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Acosta, Stefan; Dizeyi, Nishtman; Feinstein, R; Pierzynowski, Stefan; Abrahamsson, Per-Anders

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Long-term testosterone stimulation induces hyperplasia in the guinea-pig prostate

S Acosta[1]*, N Dizeyi[1], R Feinstein[2], S Pierzynowski[3] & P-A Abrahamsson[1]

[1]Department of Urology, University Hospital of Malmö, Malmö, Sweden; [2]Section of Laboratory Animal Pathology, National Veterinary Institute, Uppsala, Sweden; and [3]Department of Animal Physiology, University Hospital of Lund, Lund, Sweden

The relation between supraphysiologic circulating testosterone levels and prostatic diseases is unclear and difficult to study in men. Animal models may be advantageous. Based on a pilot study, testosterone enanate 50 mg ($n = 12$) or 25 mg ($n = 12$) was administered to guinea-pigs intramuscularly every 3 weeks, for either 7 or 14 months. The histopathology of the prostate was described. Epithelial hyperplasia was found in 14/21 animals receiving testosterone and in 7/12 very old animals, but no such changes were found in the sham or castrated animals. Testosterone stimulation seems to induce epithelial hyperplasia, but not cancer, in the guinea-pig prostate.

Introduction

The role of androgens in the initiation of prostatic diseases is controversial. Within physiologic range, there were no differences in serum testosterone in Scandinavian studies between patients with and without prostate cancer,[1,2] but higher testosterone levels have been found in both American black and white men with prostate cancer compared to men without prostate cancer, respectively.[3] In young anabolic steroid abusers, a significant increase in central prostate volume has been found, implicating that testosterone may be involved in the pathogenesis of benign prostatic hyperplasia (BPH).[4] As testosterone supplementation therapy is increasingly being used, there are concerns about long-term side effects and development of symptomatic BPH and prostate cancer.[5]

Studies on the pathogenesis of prostatic hyperplasia and cancer have been difficult, since there is a lack of suitable animal models that can predict the human pattern of biological changes in the prostate and respond to experimental treatments similar to humans. In contrast to rat models, experimental data relating to changes in morphology or histopathology in the guinea-pig prostate are scarce.[6] However, the guinea-pig prostate serves as a model for research on neuroendocrine (NE) cells;[7] a population of epithelial cells that are thought to be involved in normal prostate growth, hyperplasia and cancer development.[8–11] The aim of this experimental study was first to establish a model for testosterone administration, and then to study the effects of long-term maximal testosterone stimulation on the guinea-pig prostate.

Materials and methods

Experimental animals

In total, 76 male albino pubertal guinea-pigs (outbred Dunkin-Hartley strain, 10–12 weeks old, weight 598–755 g) were provided by a local breeder, M&B A/S (PO Box 39, DK-8680 Ry, Denmark). The animals were kept at appropiate environmental requirements: room temperature 17–19°C, relative humidity 40–60%, ventilation changed 15 times per hour, and light/dark cycles of 12/12 h. The animals had free access to water and food supplemented with C-vitamins.

*Correspondence: S Acosta, Department of Urology, University Hospital of Malmö, Malmö, Sweden.
E-mail: stefan.acosta@telia.com

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Pilot study: creating a model for supraphysiologic testosterone levels

Central venous catheter insertion A total of 18 guinea-pigs were anaesthesized with ketamin (Ketaminol® vet 50 mg/ml; Veterinaria AG, Zürich, Switzerland) 40 mg/kg and xylazine (Rompun® vet 20 mg/ml; Bayer, FRG) 5 mg/kg intraperitoneally. Vena jugularis interna dx were exposed after a vertical incision at the right side of the neck. The vein was ligated superiorly and a polyurethane microcatheter (Micro-Renathane®, Braintree Scientific, Braintree, MA, USA) with an inner diameter of 0.64 mm and an outer diameter of 1.0 mm, was inserted through an opening in the vein and put forward until the catheter tip reached vena cava superior or the right atrium. Back-flow was confirmed with a cannula (Mediplast Metal Tip®, 0.7 x 60 mm) connected to a syringe and 1 ml heparin (Heparin Leo® 100 IE/ml; Leo Pharma, Malmö, Sweden) was infused into the catheter leaving it filled. The catheter was then fixed with a ligature. The proximal end of the catheter was then tunneled subcutaneously to the nape of the neck where it exited 1 cm above the skin. Patency was controlled again and the catheter sealed with a plug.

