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Full Length Research Paper

Sex steroid induced changes on the morphology of prostate of sprague-dawley rats

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The effects of combined administration of ethinylestradiol (E) and testosterone (T) were studied in castrated Sprague-Dawley rats. The hormones E and T were administered three times weekly on alternate days, subcutaneously in the inguinal region for 4 weeks, 30 days after castration. E- treated animals received injections of 3 g/kg body weight (B.W), T- treated animals received 30 mg/kg B.W., combined testosterone and ethinylestradiol (T & E) - treated animals received injections of 30 mg/kg B.W. of T and 2 g/kg B.W. of E. Control animals - were in two groups, castrated and intact both received injections of 5 ml/kg B.W. of normal saline. i) The T-treated prostate weight was significantly higher than in castrated control (P<0.05). (ii) The E- alone treated prostate weight was not significantly different from castrated control (P b0.05). iii) The combined T and E-treated prostate weight was significantly higher than in castrated control (P<0.05). Morphological findings: in the combined T and E-treated, the amount of connective tissue was well marked, there was an increase in the thickness of the epithelium and the size of the oval acini, relative to T-alone treated or the intact control. E-alone did not elicit any appreciable effect on the prostate, different from the castrated control. This finding demonstrates a synergistic effect of E on the prostate when administered along with T and also suggests that E may be involved in the pathophysiology of the abnormal enlargement of prostate gland.

Key words: Prostate, castration, testosterone, ethinylestradiol, histology

INTRODUCTION

Several factors are implicated in the normal prostate development and growth: androgens, growth factors, and stromoepithelial cell interactions. These factors are also speculated to play a role in benign Prostatic hyperplasia (Adesanya et al., 2006).

Sexually active male rats have larger accessory sex glands (functional hypertrophy) than sexually inexperience rats, but such increases are dependent on frequency of sexual stimulation as well as the intensity of sexual experience and this increase is reversible after sexual rest (Sodersten et al., 1977).

Androgens are required for the growth and physiology of the prostate. The circulating androgens are testosterone (T) and dihydrotestosterone (DHT). The former is produced mainly by the testis (~95%) and the adrenal gland (~5%) (Coffey, 1992). The conversion of T to the more potent androgen DHT by the enzyme -reductase occurs in the testis and other tissues, including the prostate. Although the normal prostate has been reported not to contribute to the circulating levels of DHT (Toorians et al., 2003), there may be a contribution by this organ in subjects with a hypertrophic prostate (Ghanadian et al., 1977). Both T and DHT bind to the same androgen receptor (AR) with different affinities and apparently with different transcriptional activities.

Although the prostate is an androgen-dependent tissue, its physiology and pathology are also influenced by estradiol-17-beta (E2). Accordingly, E2 receptors and ß

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are expressed in the prostatic stroma and epithelium, respectively (Leav et al., 2001, Weihua et al., 2002). Furthermore, aromatase has been identified in the human prostate, suggesting that the prostate is a site of aromatization and a possible source of E2 (Tsugaya et al., 1996) . These data suggest that E2 may be produced locally in the prostate gland and may influence both epithelial and stromal cells via its two receptors. However, E2 has a general anti-androgen effect, and negatively regulates the hypothalamus-hypophysis-testis axis, thereby reducing androgen production by Leydig cells and causing involution of the prostatic epithelium and growth of the stroma in adult animals (Weihua et al., 2002).

Brief exposure of rats to estrogens during neonatal period causes permanent disturbances in the adult prostate, including reduced size, reduced responsiveness to androgens and epithelial dysplasia with aging (Gails et al., 2001). Long-term exposure of Noble rats to T and E2 induces dysplasia in the dorsolateral lobe but not in the ventral lobe (Lane et al., 1997) . Circulating T is converted to estrogen (E2) in several tissues by the enzyme aromatase. Apart from the interest in the physiological role E2 in males, these hormones have gained importance because they may have deleterious effects on the formation of the male reproductive organ and on reproductive performance and behaviour. Indeed, a hypothesis has been proposed whereby embryonic exposure to E2 is implicated as the causative agent of cryptorchidism, testis cancer, hypospadia and a low sperm count (Sharpe et al, 2003).

Androgen deprivation elicited by surgical or chemical castration induces apoptosis in the prostatic epithelium. In the ventral prostate of the rat, epithelial apoptosis reaches its peak three days after androgen withdrawal (Kurita et al., 2001) days after surgical castration the rat prostate undergoes rapid involution. Basal and secretory cellular subtypes persist. However, the number of glandular cells is significantly reduced (a 66% decrease) (English et al., 1987) and secretory activity is severely attenuated.

