. From a public health perspective, osteoporosis is a greater problem in women than in men [3]. This explains why most research efforts to explore skeletal effects of sex steroids have been devoted to estrogens. Moreover and rogens may be converted into estrogens via the P450 aromatase enzyme complex and may therefore act as prohormones for estrogens. In this respect, there is increasing evidence that at least part of

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the effects of androgens in men can be explained by their aromatization into estrogens [4]. Epiphyseal closure at the end of puberty, for example, is now generally accepted to be estrogen-dependent in both genders [5]. In recent years, a specific role of androgens in skeletal homeostasis has even been questioned, although androgen receptors (ARs) in bone cells and AR-mediated actions on bone have been documented for more than a decade [6].

The aim of this work was to address the question whether and how (through which receptors and/or pathways) androgens may affect bone strength and provide protection against osteoporosis. The clinical relevance of this question results from the recognition that, even in men, fractures due to skeletal fragility represent a huge public health problem.

MATERIALS AND METHODS

The experiments were carried out with male Wistar rats, divided into 4 groups: sham-operated control rats; sham-operated rats receiving cyproteron at doses of 2, 5, 7 mg/kg orally after 2weeks.The body weight of each rat was determined at 3 and 10 wks of age The drug was administered for 4 weeks. After sacrification, the biceps brachii, soleus, gastronemious and tibialis cranialis muscleswere carefully dissected out. The mean weight of the muscles was determined. The bone of femur was dissected and preserved in 10% formalin and formic acid for histopathological study. After 10 weeks, blood of rats were collected by aortic puncture; the plasma was separated and testestron level has been measured with a specific radioimmunoassay.

Statistical Analysis: The data obtained for the muscle were subjected to analysis of variance using F-ratio and Duncans New Multiple Range.

RESULTS

Hormonal Results: Serum testestron levels were assayed in male rats of similar age and weight (10 week old; $n = 9$) and levels showed significant ($P<0.5$)differences among groups (Table.1).

Body Weight: The comparison of body weights in control and cyproteron exposed male rats showed significant differences among groups at 4 weeks ($P<0.05$) and in groups of 10 weeks were compared ($P<0.01$) (Table 2).

Muscles: The comparison of the absolute muscle weights of control and cyproteron exposed male rats revealed significant ($P<0.5$) differences among groups. Comparison revealed that the absolute weights of bicep brachii, triceps brachii, tibialis cranialis and gastronemius muscles of the control males were significantly superior to those of the cyproteron exposed groups ($P<0.01$). The absolute weight of soleus muscle of control males was significantly superior to that of the cyproteron exposed male rats ($P< 0.05$) (Table 3).

Bones: The bone lesions included enlarged marrow cavity with thin bone trabeculae, wide seams of pale staining osteoid on bone. Hypertrophic zone of growth cartilage narrowed and distorted (Fig. 1).

Table 1: Testosterone (T) serum level (ng/ml)in control rats and in rats cyproteron-exposed groups at 4th and 10th week

	Treatments					
	T1-Control	T2	T3	T4	SE	Significance
4th week	0.309 ±0.09[a]	0.296±0.15[b]	0.280±0.16[ab]	0.275±0.18[b]	0.17	*
10th week	3.25±0.49[a]	2.96±0.16[b]	2.79±0.10[c]	2.69[c]±0.15	0.22	**

Table 2: Comparison of body weights (g) of three groups of male rats of Control and cyproteron exposed at 4th and 10th week

	Groups	
	10th week	4th week
Control	58.31±0.28[a]	196.25±0.49[a]
T2	56.94±0.24[a]	182.56±0.35[b]
T3	55.1±0.22[ab]	172.15±0.54[c]
T4	53.54±0.31[b]	163.23±0.43[d]
SEM	0.86	0.58
Significance	*	**

[ad]: Values in the same row and variable with no common superscript differ significantly. *: $P<0.05$, **: $P<0.01$, NS: Not Significant. [1] Values are means of six observations per treatment and their pooled SEM. [2] T1 = control males, T2 =group II., T3 =group3, T4=groupIV

Table 3: Comparison of muscle weights (g) of four groups of male rats of control and cyproteron-exposed

	Treatments					
Groups	T1-Control	T2	T3	T4	SEM	Significance
Biceps brachii	0.185 ± 0.27[a]	0. 167 ± 0.12[a]	0.148 ± 0.38[b]	0.135± 0.21[b]	0.22	**
Triceps brachii	0.630 ± 0.22[a]	0.584 ± 0.16[b]	0.541 ± 0.24[c]	0.508± 0.33[d]	0.60	**
Soleus	0.108± 0.23[a]	0.097± 0.26[ab]	0.086± 0.33[b]	0.081 ±0.27[b]	0.31	*
Tibialis cranialis	0.511± 0.26[a]	0.478± 0.31[b]	0.459 ± 0.35[bc]	0.447 ± 0.02[c]	0.28	**
Gastrocnemius	0.740± 0. 16[a]	0.722 ± 0. 25[a]	0.706 ± 0.05[b]	0.679 ±0.03[b]	0.342	**

