Low Tumorigenic Response to 3,2'-Dimethyl-4-aminobiphenyl Administration in the Prostate of Rats Castrated at Birth

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Six-week-old rats which had been orchietomized at birth were given 3,2'-dimethyl-4-aminobiphenyl (DMAB) at various doses combined with a stimulus to prostate epithelial cell proliferation in the form of oral administration of methyltestosterone (MT) for 4 weeks. Thereafter MT treatment was continued or the animals received subcutaneous implants of testosterone propionate (TP) and were maintained until sacrifice at week 60. Although prostatitis and prostatic enlargement were frequently observed, especially in the TP group, numbers of atypical hyperplastic lesions were low and only one prostatic carcinoma *in situ* developed. Thus, despite the presence of proliferation, castration brought about a significant reduction in susceptibility to DMAB.

Key words: DMAB — Prostate — Carcinogenesis — Castration — Newborn rat

Recent investigations of chemically induced prostate carcinoma development in rodents have concentrated attention on the effects of cell proliferation in the gland during the period of carcinogen administration. Thus good yields of prostatic cancers resulted when rats were treated with N-methyl-N-nitrosourea (MNU),¹⁾ 3,2'-dimethyl-4-aminobiphenyl (DMAB)^{2,3)} or N-nitrosobis(2-oxopropyl)amine (BOP)⁴⁾ in combination with exogenous or endogenous androgenic stimulation after chemical or surgical castration. However, a previous experiment in this laboratory suggested that testosterone-induced glandular proliferation 3 weeks after surgical castration of adults was not effective for enhancement of DMAB-induction of prostatic carcinogenesis.⁵⁾ Chung and Ferland-Raymond⁶⁾ compared the rate of [³H]thymidine incorporation into tissue DNA from the ventral prostate *in vitro* after testosterone propionate (TP) between neonatally castrated rats and rats castrated in adulthood and found that the rate in the former was higher than in the latter.

The present study was therefore performed to see whether the neonatally castrated rat demonstrates a similar low susceptibility of prostate epithelium to DMAB carcinogenicity.

MATERIALS AND METHODS

Pregnant F344 strain rats were purchased from Charles River Japan Inc., Kanagawa, and all newborn males were orchietomized 2 days after birth under a dissecting microscope. Animals were housed in plastic cages with hard wood chip bedding, in an air-conditioned room with a 12 hr-12 hr light-dark cycle, and given food (Oriental MF; Oriental Yeast Co., Ltd., Tokyo) and water *ad libitum*. Methyltestosterone (MT) and TP were purchased from Sigma Chemical Co., St. Louis, MO and DMAB was obtained from Matsugaki Pharmaceutical Co., Osaka. MT was supplemented into the diet at a concentration of 300 ppm. Powder TP was pelleted into a tablet weighing about 20 mg using a press with a 4 mm diameter hole. All rats were given MT for 4 weeks, starting at 6 weeks of age. Subcutaneous injections of DMAB at a dose of 200 mg/kg body weight in 0.5 ml of corn oil were administered to rats on the 3rd and the 5th days after the commencement of MT diet in groups 1 and 2 and on the 3rd, 5th, 14th and 21st days in groups 3 and 4. Rats in the non-carcinogen control groups 5 and 6 received vehicle alone in place of carcinogen. After the initial 4-

Abbreviations used are: DMAB; 3,2'-dimethyl-4-aminobiphenyl, MNU; N-methyl-N-nitrosourea, BOP; N-nitrosobis(2-oxopropyl)amine, MT; methyltestosterone, TP; testosterone propionate.

week administration of MT, animals in groups 1, 3 and 5 were continuously maintained on diet supplemented with MT for 56 weeks, but rats in groups 2, 4 and 6 received a subcutaneous implant of a TP pellet in the scapular area under ether anesthesia instead of dietary MT administration. New pellets of TP were implanted every 2 months until the end of the experiment at week 60, when all animals were sacrificed and subjected to routine histopathological examination.

