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Direct Effects of Testosterone, Dihydrotestosterone and Estrogen on 3,2′-Dimethyl-4-aminobiphenyl-induced Prostate Carcinogenesis in Castrated F344 Rats

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The present experiment was carried out to explore the effect of endogenous androgen on rat prostate carcinogenesis induced by 3,2′-dimethyl-4-aminobiphenyl (DMAB) and testosterone propionate (TP) or 5α-dihydrotestosterone (DHT) with or without ethinyl estradiol (EE). In order to eliminate the influence of endogenous androgen, F344 rats were orchiectomized just after initiation with the prostate carcinogen, DMAB, and then given TP, DHT, TP plus EE or DHT plus EE for 40 weeks. The results demonstrated that while administration of TP following DMAB treatment causes invasive carcinomas in the lateral and anterior prostate and seminal vesicles, DHT does not exhibit equivalent effects. Synergistic enhancement was also evident with TP plus EE, but not with DHT plus EE. The incidences of prostatic and seminal vesicle lesions in all groups of the present experiment, except for the group given castration without hormonal supplement, were equivalent to those previously found in non-castrated animals. Therefore, the present findings indicate that endogenous testosterone may not be required for promotion by TP/EE of DMAB-initiated prostate carcinogenesis and that it may not contribute to the actions of DHT.

Key words: Prostate carcinogenesis — Androgen — Ethinyl estradiol — 3,2′-Dimethyl-4-aminobiphenyl — Castration

Epidemiological studies of castrates strongly support a key role for the testis in the pathogenesis of prostate cancer. In rodents, orchiectomy or administration of estrogen also inhibits development of chemically induced prostate carcinomas. Thus, at least physiological levels of circulating androgens appear to be required for prostate neoplasia. In fact, pharmacological doses of testosterone have been demonstrated to increase the numbers of naturally occurring or chemically induced prostate carcinomas in rats;[1–5] with estrogen being found to enhance this testosterone action on spontaneous prostate carcinogenesis in Noble rats.[1–3] In contrast, 5α-dihydrotestosterone (DHT) was without influence.[6] In our recent experiment, combined administration of testosterone propionate (TP) and ethinyl estradiol (EE) resulted in a high incidence of lateral and anterior invasive carcinomas and a decrease of seminal vesicle carcinomas.[7] A similar combined effect of testosterone and estrogen was reported by Bosland *et al.*[8] However, in our experiment, a combination of DHT and EE did not exert such carcinogenic effects.[7] Thus, we can conclude that exogenous sex hormones may exert their effects by complex modulation of endogenous hormonal conditions. DHT decreases serum endogenous testosterone levels by a homeostatic mechanism[7] and EE suppresses release of testosterone from the testis by acting on the hypothalamus-pituitary-gonadal axis. In the present experiment, in order to explore direct actions of TP, DHT and EE, the role of endogenous testosterone was eliminated. For this purpose, castrated male rats were treated with TP, DHT, TP plus EE or TP plus EE after 3,2′-dimethyl-4-aminobiphenyl (DMAB)-initiation[9–11] and the effects on prostate carcinogenesis were ascertained.

MATERIALS AND METHODS

A total of 100 male F344 rats (purchased from Charles River Japan. Inc., Kanagawa), 6 weeks old and weighing approximately 120 g at the beginning of the experiments, 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*. DMAB was obtained from the NARD Institute, Osaka. Its purity was more than 98%. TP was purchased from Tokyo Kasei, Chemical Co., Tokyo. DHT and EE were from Sigma Chemical Co., St. Louis, MO.

