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Effects of Tamoxifen, an Antiestrogen, on Rat Prostate Carcinogenesis by 3,2′-Dimethyl-4-Aminobiphenyl and Testosterone do not Support an Estrogen Role in Testosterone Promotion

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BACKGROUND. Our previous data suggest that estrogen plays an important role in rat prostate carcinogenesis, particularly in promotion by testosterone. Therefore, in the present experiment, effects of an antiestrogen, tamoxifen (TAM), were investigated.
METHODS. Male F344 rats initially received 3,2′-dimethyl-4-aminobiphenyl (DMAB) at 50 mg/kg bw every 2 weeks for 20 weeks and then TAM in Silastic tubes was subcutaneously given alone or together with testosterone propionate (TP) for 40 weeks.
RESULTS. TAM significantly suppressed prostate weights, suggesting an estrogenic action, but the development of preneoplastic and/or neoplastic lesions of the prostate or seminal vesicles in rats given DMAB alone or DMAB and TP was not altered. TAM reversed the suppression of development of ventral atypical hyperplasias by TP.
CONCLUSIONS. These findings suggest that estrogen, which is derived from testosterone by the action of aromatase, is not involved in the strong promotion by TP of DMAB prostate carcinogenesis. *Prostate 31:9–13, 1997.*

KEY WORDS: **tamoxifen; prostate carcinogenesis; rat; testosterone**

INTRODUCTION

In the rat, it has been clearly demonstrated that exogenous testosterone at a pharmacological dose can strongly enhance the induction of invasive carcinomas of the prostate and seminal vesicles [1–6]. Furthermore, estrogen given together with testosterone results in greater induction of prostate carcinomas [6,7]. These findings indicate that estrogen also plays an important role in prostate carcinoma development, and that the enhancing influence of testosterone itself might partly be due to conversion to estrogen by aromatase.

Tamoxifen (TAM), which acts as an antiestrogen by competitive binding to the estrogen receptor, has been shown to inhibit experimental mammary carcinogenesis [8] and is currently widely used in endocrine therapy for breast cancer [9]. In the present experiment, as one of a series of studies aimed at elucidation of the mechanism of testosterone-promotion potential of rat prostate carcinogenesis, TAM was given together with a pharmacological and promoting dose of testosterone in order to explore the possibility of estrogen involvement in this action. The 3,2′-dimethyl-4-aminobiphenyl (DMAB)-prostate cancer model developed in our laboratory was used for this purpose [5,6].

Abbreviations: DMAB, 3,2′-dimethyl-4-aminobiphenyl; TP, testosterone propionate; TAM, tamoxifen.

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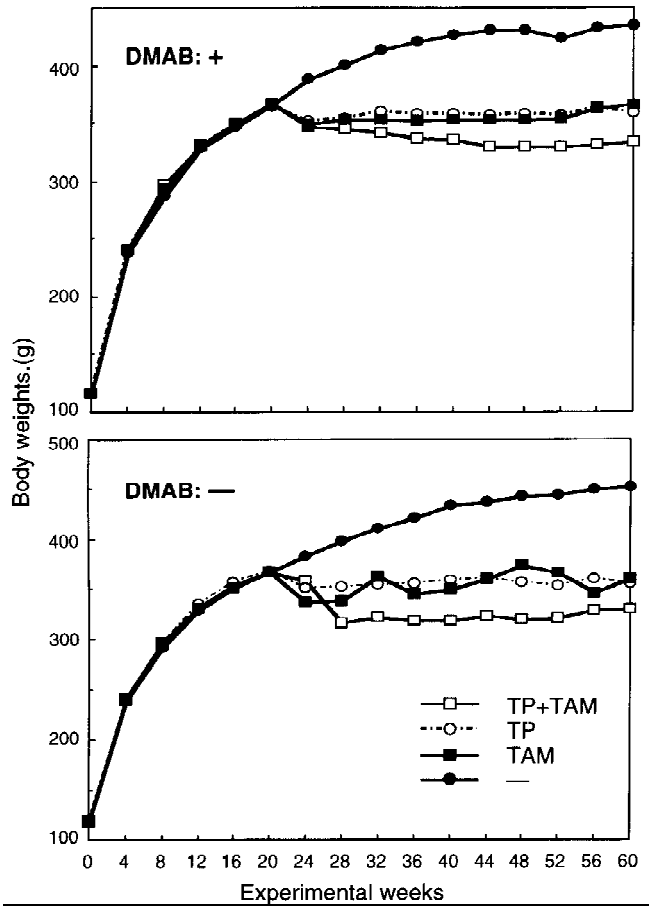

Fig. 1. Growth curve of rats given DMAB with/without TP and/or TAM.