The morphological study of normal prostate in a laboratory animal is important in relation to the studies of spontaneous and experimentally induced prostatic hypertrophy. Presently, the only available model for human prostatic hypertrophy is the spontaneous and experimentally induced prostatic hypertrophy in the dog prostate (Walsh et al., 1976; Huttunen et al., 1981) Benign hypertrophy of the dog prostate is produced by the chronic administration of androstane-diol or a combination of androstane-diol and estradiol. The castration of experimental animals also induces prostatic atrophy that can be reversed by testosterone (T) treatment (Huttunen et al., 1981). Conflicting reports, however, are available on the effects of androgens and estrogen on the rat prostate. This paper reports the effects of interaction of T with E on morphology of the prostate gland.

MATERIAL AND METHODS

Animals: Adult Sprague-Dawley rats were supplied by the animal house of the College of Medicine, University of Lagos. The rats were kept in rat control room of the Anatomy department, acclamatized for two weeks before the experiment commenced. The rats were fed a standard diet (Pfizer pellet, Pfizer Nigeria Limited, Ikeja), given water *ad libitum* and maintained under standard conditions. The room was well ventilated with a temperature range of 25 - 27^{O}C under day/night 12 - 12 h photoperiodicity. All animals were castrated via the scrotal route under anaesthesia, 30 days prior to hormone treatment (Huttunen et al., 1981; Eero et al., 1982). A total of 25 adult male Sprague-Dawley rats weighing between 180 – 200 g were used for this experiment. Hormones, Testosterone propionate (T) (Shanghai Medicine and Health export, Republic of China) and Ethinylestradiol (E) (Organon Pakistan, Private Limited) were used in the experiment. The rats were divided into intact and castrated groups and hormone treatment lasted for 4 weeks (Huttunen et al., 1981). Depending upon type of hormone treatment, the animals were divided into five subgroups: Each group consists of 5 animals.

Subgroup I: Intact control animals received 5 ml/kg B.W normal saline on alternate days three times weekly.

Subgroup II: Castrated control received 5 ml/kg B.W normal saline on alternate days three times weekly.

Subgroup III: (T treated) Animals received 30 mg/kg B.W. T on alternate days three times weekly subcutaneously in the inguinal region (Hutttunen et al., 1981; Eero et al., 1982) . This dose was chosen to exert a strong action on the prostate with substantial changes of the tissue compartments.

Subgroup IV: (E treated) Animals received 3 g /kg B.W .E on alternate days three times weekly subcutaneously in the inguinal region.

Subgroup V: (T&E treated) castrated Animals received 30 mg/kg B.W. T and 2 g/kg B.W.E on alternate days three times weekly subcutaneously in the inguinal region.

The treatment lasted for 4 weeks. The animals were sacrificed by decapitation under guilotine, and the paired prostate and seminal vesicle were dissected free from fascia, weighed in a torsion balance and fixed in Bouins solution, and stained with haematoxylin and eosin (Srinivasan et al., 1986), The weights of the prostate in the treated and control groups were recorded and statistical analysis was performed using the student t-test. Data on group experimental data were presented as mean ± standard deviation of mean (SDM) . Values of probability were taken to be statistically significant at $P<0.05$.

RESULTS

The average weight gain per week of the castrated rats was significantly higher ($P<0.05$) than that of the intact control at pretreatment period (Table 1). During treatment the average weight gain per week of the T alone treated and T and E combined treated were significantly lower than that of the intact control ($P< 0.05$). There was no

Table 1. Average weight gain in grams during pre-treatment and treatment periods.

	Average weight gain per week (grams)	
	Pre-treatment	**Treatment**
Intact Control (I)	4.5 ± 4.20*	6.0 ± 4.20
Castrated Control (C)	7.0 ± 5.42	3.75 ± 3.0
Testosterone-Treated (T)	7.7 ± 2.4	4.90± 3.0
Ethinylestradiol-treated (E)	8.3 ± 2.4	9.00 ± 1.20*
Testosterone and Ethinylestradiol-treated (T&E)	7.0 ± 1.4	5.90 ± 3.50

Values are given as Mean ± S.D. n = 5 For each group *values are significantly different from castrated control at P < 0.05.

Table 2. Effect of testosterone and ethinylestradiol on the average weight of prostate and seminal vessicle.

	Prostate (mg/kg B.W.)	**Seminal Vesicle (mg/kg B.W.)**
Intact Control (I) [c]	150 ± 11*	190 ± 12*
Castrated Control (C)	25±18	25 ± 1.6
Testosterone-treated (T)	120 ± 21*	240 ± 12*
Ethinylestradiol-treated (E)	22 ± 1.9	21 ± 1.7
Testosterone and Ethinyl-estradiol-treated (T&E)	140 ± 23*	250 ± 11*

Values are given as Means ± S.D. n = 5. For each group, *Values are significantly different from castrated control at p<0.05

significant difference in average weight gain per week of the T alone treated and T and E combined treated as compared with the castrated control (p>0.05). However the average weight gain by the E alone treated was significantly higher than that of the castrated control and intact control (P<0.05) Table 1.

