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Site-specific Effects of Testosterone Propionate on the Prostate of Rat Pretreated with 3,2′-Dimethyl-4-aminobiphenyl: Dose-dependent Induction of Invasive Carcinomas

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It has been shown that testosterone propionate (TP) strongly promotes induction of invasive carcinomas in previously initiated accessory sex organs. In this study, in order to clarify the dose-dependence of this promotion, TP was given at 3 different levels (high, medium or low doses) using different sizes (2, 1 and 0.5 cm long) of Silastic tube for 40 weeks after administration of 3,2′-dimethyl-4-aminobiphenyl to male F344 rats. The data showed development of invasive carcinomas in the dorso-lateral and anterior prostate and in the seminal vesicle to be dose-dependent with the high dose of TP being most effective for tumor induction. Average levels of serum testosterone were approximately 800, 600, 300 and 150 ng/dl in rats given the high to low doses and in control rats, respectively. Development of neoplastic lesions in the ventral prostate demonstrated an inverse dependence on the dose of TP. These findings, together with previous data, suggest that the tumor-promoting potential of TP on rat prostate is unlikely to be simply due to its androgenic action and other factors should also be considered.

Key words: Prostate carcinogenesis — Rat — Testosterone — Dose dependency

It is well known that continuous exposure to pharmacological doses of testosterone propionate (TP) or testosterone induces prostate carcinomas when given after carcinogen treatment.[1–4] These tumors are characterized by invasive growth and the potential for metastasis. Our recent study demonstrated that longer periods of administration are more effective for induction of carcinomas.[5] Serum levels of testosterone in our experiments were 10-fold higher than the normal value. It is likely that for induction of invasive carcinomas of the prostate, high doses and extended administration periods of TP are most effective, if not essential. However, a recent experiment by Bosland *et al.*[6] suggested that even low doses of TP, elevating circulating testosterone to only 2- or 3-fold the normal male levels, can cause invasive prostate carcinoma development in rats pretreated with N-methylnitrosourea. They suggested the involvement of enhancing effects through TP-mediated receptor action. Here we present findings indicating that TP at pharmacological doses is required for induction of invasive carcinomas in rats pretreated with 3,2′-dimethyl-4-aminobiphenyl (DMAB).

MATERIALS AND METHODS

A total of 100 male F344 rats (purchased from Charles River Japan, Inc., Kanagawa), 6 weeks old and weighing approximately 123 g at the beginning of the experiments, was used. They were housed in plastic cages with hard wood chips in an air-conditioned room with a 12 h–12 h light-dark cycle and given food (Oriental MF; Oriental Yeast Co., Ltd., Tokyo) and water *ad libitum*. TP was purchased from Sigma Chemical Co., St. Louis, MO, USA and DMAB (>98% purity) was obtained from NARDO Institute, Amagasaki. The animals were divided into 4 groups of 25 rats each. All animals were given DMAB subcutaneously at a dose of 50 mg/kg body weight 10 times at 2-week intervals. TP-containing Silastic tubes were then implanted into the subcutis of the interscapular region of animals in groups 2–4 until the end of the experiment at week 60. The TP-implants were replaced at 6-week intervals. In order to change the release of testosterone from the tube, three sizes of tube were prepared; 0.5, 1.0 and 2.0-cm long, all with a 0.2 cm inner diameter and 0.3 cm outer diameter, sealed at both ends with silastic medical grade adhesive (Dow Corning Co., MI, USA). The amounts of TP in the tubes were 10, 20 and 40 mg per tube, respectively. Group 1 served as the carcinogen control given only DMAB.

All surviving rats were killed at experimental week 60 and subjected to complete autopsy. Animals that died earlier or were killed upon becoming moribund were also autopsied. All organs were examined for gross abnormalities and fixed in 10% buffered formalin. For tissue preparation of the accessory sex organs, two sagittal slices of the ventral prostate, sagittal samples of the dorsolateral

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prostate, including the urethra, and transverse samples from each side of the seminal vesicles including the anterior prostate (coagulating glands) were embedded in paraffin. Single sections (4 μm) through all tissues were cut and stained with hematoxylin and eosin for histological examination.

An additional 20 rats, divided into 4 groups, were given 3 different sizes of Silastic tube containing TP without DMAB treatment, starting at the same age as in the main experiment. About 5 ml of blood per rat was collected from the orbital sinus 3, 20 and 40 weeks after the beginning of the treatment and the serum levels of testosterone were measured by radioimmunoassay.