RESULTS

Fifteen percent of the rats given DMAB died within the first week of the experiment and many other animals died from urinary obstruction or for unclear reasons during the study. The final survival rates of 47, 35, 56, 32, 47 and 47% in groups 1 to 6, respectively, were thus very low. All animals which survived beyond week 50 of the experiment were included in the effective numbers (Table I). The average weights of the ventral prostate in animals receiving TP were about twice

those of animals given MT. Inclusion of the seminal vesicles and all parts of the prostate gave combined weights in the groups given TP approximately 3 times heavier than those of the groups receiving MT (Table I).

Histopathological lesions observed in the accessory sex organs are summarized in Tables II and III. The most prevalent change was chronic inflammation, i.e., focal infiltration of polynuclear neutrophilic leukocytes, lymphocytes and plasma cells, which was particularly frequent in rats administered TP. Squamous metaplasia was also noted in the coagulating glands and seminal vesicles of rats treated with TP. Only a few cases of atypical hyperplasia were observed in the ventral prostate, seminal vesicles and coagulating glands of animals treated with DMAB, no significant inter-group differences being apparent. Only one carcinoma *in situ* was observed in group 3 and no other prostate tumors were induced.

Table I. Mean Prostate and Seminal Vesicle Weights of Rats Castrated at Birth and Subsequently Treated with DMAB Combined with Testosterone

Group	Treatment ^{a)}	Effective No. of rats	Prostate + seminal vesicle	Ventral prostate
1	DMAB (2) — MT	16	1.04 ± 0.36 ^{b)}	0.21 ± 0.09 ^{a)}
2	DMAB (2) — TP	8	3.63 ± 1.41	0.56 ± 0.26
3	DMAB (4) — MT	18	1.14 ± 0.38	0.27 ± 0.10
4	DMAB (4) — TP	12	2.97 ± 0.95	0.47 ± 0.20
5	MT	7	1.31 ± 0.41	0.24 ± 0.13
6	TP	7	4.86 ± 1.22	0.64 ± 0.30

a) Numbers in parentheses, number of injections.

b) Means ± SD (g).

Table II. Histopathological Findings in the Prostate of F344 Rats Castrated at Birth and Then Treated with DMAB Combined with Testosterone

Group	Treatment ^{a)}	No. of rats	No. of rats demonstrating lesions in the prostate (%)				
			Ventral			Dorso-lateral	
			Atypical hyperplasia	Carcinoma	Inflammation	Atypical hyperplasia	Inflammation
1	DMAB (2) — MT	16	2 (12.5)	0	7 (43.8)	0	0
2	DMAB (2) — TP	8	2 (25.0)	0	6 (75.0)	0	5 (62.5)
3	DMAB (4) — MT	18	6 (33.3)	1 (5.6)	10 (55.6)	0	4 (22.2)
4	DMAB (4) — TP	12	0	0	9 (75.0)	0	9 (75.0)
5	MT	7	0	0	2 (28.0)	0	0
6	TP	7	0	0	6 (85.7)	0	6 (85.7)

a) Numbers in parentheses, number of injections.

Table III. Histopathological Findings in the Seminal Vesicles and Coagulating Glands of Rats Castrated at Birth and Then Treated with DMAB Combined with Testosterone

Group	No. of rats	No. of rats with lesions (%)					
		Seminal vesicles			Coagulating gland		
		Atypical hyperplasia	Squamous metaplasia	Inflammation	Atypical hyperplasia	Squamous metaplasia	Inflammation
1	16	3 (18.8)	0	2 (12.5)	0	0	3 (18.8)
2	8	1 (12.5)	1 (12.5)	4 (50.0)	0	3 (37.5)	6 (75.0)
3	18	2 (11.1)	0	2 (11.1)	0	0	0
4	12	4 (33.3)	2 (16.7)	9 (75.0)	1 (8.3)	1 (8.3)	9 (75.0)
5	7	0	0	1 (14.3)	0	0	0
6	7	0	0	3 (42.9)	2 (28.6)	2 (28.6)	3 (42.9)

Table IV. The Incidence of Tumors in Organs Other Than the Prostate of Rats Castrated at Birth and Then Treated with DMAB Combined with Testosterone