MATERIALS AND METHODS

A total of 80 male F344 rats (purchased from Charles River Japan, Inc., Kanagawa, Japan), 6 weeks old and weighing approximately 120 g at the beginning of the experiment, were housed in plastic cages on hard wood chips in an air-conditioned room with a 12 hr-12 hr light-dark cycle and given food (Oriental MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. DMAB was obtained from Matsugaki Pharmaceutical Co., Osaka, Japan. Its purity was more than 98%. Testosterone propionate (TP) was purchased from Tokyo Kasei, Chemical Co., Tokyo, Japan. TAM was from Aldrich Chemical Co., Milwaukee, WI.

Animal Experimentation

The animals were divided into eight groups; four groups of 20 rats each and another four groups of 15 each. Rats in groups 1–4 were given DMAB subcutaneously at a dose of 50 mg/kg bw 10 times at 2-week intervals and then from week 20. Groups 1–3 underwent exogenous hormonal administration for 40 weeks: TP plus TAM for group 1, TP for group 2, and TAM for group 3. TP and TAM were introduced into 2 cm-long Silastic tubes, the respective amounts being approximately 40 and 28 mg. The Silastic tubes (inner diameter, 0.2 cm; outer diameter, 0.3 cm) were sealed at both ends with Silastic medical grade adhesive (Dow Corning Co., Midland, MI) and implanted into the subcutis of the interscapular region under anesthesia with ethyl ether [5]. The hormone-filled implants were replaced at 6-week intervals for TP and 12-week intervals for TAM. Group 4 served as a carcinogen control given only DMAB. Groups 5–7 were control groups corresponding to groups 1 to 3, respectively, given hormone(s) without prior administration of DMAB. Group 8 was a complete control and no treatment was applied. All surviving rats were sacrificed at experimental week 60 and subjected to complete autopsy. Animals that died earlier or became moribund were also autopsied. All organs were examined for gross abnormalities and fixed in 10% buffered formalin. After fixation, the ventral prostate removed from the base of the bladder neck and the seminal vesicles and anterior prostate removed together were weighed. The weight of the seminal vesicles included the enclosed secretion and the anterior prostate. The dorsolateral prostate was weighed together with the part of the urethra surrounded by its two lobes. For tissue preparation of the accessory sex organs, two sagittal slices of the ventral prostate, three sagittal samples of the dorsolateral prostate, including the urethra, and four 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. At the final sacrifice, blood was collected from the aorta under ether anesthesia from five animals of each group for measurement of serum levels of testosterone and estrone. Their levels were analyzed by radioimmunoassay. Differences in body and organ weights and serum levels of hormones were analyzed by means of the Student's t test. Incidences of tumors and other histopathological lesions were analyzed by the Fisher's exact probability test (two tailed).

RESULTS

Administration of DMAB did not markedly alter the growth rate of rats but hormone treatment retarded growth. TP and TAM showed similar suppression effects on body weight gain (about 12–17% suppression as compared to control values) and the coadministration of TP and TAM caused an addi-