Differences in data for body and organ weights and serum levels of testosterone were analyzed by means of Student's t test. Incidences of tumors and other histopathological lesions were analyzed by the Fisher exact probability test (two-tailed). The Cochran-Armitage analysis was also applied to assess the presence of dose-response relation for TP.

RESULTS

The additional experiment showed that implantation of Silastic tubes containing TP was associated with the size-dependent elevation of serum testosterone concentration over the control values at all 4 time points examined; the average levels in the high, medium and low dose groups were about 6, 4 and 2 times the control values, respectively (Fig. 1).

The administration of TP suppressed body weight gain by about 20% at the high and medium doses and about 12% at the low dose. In contrast, the weights of the ventral prostate and seminal vesicles were significantly

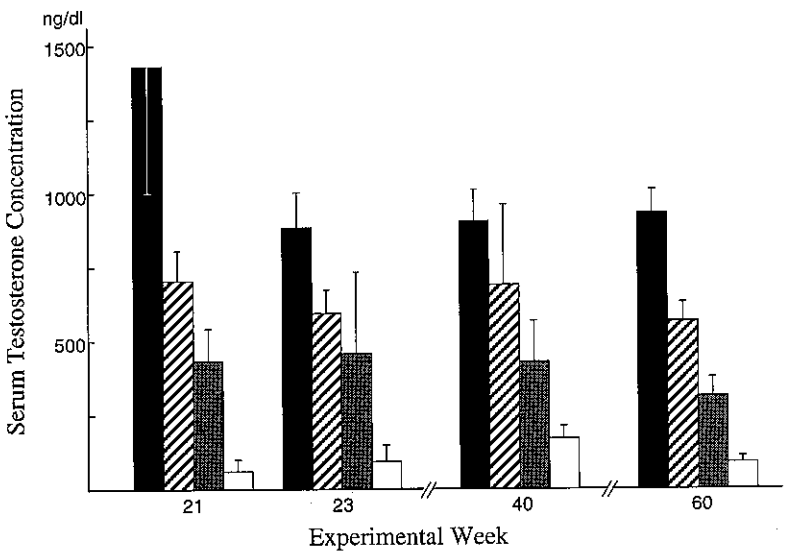

Fig. 1. Sequential changes in serum testosterone levels (ng/dl), measured at weeks 21, 23, 40 and 60. ■, TP in a 2 cm long Silastic tube; ▨, TP in a 1 cm long Silastic tube; ▩, TP in a 0.5 cm long Silastic tube; □, no TP control. Data are the mean values for 5 rats and bars indicate the SD.

Table I. Average Final Body and Organ Weights for Rats Given DMAB and TP

Group	Treatment	No. of rats[a)]	Body weight[b)]	Organ weights[c)]		
				Ventral prostate	Seminal vesicles	Testis
1	DMAB→none	17	429±41	0.45±0.10	1.17±0.29	3.46±0.48
2	DMAB→TP(L)	7	377±22[d)]	0.73±0.13[e)]	2.05±0.29[e)]	1.69±0.18[e)]
3	DMAB→TP(M)	18	343±22[e)]	0.99±0.12[e)]	3.24±0.48[e)]	2.15±0.11[e)]
4	DMAB→TP(H)	9	346±45[e)]	1.17±0.24[e)]	3.59±0.43[e)]	2.19±0.17[e)]

a) Numbers of rats include all animals which survived for 60 weeks.
b) Weights are mean(g)±SD values.
c) Weights represent mean relative weights (% of body weight). The weights of the seminal vesicles include those of the anterior prostate.
Significantly different from the group 1 values: *d*) $P<0.01$ and *e*) $P<0.001$.

Table II. Incidences (%) of Atypical Hyperplasias and Carcinomas in the Prostate and Seminal Vesicles of Rats Given DMAB and TP

Treatment	Effective No. of rats	Prostate[a]						Seminal vesicles	
		Ventral		Lateral		Anterior			
		AH[b]	CA[c]	AH[b]	CA	AH	CA[b]	AH	CA[c]
1 DMAB→none	24	20 (83.3)	8 (33.3)	0	0	8 (33.3)	0	19 (79.2)	0
2 DMAB→TP(L)	24	20 (83.3)	0[e]	1 (4.2)	1 (4.2)	5 (20.8)	0	17 (70.8)	2 (8.3)
3 DMAB→TP(M)	24	17 (70.8)	0[e]	3 (12.5)	1 (4.2)	6 (25.0)	2 (8.3)	18 (75.0)	9 (37.5)[e]
4 DMAB→TP(H)	23	12 (52.2)[d]	0[e]	4 (17.4)[d]	1 (4.3)	4 (17.4)	4 (17.4)[d]	16 (69.6)	10 (43.5)[f]

a) AH, atypical hyperplasia; CA, carcinoma.
b) Dose-related response at $P<0.05$ by the Cochran-Armitage analysis.
c) Dose-related response at $P<0.001$ by the Cochran-Armitage analysis.
d) Significantly different from the value of Group 1 at $P<0.05$.
e) Significantly different from the value of Group 1 at $P<0.01$.
f) Significantly different from the value of Group 1 at $P<0.001$.