Injections, blood sampling and analysis In total, 12 and six guinea-pigs were given 0.1 ml testosterone enantate (Testoviron®-Depot 250 mg/ml; Schering Nordiska, Järfälla, Sweden) and 0.1 ml sodium chloride 9 mg/ml intramuscularly (i.m.) (0.60 × 25 mm needle) in the thigh, respectively (Testoviron®-Depot 250 mg i.m. is given as substitution therapy every 3 weeks to human males with hypogonadism). While one person held the guinea-pig still, the other first aspirated 1 ml of blood from the catheter and then refilled it with heparin. The blood sample was then flushed through a cannula perforating into Vacutainer®-sealed heparinised 4 ml tubes (Hemogard® SST). Blood was sampled at baseline, and after 1–5, 7, 14 and 21 days. The samples were immediately transported to the Department of Clinical Chemistry, University Hospital of Malmö, Sweden. Serum testosterone was analysed using competetive radioimmunoassay (RIA) (Figure 1). No side effects were observed and these animals were put to death under a narcosis of carbon dioxide.

Experimental design

Based on this pilot study, we decided to administer Testoviron®-Depot 50 mg ($n = 12$), 25 mg ($n = 12$) or sodium chloride ($n = 12$) im every 3 weeks, alternating between the thighs. In addition, 10 guinea-pigs were castrated by bilateral orchidectomy, which was performed through a midline incision in the lower abdomen after administration of anaesthesia with Ketaminol® vet and Rompun® vet intraperitoneally. Two equal subgroups, each consisting of 23 animals, were put to death after 7 and 14 months, respectively. Each animal was weighed and thereafter each prostate was immediately dissected, weighed and fixed in 4% formaldehyde.

In all, 12 guinea-pigs were housed until they died from natural causes to provide information on median (range) life expectancy, body and prostate weight, and sponta-
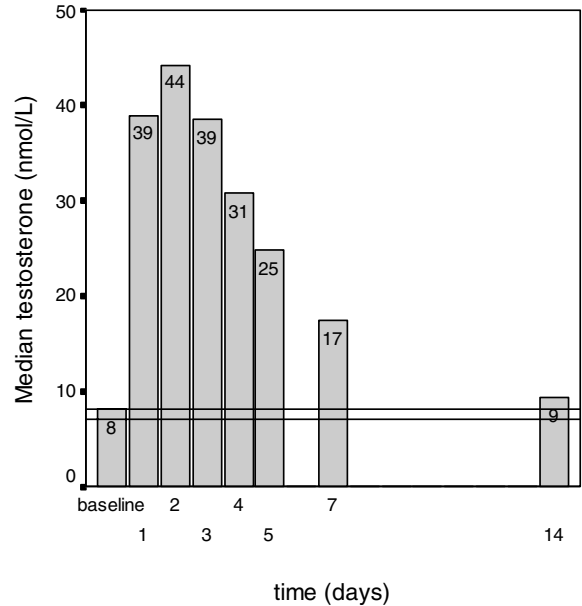

Figure 1 *Median serum testosterone levels in 12 guinea-pigs after injection of testosterone enantate 25 mg i.m., compared to six control animals (median testosterone levels within reference lines). Testosterone levels were increased for 14 days, showing an early peak followed by a moderate elevation.*

neous age-related changes of the prostate, under the same conditions (Table 1).

Our experienced animal keeper observed changes in behaviour or physical appearance in the animals throughout the study.

Diagnostic autopsy Three testosterone-stimulated (50 mg) guinea-pigs died 4–8 months after the start of study. These animals underwent autopsy, including histopathology, of the prostate, urogenital organs and tracts, adrenal gland, bone, heart, liver, lung, lymph nodes, kidney and spleen, at the section of Laboratory Animal Pathology, National Veterinary Institute, Uppsala, Sweden. The main findings in all three animals were severe chronic urinary lesions with partial obstruction of urinary ways with moderate hydronephrosis and ureterolithiasis. Histological examination revealed severe tubular necrosis with extensive deposition of mineral crystals and moderate interstitial fibrosis (nephrosis, nephrocalcinosis). The renal pelvis and urinary tract displayed necrosis of epithelium and rich amounts of mineral casts.