The weights of the prostate and seminal vesicles of all rats used (control and experimental) are shown in Table 2. The prostate and seminal vesicles of the rats in testosterone T-alone treated have a significantly higher weight than the castrated control (P<0.05) respectively. The prostate and seminal vesicles weights of ethinylestradiol (E) - alone treated and the castrated control are not significantly different (P>0.05) respectively. The weights of prostate and seminal vesicles in combined testosterone and ethinylestradiol T and E - Treated were significant higher than the weights of the prostate and seminal vesicles of the castrated control rats respectively (P<0.05).

HISTOLOGY

Intact control

The connective tissue between the acini and the tubules were thin and condensed around the acini and tubules of the gland. The tissues were tightly packed. The epithelium was cuboidal and regular in size in the tubules, and columnar with infolding into the lumen in the oval acini (Figure 1).

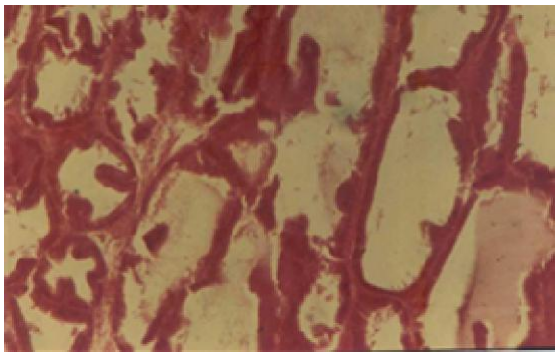

Figure 1 - Light micrograph of intact control prostate (x-100).

Castrated control

There were considerable changes in the histoarchitecture of the prostate gland, the connective tissue stroma was well marked (uncondensed) and the number of tubules and oval acini increased relative to the intact control. The epithelium was predominantly flat. The tubules had different shapes and the oval acini were small in size (Figure 2).

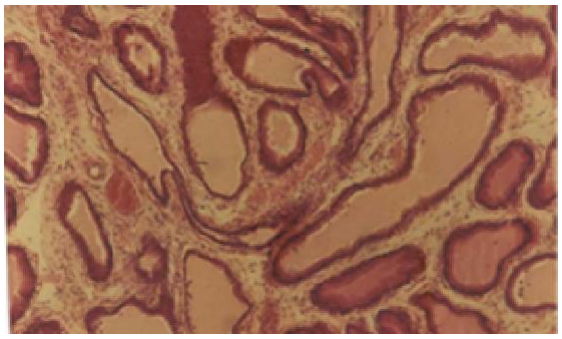

Figure 2. Light micrograph of castrated control prostate (x-100).

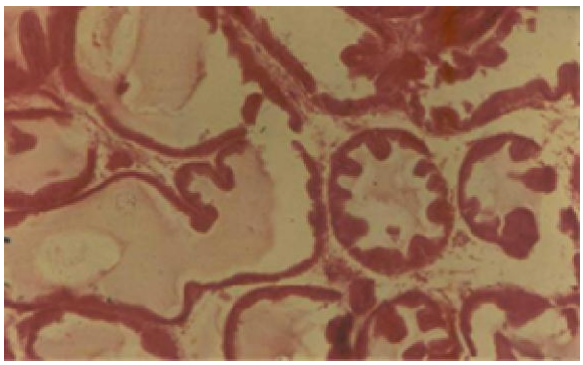

Figure 3. Light micrograph of testosterone treated prostate (x-100).

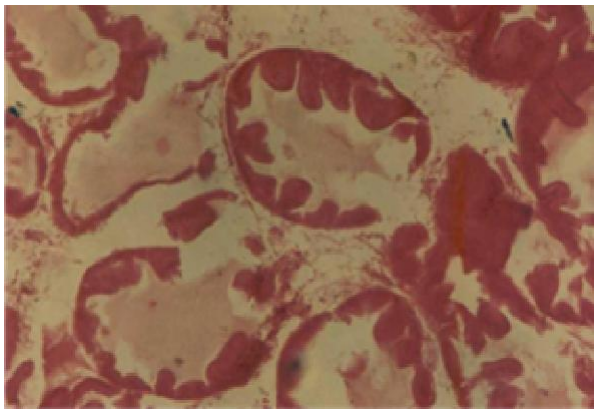

Figure 4. Light micrograph of combined testosterone and oestrogen treated prostate (x-100).