Animal experimentation The animals were divided into 5 groups. Rats in all groups were given DMAB subcutaneously at a dose of 50 mg/kg b.w. 10 times at 2-week intervals and then subjected to orchiectomy. Under ether anesthesia, both testes were removed through incisions in the scrotal sacs. After this surgical castration at week 20, groups 1 to 5 received exogenous hormonal

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administration for 40 week: TP for group 1; DHT for group 2; TP plus EE for group 3, DHT plus EE for group 4; and no treatment for group 5. The hormone-containing Silastic tubes (Dow Corning Co., MI) were prepared as previously described[7] and implanted into the subcutis of the interscapular region under anesthesia with ethyl ether.[12] The hormone-filled implants were replaced at 6-week intervals. All surviving rats were killed at experimental week 60 and subjected to complete autopsy. Animals that died earlier or that were killed on becoming moribund were also autopsied. The protocol of the present experiment was exactly the same as that used in our earlier work[7] except for orchiectomy at experimental week 20 and the lack of an EE-alone group. All organs were examined for gross abnormalities and fixed in 10% buffered formalin. For tissue preparation of the accessory sex organs, two sagittal slices through the ventral prostate, 3 sagittal samples of the dorsolateral prostate, including the urethra, and 4 transverse samples from each side of the seminal vesicle 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.

Statistics Differences in body and organ weights and serum levels of hormones were analyzed by means of Student's t test. Incidences of tumors and other histopathological lesions were analyzed by use of Fisher's exact probability test (two tailed).

RESULTS

Animal growth EE markedly suppressed body weight gain regardless of whether it was combined with an androgen, TP or DHT. TP also markedly retarded animal growth but DHT did not show any suppression (Fig. 1). EE significantly increased pituitary weight, as found in previous experiments.[12] The weights of the prostate and seminal vesicles of castrated rats were significantly increased by TP, but weight restoration by DHT was not complete. For reference, our previous data on prostate and seminal vesicle weights of rats that received the same treatments but without orchiectomy[7] are also shown in Fig. 2. The weights of the prostate and seminal vesicles of orchiectomized rats receiving TP or TP plus EE in the present experiment were very similar to those of non-orchiectomized rats (Fig. 2), suggesting that restoration of the accessory sex organs by exogenous hormone was not affected by the presence of testicular androgen.

Incidences of prostate and seminal vesicle lesions All parts of the accessory sex organs of rats in group 5

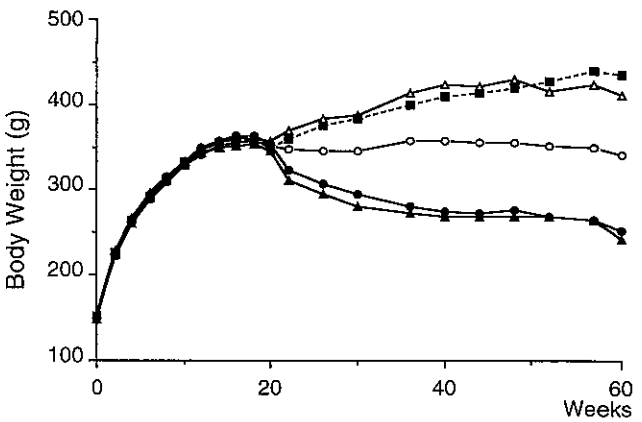

Fig. 1. Growth curves of rats given DMAB and TP, DHT and/or EE.
○, TP; △, DHT; ●, TP + EE; ▲, DHT + EE; ■, control.