Histology Tissue specimens were fixed overnight in 10%, neutral formaline. After standardised histological processing to paraffin, two parallel sections (4 μm thick) were cut transverse to the urethra, including the large dorsolateral and small ventral lobes, as recommended for screening in toxicological studies by Bahnemahn et al,[12] followed by staining with haematoxylin and eosin.

Immunohistochemistry Serotonin positive cells in sections were visualised using rabbit antibodies to serotonin

Table 1 Influence of testosterone and age on the occurrence of hyperplasia in the guinea-pig prostate

Intervention (n)	Body weight[a] (g) (end of study)	Prostate weight[a] (g)	Squamous metaplasia (share of animals)	Hyperplasia (share of animals)
Testosterone (n = 21)				
50 mg for 14 months (5)	1195 (1056–1323)	2.7 (1.9–3.6)	0/5	3/5
50 mg for 7 months (4)	1145 (783–1230)	2.4 (1.7–3.6)	0/4	3/4
25 mg for 14 months (6)	1160 (989–1618)	2.6 (2.0–3.2)	2/6	4/6
25 mg for 7 months (6)	1016 (673–1075)	2.5 (2.2–2.9)	0/6	4/6
Sham (n = 12)				
14 months (6)	1204 (1087–1341)	1.9 (1.6–2.2)	1/6	0/6
7 months (6)	950 (886–1033)	2.2 (1.6–2.9)	0/6	0/6
Castrated (n = 10)				
14 months (5)	1120 (968–1136)	0.7 (0.6–1.2)	0/5	0/5
7 months (5)	1075 (972–1283)	0.8 (0.7–1.4)	0/5	0/5
No intervention (n = 12)				
44 months (37–48)[b]	950 (766–1186)	2.2 (1.9–2.5)	3/12	7/12

[a]Median (range).
[b]The median (range) life expectancy in guinea-pigs dying from natural causes.

(HT, Dia Sorin, Stillwater, MN, USA) and a DAKO LSAB® Kit, based on the sequential application of a biotinylated link antibody and streptavidin labelled with horseradish peroxidase (HRP). Sections were deparaffinised in xylene, rehydrated through graded alcohol to distilled water and incubated with a 1% solution of hydrogen peroxide for 20 min to inhibit endogenous peroxidase activity. The sections were then incubated with the primary HT-antiserum diluted 1 : 25 000 for 24 h at 4°C. After rinsing, the sections were incubated for 15 min with DAKO LSAB® Biotynilated Link antibody Rabbit/Mouse (DAKO, K1015), rinsed and incubated with ready-to-use DAKO LSAB® Streptavidin/HRP (DAKO, K1016) for 15 min. A 5% solution of 3-amino-9-ethylcarbazole (A5754, Sigma Chemical, St Louis, MO, USA) was used as a chromogen, and 0.05 M Tris-HCl buffer pH 7.6 was used for dilution of reagents and rinsing. In control sections, the primary antibody was substituted by 2% BSA. The specimens were counterstained with haematoxylin for 2 min, rinsed, and mounted in glycerine–gelatin.

For observation and photography, a Nikon Eclipse E600 microscope equipped with a Nikon dxm 1200 digital camera was used. A pathologist (RF), blinded to the performed interventions, evaluated the histopathology findings descriptively.

Ethics

The experimental procedures were approved by the Ethics Committee, Lund University, Sweden.

Results

Behaviour

In testosterone-exposed guinea-pigs, five pairs who were housed in the same cages, had to be separated due to signs of fights and wounds. All testosterone-exposed animals had a more greasy and sprawling fur. The testosterone-exposed animals that died before the end of the study, were ill for several weeks and exhibited the same symptoms with low volume of urine output and stools, and loss of weight.

Histopathology of the prostate

Epithelial hyperplasia of acinar prostatic cells was the most common finding, occurring in the testosterone-exposed animals and in the very old animals (Table 1). The term epithelial hyperplasia denotes here acini lined by crowded, tall columnar epithelium having increased basophilia and forming few or simple papillary projections (Figure 2a,b). Epithelial hyperplasia were found in both the ventral and dorsolateral lobes, and was occasionally multifocal. Unrelated to the presence of epithelial hyperplasia, increased fibrous stroma, rich in collagen, were frequently found in all animals, especially among the oldest. Metaplasia denotes differentiation in squamous epithelium (Figure 2a) and it usually involved a few peripheral acini. In affected areas, the metaplastic epithelium in occasional acini were also hyperplastic. Cancer or changes such as deformation, compression, atrophy or inflammation (attributable to a potential adjacent prostate cancer), was not observed.