Group	No. of rats	No. (%) of rats with tumors in						
		Small intestine ^{a)}	Large intestine ^{a)}	Mammary gland ^{a)}	Zymbal gland ^{a)}	Preputial gland ^{a)}	Subcutis ^{a)}	Urinary bladder ^{a)}
1	16	1 (6.3)	1 (6.3)	0 —	0 —	2 (12.5)	1 (6.3)	0 —
2	8	1 (12.5)	1 (12.5)	3 (37.5)	0 —	1 (12.5)	3 (37.5)	2 (25.0)
3	18	1 (5.6)	1 (5.6)	0 —	0 —	0 —	2 (11.1)	0 —
4	12	1 (8.3)	3 (25.0)	2 (16.7)	1 (8.3)	2 (16.7)	2 (16.7)	3 (25.0)
5	7	0 —	0 —	0 —	0 —	0 —	0 —	0 —
6	7	0 —	0 —	0 —	0 —	0 —	0 —	1 (14.2)

a) Adenocarcinomas. b) Fibroadenomas. c) Sebaceous carcinomas.

d) Neurinomas. e) Transitional papillomas or transitional cell carcinomas.

Development of tumors in organs other than the accessory sex organs was observed in the small intestine, large intestine, urinary bladder, preputial gland, mammary gland, Zymbal gland and subcutis (Table IV). The incidences were generally low (6.5 to 37.5%) and the lesions of the urinary bladder which were most frequently observed (6.3 to 33%), in both DMAB and non-DMAB groups, were associated with the presence of bladder calculi in 80% of cases. There were no clear difference in tumor incidence for any organ between the groups.

DISCUSSION

It was earlier reported that administration of testosterone to 9-week-old rats which had been castrated for 3 weeks induces DNA synthesis in the accessory sex organs.^{6,7)} Although DMAB was given at the time of en-

hanced DNA synthesis, only very little induction of preneoplastic and neoplastic lesions of the prostate was observed in the present study. The total dose of DMAB per rat in the present experiment was 400 or 800 mg/kg body weight, levels comparable to or even higher than those administered in our recent studies which resulted in successful induction of prostate carcinomas at high yields.^{2,3)} The present finding of low tumorigenic response in the prostate is, however, in accordance with the results of a previous experiment performed in our laboratory in which a total dose of 1200 mg/kg body weight combined with testosterone administration failed to induce prostate carcinomas in rats that had been castrated at 6 weeks of age.⁵⁾ Katayama *et al.*⁸⁾ also reported that DMAB failed to induce carcinoma development in the prostate of orchiectomized rats. Thus the situation

with surgical castration is in clear contrast to that with chemical castration by estrogen, which gives rise to a temporary atrophy. In this latter case, subsequent regenerative cell proliferation in response to androgen stimulus was found to be very effective for the induction of prostate tumors by DMAB.

Chung and MacFadden⁷⁾ reported that the ventral prostate of neonatal castrates treated with testosterone during the weaning period showed loss of normal acinar structure probably resulting from proliferation of epithelial cells and expansion of connective tissues elements. Atrophy of the prostate caused by surgical castration was severer than that by chemical castration. It is thus conceivable that prostate epithelial cell under these conditions are immature and lack sufficient metabolizing enzymes for the effective activation which is essential to the carcinogenicity of DMAB. We recently established using sepecific antibody that the formation of DMAB-DNA base adducts can be clearly detected in the epithelial cells of the prostate of normal and estrogen-treated rats but not in orchiectomized animals (manuscript in preparation). Thus, while previous results have demonstrated that cell proliferation in target tissues plays an important role in determining the level of carcinogenic response, this is qualified by the proviso that initiation be effective.

The reasons for the chronic inflammation observed in the prostate and other accessory organs in the present study are unclear, although surgical trauma to the urogenital region immediately after birth is presumably involved. However, since this prostatitis developed more frequently in rats treated with TP than in those given MT, it presumably is also a reflection of androgen-induced changes.

In conclusion, use of rats castrated at birth for long-term prostate carcinogenicity experiments is not to be recommended because of urogenital damage resulting from the operative procedure.

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