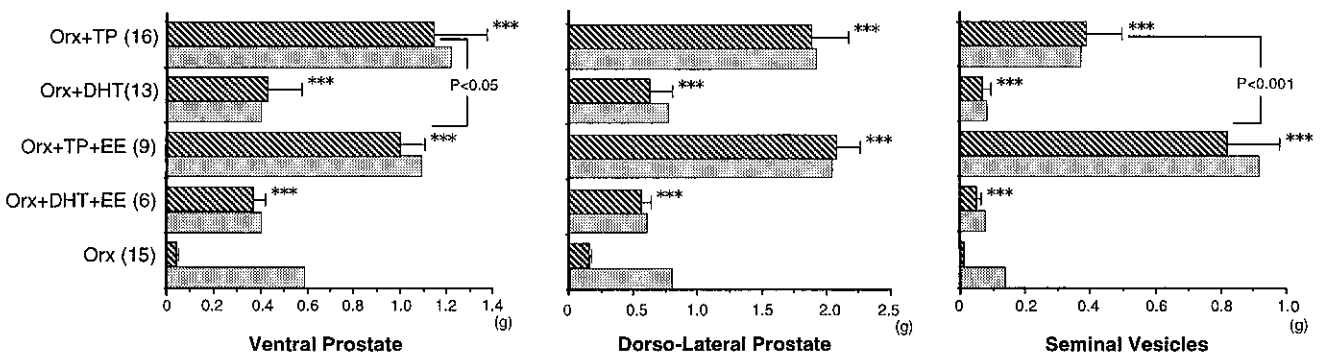

Fig. 2. Weights of prostate and seminal vesicles in rats given sex hormone(s) following DMAB administration and then castrated. (means ± SD). The narrow stippled bars represent data from our previous paper (ref. 7) for comparison. Orx, orchiectomy; TP, testosterone propionate; DHT, 5 α-dihydrotestosterone; EE, ethinyl estradiol.
*** Significantly different from the value of the orchiectomized rats without external hormonal treatment at $P<0.001$.

Table I. Tumor Incidences (%) in the Prostate and Seminal Vesicles

Treatment	No. of rats	Prostate								Seminal vesicle		Other tumors
		Ventral		Lateral		Dorsal		Anterior				
		AH	Ca	AH	Ca	AH	Ca	AH	Ca	AH	Ca	
Orx+TP	18	6 (33)	1 (6)	5 (28)	3 (17)	3 (17)	1 (6)	12 (67)	8 (44)	15 (83)	8 (44)	0
Orx+DHT	16	10 (63)	1 (6)	2 (19)	0	1 (6)	0	2 (13)	0	12 (75)	0	0
Orx+TP+EE	13	12 (92)*	0	2 (15)	7 (54)	8 (62)	0	7 (54)	9 (69)	7 (54)	1 (8)	3[a]
Orx+DHT+EE	13	10 (77)	1 (8)	1 (8)	0	2 (15)	0	4 (31)	0	7 (54)	0	1[b]
Orx	20	0	0	0	0	0	0	0	0	0	0	0

AH, atypical hyperplasia; Ca, carcinoma; Orx, orchiectomy; TP, testosterone propionate; DHT, 5α-dihydrotestosterone; EE, ethinyl estradiol.
a) Sarcoma, mesothelioma and schwannoma. *b*) Sarcoma.
* $P<0.01$ vs. the Orx+TP group.

Table II. Tumor Incidences (%) in Other Organs

Treatment	No. of rats	Small intestine[a]	Large intestine[a]	Lung[b]	Liver[c]	Preputial gland[a]	Subcutaneous[a]	Pituitary[d]
Orx+TP	18	2 (11)	2 (11)	5 (28)	8 (44)*	3 (17)	4 (22)	0
Orx+DHT	16	2 (13)	2 (13)	7 (44)	4 (25)	0	1 (6)	2 (13)
Orx+TP+EE	13	0	0	1 (8)	12 (92)***	0	1 (8)	10 (77)***
Orx+DHT+EE	13	1 (8)	0	3 (23)	11 (85)***	1 (8)	3 (23)	11 (85)***
Orx	20	1 (5)	0	8 (40)	2 (10)	2 (10)	7 (35)	0

Orx, orchiectomy; TP, testrosterone propionate; DHT, 5α-dihydrotestosterone; EE, ethinyl estradiol.
a) Adenomas/carcinomas. *b*) Hyperplasia/adenoma. *c*) Foci/nodules. *d*) Adenomas.
* $P<0.05$, *** $P<0.001$ vs. orchiectomy alone.