Immunohistochemistry (IH) showed low number of serotonin-positive NE cells interspersed within the acinar cells. Serotonin cells appeared singly, most often as basal cells, but most basal cells were not stained by IH. The NE cells often had cytoplasmatic extensions reaching the acinar lumen. The number of serotonin-positive cells/acinus varied from none to about eight, but was unrelated to hyperplasia or metaplasia. Serotonin-positive NE cells appeared to be more common in acini located closer to the urethra.

Discussion

The created model for achieving long-term intermittent supraphysiologic circulating testosterone levels was found to be suitable to test our hypotheses. The present study suggests that high doses of testosterone are able to
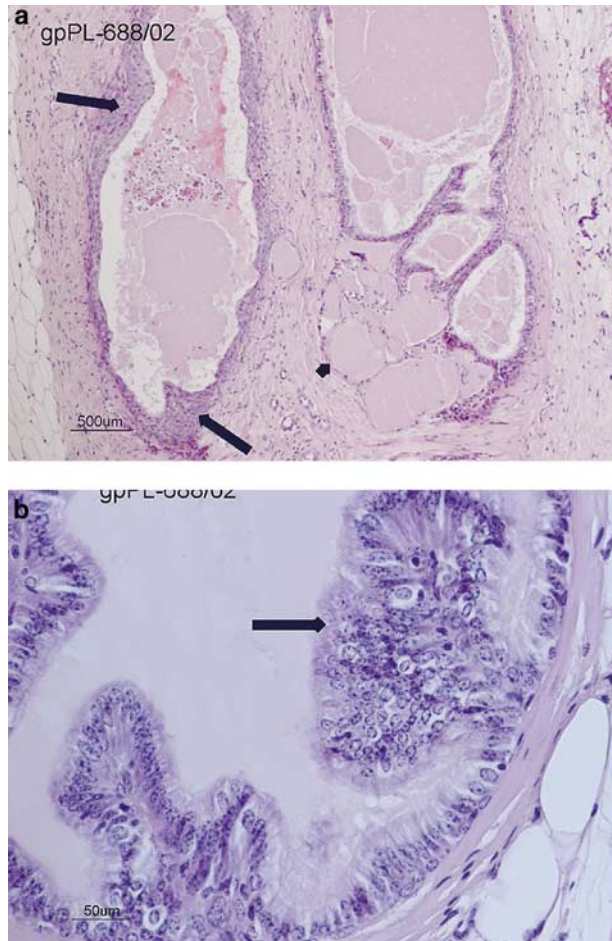
Figure 2 *Prostatic hyperplasia in long-term testosterone-stimulated guinea-pigs. (a) Large acini displaying hyperplasia and squamous metaplasia (arrows). Acini having flattened, degenerate or atrophic epithelium (short arrow). Increased fibrous stroma. (b) Epithelial hyperplasia forming a papillary projection in an acinus (arrow).*

induce hyperplasia in the guinea-pig prostate, but not prostate cancer. Neither doubling of testosterone dose to 50 mg, nor doubling of time increased the occurrence of epithelial hyperplasia. Instead intolerable side effects and deaths occurred in three cases. A shorter administration interval might have increased morbidity and mortality.

Castration of guinea-pigs has been shown to have dramatic effects within weeks, and apart from reducing prostate weight,[7] it also reduces plasma levels of testosterone and dihydrotestosterone (DHT) and prostatic DHT, to undetectable levels.[13] Prostatic DHT is the main local effector of prostate anabolism,[14] and circulating testicular androgen is a prerequisite for prostate formation in guinea-pigs; however, it seems that adrenal androgens may have a role in humans.[13] Moreover, both onset of puberty and DHT administration have been shown to decrease prostatic oestrogen receptor levels in the guinea-pig.[15] Thus, our model involving long-term toxic doses of anabolic testosterone seemed suitable to provoke histological changes in the guinea-pig prostate. The microscopy findings suggested that the pathophysiological process associated with epithelial hyperplasia

were accelerated in relation to the late occurrence of spontaneous epithelial hyperplasia. The risk to underestimate pathological findings in the prostate could probably have been decreased by using a serial cutting technique. Further studies are needed to better define the sufficient testosterone exposure, in terms of dosage and time, to achieve hyperplasia in the guinea-pig prostate.