TABLE I. Final Body and Organ Weights of Rats Given DMAB and/or TP and TAM†

				Organ weights(g)				
				Prostate				
Group	Treatment	No. of rats	Final bw(g)	Ventral	Dorso-lateral	Seminal vesicles	Testes	Pituitary $\times 10^{-3}$
1	DMAB→TP+TAM	10	317**	1.34 (0.42)**	1.48 (0.47)**	3.58 (1.13)**	1.99 (0.63)	7.8 (2.5)
2	DMAB→TP	12	361**	1.23 (0.34)**	1.53 (0.42)**	3.70 (0.91)**	2.20 (0.61)	10.7 (3.0)
3	DMAB→TAM	14	350**	0.16 (0.05)**	0.29 (0.08)**	0.33 (0.09)**	2.59 (0.74)	10.3 (3.0)
4	DMAB→Control	16	416	0.50 (0.12)	0.61 (0.15)	1.16 (0.28)	2.94 (0.72)	12.4 (3.0)
5	TP+TAM	11	316**	1.38 (0.44)**	1.36 (0.43)**	3.38 (1.07)**	1.95 (0.62)**	10.3 (3.4)
6	TP	13	343**	1.23 (0.36)**	1.53 (0.45)**	3.98 (1.17)**	2.33 (0.68)*	11.5 (3.5)*
7	TAM	15	349**	0.16 (0.05)**	0.33 (0.09)**	0.29 (0.08)**	2.84 (0.81)*	10.5 (3.0)
8	Control	15	439	0.64 (0.15)	0.71 (0.13)	1.38 (0.32)	3.28 (0.75)	11.9 (2.9)

†Numbers in parenthesis represent percentage of b.w.
*$P < 0.05$, **$P < 0.01$.

tional decrease (20–23%; Fig. 1). TAM caused slightly but statistically significantly increased liver, kidney, and adrenal gland weights, but significantly (about 70%) reduced the weights of the prostate and seminal vesicles (Table I). Histological examination revealed the glands or acinar structures of accessory sex organs to be moderately atrophied, but not to the same extent as in orchiectomized or estrogen-medicated chemically castrated rats. TP, however, significantly increased prostate and seminal vesicle weights, two and three times, respectively, as well as kidney values. No increase of the pituitary weight was observed in rats given TAM. Coadministration of TP and TAM resulted in an increase in the weights of the liver, kidney, adrenal glands, prostate, and seminal vesicles.

The serum levels of testosterone and estrone are shown in Table II. Subcutaneous implantation of TP-containing Silastic tubes increased the testosterone level to about 600 mg/dl and coadministration of TAM with TP did not affect this. Estrone levels in each group were similar without any TAM influence.

Development of carcinomas of the accessory sex organs in rats treated with DMAB alone or DMAB and TAM was confined to the ventral prostate, but it was shifted to the dorsolateral and anterior prostate and seminal vesicles when animals were treated with TP or TP plus TAM after carcinogen application (Table III). The former tumors were noninvasive carcinomas while the latter were invasive as observed in previous experiments [5,6,10,11]. Administration of TP significantly suppressed development of atypical hyperplasia of the ventral prostate ($P < 0.05$; group 2 vs. group 4). However, the incidences of prostatic and seminal vesicle lesions in rats given DMAB and TAM did not differ from those in animals given DMAB alone. When compared between groups 1 and 2, the incidences of prostatic and seminal vesicle lesions were not altered by administration of TAM, except for the incidence of ventral atypical hyperplasia, which was significantly increased. There is no difference in the overall incidence of invasive carcinomas between groups 1 and 2. The incidence of atypical hyperplasias of the ventral and anterior prostate and that of ventral carcinoma in animals given DMAB plus TAM did not differ from those in group 4 given DMAB alone. The incidences of invasive carcinomas in group 1 which received DMAB and then TP plus TAM were not statistically different from those in group 2 given DMAB and TP. No neoplastic lesions were noted in groups to which DMAB was not administered.

Tumor development was also noted in the subcutis, Zymbal glands, intestine, preputial glands, and liver of DMAB-treated rats as shown in previous experiments [5,6,10]. The incidences of these tumors, however, were not affected by administration of TP or TAM (data not shown).