[ad]: Values in the same row and variable with no common superscript differ significantly. *: P<0.05, **: P<0.01, NS: Not Significant. [1] Values are means of eighteen observations per treatment and their pooled SEM. Mean ± S.E giveu for each measurement. [2]

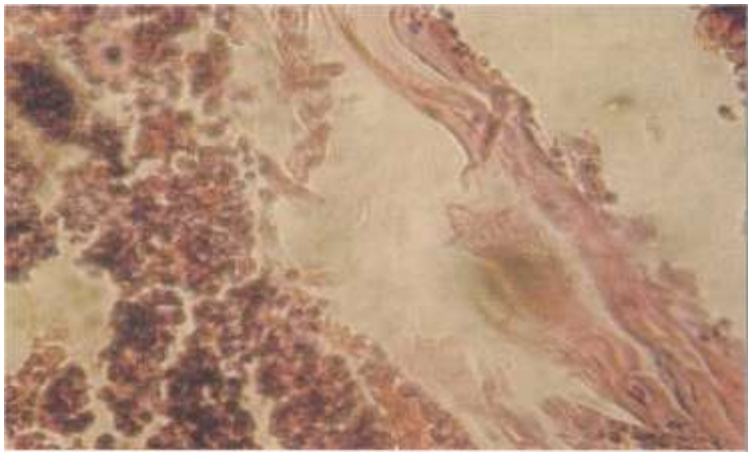

Fig. 1: Thin and discontinued bone trabeculae were evident throughout the femoral head. (Hematoxyline and Eosin, original magnification x 400)

DISCUSSION

Serum levels of testosterone at the basal state and during the postoperative period are shown in table 1. All of the serum hormonal levels were significantly affected by the operation. In this retrospective analysis, we demonstrated a significant association between lower serum level of androgen and bone disease in male rats. In these animals and rogen deficiency results in a menopause-like acceleration of bone turnover and bone loss which are presented by bone trabeculae reduction. Moreover and rogens are not only necessary for the (cortical) bone mass but also for the maintenance of the muscle mass.

The result of this study showed that the muscle mass indices of control were superior to those of treatment group. Muscular atrophy in castrated male animals observed in cattle [6] and rat [7]. This reduction may be attributed muscle mass of males deficient in androgen which were reported by other researcher [8-10]. To loss of influence of testosterone on muscles. On the basis of this observation suggested that androgen may be presumed to have direct or indirect affect on muscle.

Cyproterone acetate was used in order to investigate the role of testosterone in bone growth processes. The formation of Haversian systems in the growing antlers of white-tailed deer (*Odocoileus virginianus*) were substantially affected by only 3.5 mg of CA kg/wk [11].

Numerous other studies have provided evidence of an association between androgen and bone diseases in rat [12-13]. As well, numerous biochemical abnormalities have been described including hypocalcemia, hypophosphatemia. Osteoporosis is defined as a condition characterized by reduced bone mass and disruption of bone architecture, resulting in increased bone fragility.

Clinically, osteoporosis may be recognized by the presence of fragility fractures, but recently, diagnostic criteria based on bone mineral density measurements have been proposed based on the well-documented inverse relationship between bone mineral density and fracture risk [3,10,14].

Sex steroids play an important role in bone growth and the attainment of peak bone mass. They are responsible for the sexual dimorphism of the skeleton, which emerges during adolescence [15-17], the male skeleton is characterized by larger bone size (even when

corrected for body height and weight) with both a larger diameter and greater cortical thickness in the long bones. Volumetric bone mineral density is, however, very similar in young adult men and women [18-19], but the larger bone size in men confers significant biomechanical advantages and, in part, explains the lower incidence of fragility fractures compared with women.

Estrogen is essential for normal closure of the growth plates in both sexes; thus estrogen resistance and aromatase deficiency in men are associated with delayed bone age and tall stature despite normal or high circulating concentrations of testosterone.

Anumber of studies support the contention that both estrogens and androgens are required for normal skeletal health in males and females [20-21]. Thus the administration of flutamide, a specific androgen receptor antagonist, to female rats results in osteopenia, indicating a role for androgens in the female skeleton In support of these findings, [22] reported that the antiandrogen compound Casodex inhibited the protective effects of androstenedione on ovariectomy-induced bone loss, whereas administration of an aromatase inhibitor was ineffective. Furthermore, in female rats, nonaromatizable androgens have been shown to prevent or reverse bone loss induced by ovariectomy, these effects being mediated by a reduction in bone turnover in cancellous bone and increased periosteal and endostealand bone formation [23-24].

In conclusion and rogens are considered to be the key regulator of male periosteal bone expansion and essential for the increase in muscle mass. The present study was carried out to determine whether or not cyproterone acetate, as an anti-androgenic agent has an impact on muscle and bone in rat. Cyproterone acetate treatment resulted in atrophy of the muscle in rat as characterized by the loss of wet weight of muscle. Moreover, cyproteron acetate had an impact on cortical bone modeling, Trabecule and bone volume in rat.

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