Testosterone treated

There were changes in the histoarchitecture of the gland. The tubules and oval acini were both increased in size, relative to the intact control. The connective tissue stroma was marked (distinct) . The tubules of the gland were distended with secretory material and the epithelium was cuboidal. The oval acini were large, and lined by columnar epithelium with infolding into the lumen (Figure 3).

Combined testosterone and ethinylestradiol - treated

There was a disruption of the histoarchitecture of the prostate tissue, the amount of connective tissue was well marked, and the sizes of the oval acini were increased. The epithelium of the oval acini were tall columnar, with infolding into the acinar lumen. The height of the epithelial lining was irregular in the tubules. These had the highest amount of connective tissue and fewer numbers of oval acini and tubules (Figure 4).

DISCUSSION

The experimental condition of the present study was chosen to take advantage of the well-known restorative action of testosterone on prostate tissue with castration atrophy. Testosterone (T) treatment was initiated 4-weeks after castration when the prostate atrophy was complete (Hutttunen et al., 1981). In this way we could minimize the effect of the early reaction to castration. The high dose of testosterone propionate used in this study (30 mg/kg B.W.) induced rapid growth of the prostate. By selecting this dose we wanted to exert strong androgen action on the prostate with substantial changes on tissue compartments. Some previous investigators have used smaller doses of T (2.5 – 5 mg /Kg B.W/ s.c /day) (Yamanaka et al., 1975; Touhimaa et al., 1973), and others have employed larger doses 20 mg/Kg B.W/ s.c) (De-Klerk and Coffey 1978), and even as high as 40 mg/Kg B.W/ s.c (Brandes, 1974). A high dose of 30 mg/Kg B.W used in this study induced striking changes in the tissue compartment that could be easily assessed with stereologic morphometric method.

Prostatic atrophy results in decrease weight of the prostate of the castrated rats, due to the withdrawal of testicular testosterone (Mariotti et al., 1981). The weight loss may be attributed to the degradation of basement membrane and interstitial tissues and absence of cell proliferation due to depletion of androgens in castrated animals (Srinivasan et al., 1986).

Administration of androgens T,T and E (low dose) induced increases in the prostate and seminal vesicle weights in the castrated rats that may be due to cell proliferation and stromal growth. The stroma provides the infrastructure for the development of epithelial cells. The results reflected the anabolic effects of androgens reported earlier by Eero et al. (1982).

Complex interactions between androgen and estrogen regulate prostatic growth, development and physiology (Garcia-florez et al., 2005). In the present investigation we studied the late effect of T alone, E alone at low dose (2 µg/kg B.W), T and E combined on the prostate of castrated rats.

Estrogen in the present study could not restore prostatic atrophy in the E-alone, this is because it was given when the prostate have fully regressed, this is in agreement with the work of Weinzua et al. (2002), who reported that E has a general anti-androgen effect and negatively regulate the hypothalamus-hypophysis-testis axis and causing involution of the prostatic epithelium and growth of the stroma in adult rats.

E at low dose and given along with T produced a synergistic effect which is observable in the histological appearance of cells in T and E treated rat, where the acini diameter and the epithelial cell height are higher than the T alone or intact control rat. The hypertrophy of the epithelial cell of T and E treated suggests that addition of E at low dose potentiates the action of T, causing an increase in the thickness of epithelial cells and can directly stimulate the activity of secretory epithelial cells (Walsh and Wilson, 1976; Leav et al., 1978; Pelletier et al., 2002). However, E when administered to neonates caused monotonic permanent changes in prostate, with a low dose increasing prepuberal growth while a high dose has inhibitory effects (Saal et al., 1997).

This study provides a model for demonstrating the mechanism of prostatic enlargement in the aging prostate. It suggests that there is some level of conversion of T to E2 in the aging prostate by the enzyme aromatase and the estrogen so produced though at low level synergizes the effect of T and its metabolite DHT in the prostate thereby leading to prostatic hypertrophy (Wenderoth et al., 1982; Tsugaya et al., 1996). The solution to this problem is to research on substances which can selectively block estrogen receptors without affecting the androgen receptors in the aging prostate, as this may offer solution to problem of prostatic enlargement and hypertrophy in aging men. Soy-product is a natural substance containing phytoestrogen with promising future in this direction (Harris et al., 2005).

In conclusion, treatment with E-alone could not restore the weight of the atrophied prostate to normal, but treatment with T-alone restored the atrophied prostate to normal size. The combined administration of T and E caused an increase in the prostatic acini, tubules and epithelium size; but the number of the prostatic acini and tubules per unit area were reduced compared to the T-alone treated. The connective tissue accumulation was pronounced in the combines T and E treated prostate rather than multiplication of the prostatic cells (hyperplastic growth), which was evident in T-alone treated prostate.

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