DISCUSSION

Administration of testosterone alone at a very high dose for a long period has been shown to produce invasive adenocarcinomas of the prostate and seminal vesicles of rats given alone or in combination with carcinogen [1,2]. In F344 rats, initiation with a carcinogen is needed for induction of testosterone-related prostate carcinomas [5] and the mechanisms underlying testosterone-associated cancer production have yet to be detailed. Our previous studies using TP at

TABLE II. Serum Testosterone and Estrone Levels

Group	Treatment	No. of rats	Testosterone (ng/dl)	Estrone (pg/ml)
1	DMAB→TP+TAM	5	591.2 ± 197.8	30.7 ± 8.2*
2	DMAB→TP	5	630.0 ± 99.0	49.1 ± 3.2
3	DMAB→TAM	5	ND[a]	436. ± 7.2
4	DMAB→Control	5	ND	45.8 ± 6.8
5	TP+TAM	5	887.4 ± 200.3	59.9 ± 5.1
6	TP	5	944.0 ± 73.2	65.6 ± 3.2
7	TAM	5	ND	65.0 ± 2.3
8	Control	5	ND	58.8 ± 8.7

[a]ND, not detected. *$P < 0.05$.

TABLE III. Incidence of Lesions in the Prostate and Seminal Vesicles of Rats Treated With TP and TAM

			No.(%) of rats with									
			Prostate									
			Ventral		Dorsolateral		Anterior		Seminal vesicles		Large invasive	Overall invasive
Group	Treatment	No. of rats	AH[a]	CA	AH	CA	AH	CA	AH	CA	tumor[b]	carcinoma
1	DMAB→TP+TAM	20	15 (75)[c]	0 (—)	2 (10)	2 (10)	12 (60)	4 (20)	13 (65)	8 (40)	2 (10)	11 (55)
2	DMAB→TP	20	7 (35)	0 (—)	3 (15)	2 (10)	12 (60)	5 (25)	17 (85)	3 (15)	4 (20)	10 (50)
3	DMAB→TAM	20	14 (70)	6 (30)	0	0	3 (15)	0	16 (80)	0	0	0
4	DMAB→Control	23	19 (82)	7 (30)	1 (4)	0	8 (35)	0	23 (100)	0	0	0
5	TP+TAM	15	0	0	0	0	2 (13)	0	0	0	0	0
6	TP	15	0	0	0	0	0	0	0	0	0	0
7	TAM	15	1 (7)	0	0	0	0	0	0	0	0	0
8	Control	15	0	0	0	0	0	0	0	0	0	0

[a]AH, atypical hyperplasia; CA, carcinoma.
[b]Invasive carcinomas which are too large to identify their origin.
[c]Significantly different from group 2.

three different doses and different administration periods demonstrated that a high dose and a long period administration are necessary for effective promotion of invasive prostate carcinoma development [10,11]. Furthermore, dihydrotestosterone (DHT), an androgen active form which is not converted to estrogen, was not effective for promotion [6,12]. These findings suggested that estrogen, which is derived from the action of aromatase on the high levels of testosterone, is involved in the testosterone-associated induction of cancer. The fact that combined administration of testosterone and estrogen resulted in pronounced development of prostate carcinomas provided support for this hypothesis. However, TAM did not influence TP promotion on DMAB-initiated prostate carcinogenesis in the present study. Therefore, it is unlikely that estrogen production from administered testosterone plays an important role.

It has been shown that TAM is also partially estrogenic and the present experiment demonstrated that its administration in Silastic tubes caused a decrease in prostate weight, with histological evidence of atrophy of the glands. Although we failed to detect circulating testosterone in control or TAM-treated rats with our method, the data indicate that TAM exerts testosterone-lowering activity through the hypothalamus-pituitary-gonadal axis. Finding of reduced testes weights in TAM-treated rats may support this hypothesis.

Interestingly, although TAM induced atrophy of the accessory sex organs, there was no decrease in the incidences of prostate and seminal vesicle lesions. The reasons for this phenomenon remain to be clarified. Since surgical or chemical castration with estrogen, which is associated with much more marked atrophy, completely blocked the development of these lesions [11], a threshold may exist for the level of circulating testosterone.

TAM has been shown to cause liver tumors in the

rat [13], and a promotional action has been proposed to account for its hepatocarcinogenicity [14]. However, under the present experimental conditions, neither TAM by itself induced liver tumors nor did coadministration with DMAB promote liver tumor induction after initiation. However, the 28 mg of TAM in a Silastic tube represents a dose of about 1.7 mg/kg bw, per day. The dose is lower than the nonhepatocarcinogenic dose found in the 2-year carcinogenesis study [13].

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