Apart from epithelial hyperplasia, features of stromal hyperplasia such as increased mass and fibrosis have been demonstrated to be age-related, both in the guinea-pig and the human prostate.[16] Another change in ageing guinea-pigs is the increase in NE cell population and its serotonin content[17] in the prostate. However, the suggested testosterone-induced epithelial hyperplasia in the present study was found unrelated to serotonin-positive NE cells, which may be explained by the lack of androgen receptors in prostatic NE cells.[18]

In conclusion, high doses of testosterone stimulation seems to induce epithelial hyperplasia, but not cancer, in the guinea-pig prostate. This animal model seems suitable for research on hyperplasia of the prostate.

Acknowledgements

References

1 Gustafsson O *et al.* Dihydrotestosterone and testosterone levels in men screened for prostate cancer: a study of a randomized population. *Br J Urol* 1996; **77**: 433–440.

2 Stattin P *et al.* High levels of circulating testosterone are not associated with increased prostate cancer risk: a pooled prospective study. *Int J Cancer* 2004; **108**: 418–424.

3 Mydlo JH *et al.* A pilot study analyzing PSA, serum testosterone, lipid profile, body mass index and race in a small sample of patients with and without carcinoma of the prostate. *Prostate Cancer P D* 2001; **4**: 101–105.

4 Jin B, Turner L, Walters W, Handelsman J. Androgen or estrogen effects on human prostate. *J Clin Endocrinol Metab* 1996; **81**: 4290–4295.

5 Kaufman JM. The effect of androgen supplementation therapy on the prostate. *Aging Male* 2003; **6**: 166–174.

6 Maini A, Archer C, Wang C, Haas G. Comparative pathology of benign prostatic hyperplasia and prostate cancer. *In vivo* 1997; **11**: 293–300.

7 Acosta S *et al.* Neuroendocrine cells and nerves in the prostate of the guinea pig: effects of peripheral denervation and castration. *The Prostate* 2001; **46**: 191–199.

8 Cockett ATK *et al.* Relationship of neuroendocrine cells of prostate and serotonin to benign prostatic hyperplasia. *Urology* 1993; **42**: 513–519.

9 Abrahamsson PA *et al.* Peptide hormone and serotonin immunoreactive cells in normal and hyperplastic prostate glands. *Pathol Res Pract* 1986; **181**: 675–683.

10 Abrahamsson PA, Falkmer S, Grimelius L. The course of neuroendocrine differentiation in prostate carcinomas. An immunohistochemical study testing chromogranin A as an 'endocrine marker'. *Pathol Res Pract* 1989; **185**: 373–380.

11 Di Sant'Agnese PA. Neuroendocrine cells of the prostate and neuroendocrine differentiation in prostatic carcinoma: a review of morphological aspects. *Urology* 1998; **52**: 121–124.

12 Bahnemahn R *et al*. RITA—registry of industrial toxicology animal-data. Guides for organ sampling and trimming procedures in rats. *Exp Toxicol Pathol* 1995; **47**: 247–266.

13 Benoit B *et al*. Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men. *J Steroid Biochem* 1989; **32**: 695–698.

14 Carson C, Rittmaster R. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology* 2003; **61**: 2–7.

15 Tilley WD *et al*. Effects of ageing and hormonal manipulations on the level of oestrogen receptors in the guinea-pig prostate. *J Endocrinol* 1987; **112**: 139–144.

16 Horsfall D *et al*. Age-related changes in guinea pig prostatic stroma. *Lab Invest* 1994; **70**: 753–763.

17 Di Sant'Agnese, Davis N, Chen M, Jensen K. Age-related changes in the neuroendocrine cell population and the serotonin content of the guinea pig prostate. *Lab Invest* 1987; **57**: 729–736.

18 Bonkhoff H. Neuroendocrine cells in benign and malignant prostate tissue: morphogenesis, proliferation, and androgen receptor status. *Prostate* 1998; **8** (Suppl): 18–22.