undergoing orchiectomy without hormone supplement demonstrated involution and no proliferative lesions could be detected in the prostates and seminal vesicles of these animals. Carcinomas of the ventral prostate in the other group were intraductal or intraacinar, forming cribriform patterns, and their yields were low, being 0 to 8% (Table I). The incidence of atypical hyperplasia in this lobe in rats given TP was significantly lower than that for rats given TP plus EE. Carcinomas of the lateral and anterior prostate and seminal vesicles, characterized by invasive growth, were only present in groups given both DMAB and TP, the respective values for animals given DMAB and TP being 17 and 44%, while addition of EE lifted the yields to 54 and 69%, respectively. Lateral carcinomas developing in groups given DMAB and then TP plus EE were mainly located in the central area near the ducts and ureter. The dorsal lobe of the prostate was considered separate from the lateral prostate, because the rats receiving TP and EE after DMAB administration developed dorsal atypical hyperplasia at an incidence of 62%. However, in contrast to previous work, this was not associated with carcinoma development, only one dorsal carcinoma being found in a rat given DMAB and TP. This was an *in situ* lesion growing inside acini, like those observed in the ventral prostate. Seminal vesicle carcinomas were found in 44% of rats given DMAB and TP but in only 8% of animals given DMAB and TP plus EE. Administration of DHT resulted in development of atypical hyperplasia in all regions, but only one carcinoma, in the ventral prostate. Addition of EE did not alter the influence of DHT.

Tumors in other organs Tumor development was also observed in organs other than the accessory sex organs (Table II). Pituitary gland tumors and liver cell tumors were frequently observed in animals given EE with TP or DHT. The incidences in the TP + EE and DHT + EE groups were 77 and 85%, and 92 and 85% respectively, those values being statistically significantly higher than those without EE treatment. In contrast, lung tumor development was clearly suppressed by administration of EE. The other types of tumors observed did not appear to be influenced by sex hormone administration.

DISCUSSION

The present experiment was performed to investigate the role of endogenous testosterone in the development of non-invasive and/or invasive carcinomas of the

prostate and seminal vesicles of rats given DMAB, TP, DHT and/or EE. In our previous studies, even though DHT administration produced marked testicular atrophy and suppressed the levels of circulating androgen in non-castrated rats, a low level of plasma androgen still existed.[7] Therefore, in order to ascertain the effects of exogenous hormones, endogenous androgen was eliminated by castration in the present study.

In comparison with the data in our previous experiment using non-castrated rats,[7] the incidences of atypical hyperplasias and carcinomas of the prostate and seminal vesicles in the present study were essentially the same, except for the case of animals given DMAB and then castrated, where no proliferative lesions were observed. Thus, under the present experimental conditions, endogenous testosterone was revealed to be essential for the development of prostatic proliferative lesions initiated by DMAB, but not for the lesions induced by the combined treatment with DMAB, TP, DHT and/or EE.

Since administration of DHT to normal rats results in slight atrophy of the prostate complex[7] and suppression of plasma testosterone levels, this might be the reason for its lack of enhancing effects. In this case, the levels of DHT in the blood were twice those for TP,[7] and therefore it is likely that, contrary to the *in vitro* case,[13] DHT is not effectively utilized by the prostate epithelial cells *in vivo*. It is concluded that failure of DHT promotion or cooperative action with EE is probably simply due to insufficient androgen levels in the target cells.

Estrogen has been reported to cooperate with androgen to enhance prostate growth in dogs[14] and this action has been postulated to be due to an increase in the androgen content in prostatic cells.[15] It was also shown that estrogen increases androgen receptor content in human prostate cancers.[16] In the present study, a comparison of the actions of TP and DHT was included to assess possible involvement of estrogen which could be derived from testosterone by the action of 5α-reductase. However, since DHT did not exert a sufficient androgenic function in the prostate complex, as mentioned above, we could not evaluate the possible role of TP-derived estrogen under the present conditions. In another experiment, tamoxifen, an antiestrogen was administered together with a pharmacological and promoting dose of TP in order to explore the possibility of estrogen involvement. The data showed that estrogen derived from testosterone by the action of aromatase is not involved in the strong promotion by TP of DMAB prostate carcinogenesis.[17]

The reason why estrogen simultaneously administered with TP enhanced the induction of invasive carcinomas in the anterior and lateral prostate remains to be clarified.

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