increased in a TP-dose dependent manner (Table I). Histopathologically, prostate and seminal vesicles of rats receiving TP particularly at the higher 2 doses showed dilatation of acini filled with fluid, this findings reflecting the increases of the organ weights, and the epithelial lining cells of the acini were less atrophic compared to those of rats not receiving TP.

Development of atypical hyperplasias and/or carcinomas in the prostate and seminal vesicles was influenced by administration of TP in a dose-related fashion (Table II): those of the ventral prostate were decreased while atypical hyperplasias of the dorso-lateral prostate, carcinomas of the anterior prostate and carcinomas of the seminal vesicles were increased. Non-invasive intra-acinar carcinomas of the ventral prostate disappeared with administration of TP at any dose. Development of invasive carcinomas in the lateral and anterior prostate and seminal vesicles was most pronounced with the high dose.

No influence of TP on tumor development was noted in organs other than the prostate and seminal vesicles.

DISCUSSION

It is generally thought that androgenic stimuli play a critical role in the causation and development of prostate cancer in man. A comparative study revealed variation in serum testosterone levels in different young populations, African-American and Caucasian-American, in the USA, with the former group being shown to have a higher prevalence of prostate cancer as well as higher serum testosterone levels (3.75 pg/ml for Caucasian-American and 3.82 pg/ml for African-American).[7] In several animal experimental systems, administration of testosterone or testosterone propionate for a prolonged period of time after carcinogen treatment results in the development of invasive adenocarcinomas of the prostate.[1-4] In these experiments, with one exception, testosterone was administered in one or two Silastic tubes with a length of 2 cm. Serum testosterone levels rose to approximately 4 to 20 times the normal physiological values.

Bosland *et al.*[6] stated that development of invasive carcinomas arising from dorso-lateral prostate of rats given testosterone was probably due to receptor-mediated action, because even a 2- to 4-fold elevation of testosterone in the serum over the normal control levels exerted a significant effect on induction of carcinomas. In contrast, the present data indicated that a 6 times higher level of testosterone was required to effectively induce invasive carcinomas in rats pretreated with DMAB. This hormone level is very much higher than that under physiological conditions. Furthermore, no obvious increase in cell proliferation in the epithelial cells of the accessory sex glands was noted after administration of a pharmacological dose of testosterone propionate.[4] Therefore, these findings and the site dependence suggest that the mechanism of tumor-promoting potential of testosterone propionate on the rat prostate is unlikely to involve simply its androgenic action; the involvement of other factor(s) seems probable.

In humans, estrogen formed from testosterone by aromatase is presumed to play an important role in prostate carcinogenesis. Experiments to clarify the possible role of estrogen in prostate carcinogenesis have also been conducted. Dihydrotestosterone (DHT), which is an active form of androgen, but can not be converted to estrogen, failed to induce or to promote the development of prostate carcinomas when administered to testosterone-responsive[8] or carcinogen-pretreated rats.[9] In the latter case,[9] DHT induced atrophy of the prostate, probably due to feed-back decrease in serum levels of testosterone,

indicating that the experimental design was inappropriate and a much higher dose of DHT should have been applied. External hormonal manipulation evokes very complicated reactions *in vivo* because of homeostatic control. Nevertheless, co-administration of estrogen with TP induced invasive carcinomas in the lateral prostate, particularly in the central area, and non-invasive carcinomas in the dorsal prostate,[10] with such estrogenic action being effective only at pharmacologically high dose.[11] These findings suggest that it is at least possible that aromatase-mediated estrogen production plays an important role in induction of invasive carcinomas by high doses of testosterone. Recent studies on estrogen-related carcinogenesis suggest that reactive oxygen species and lipid peroxidation, which arise during metabolization of estrogen, contribute at least in part to its actions. Furthermore, non-infectious inflammation is commonly seen bilaterally in the lateral prostate in rats receiving TP for a long period of time at high doses (T. Shirai *et al.*, unpublished data). Therefore, the involvement of factors, including oxygen radicals, rather than direct hormonal stimulation via receptors should be clarified in further studies of tumor promotion by testosterone propionate.

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