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Induction of Prostate Carcinoma *in Situ* at High Incidence in F344 Rats by a Combination of 3,2′-Dimethyl-4-aminobiphenyl and Ethinyl Estradiol[1]

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ABSTRACT

Male F344 rats were given a diet containing 0.75 ppm of ethinyl estradiol for 3 weeks and a basal diet for 2 weeks alternately 10 times, and 2 days after each change to basal diet (on the third day of feeding of the basal diet), a single s.c. injection of 50 mg/kg body weight of 3,2′-dimethyl-4-aminobiphenyl. Then they were given the basal diet until week 60 and sacrificed for histological examination. Prostatic carcinomas were found in 18 of 21 rats (85.7%) after this treatment but in only 1 of 19 rats (5.2%) maintained on normal diet throughout the experiment but given 3,2′-dimethyl-4-aminobiphenyl at the same times as the diet given to test rats. All the carcinomas were microscopic and developed in the ventral lobe of the prostate. This method may ultimately lead to a useful animal model of prostatic carcinoma.

INTRODUCTION

Establishment of an appropriate animal model of inducible prostate cancer is required for a better understanding of prostatic carcinogenesis in humans (1, 2). The chemical carcinogens that can induce prostatic adenocarcinoma in rats include DMAB[3] (3, 4) and *N*-methyl-*N*-nitrosourea (5). *N*-Nitrosobis(2-oxopropyl)amine was reported to induce prostatic squamous cell carcinoma in rats (6). However, on systemic administration of DMAB to adult rats, the incidence of prostatic carcinoma is low, being less than 32% (3), and at the same time many tumors develop in other organs also. Thus a method was required for inducing a high incidence of prostatic carcinomas with low incidences of tumors in other organs.

Bosland *et al.* (5) recently reported that pretreatment of rats with antiandrogen to achieve chemical castration followed by short-term proliferative stimulation with testosterone together with a single injection of *N*-methyl-*N*-nitrosourea resulted in development of prostatic cancer in 35% of the animals. Similarly, Katayama *et al.* (7) found that microscopic prostatic carcinomas could be induced at high incidence 77 weeks after multiple injections of DMAB at a high dose into F344 rats of initially 3 or 4 weeks old in which DNA synthesis in the prostate was found to be very high.

Estrogen causes reversible atrophy of the prostate gland of rats, probably by blocking testicular synthesis of testosterone via the hypothalamic-pituitary-gonadal axis and direct effects on the prostate glands (8). Previously we found that addition of EE to the diet of rats for 3 weeks induced atrophy of the prostate gland and that cells in the atrophied gland began to proliferate rapidly on treatment of the rats with methyltestosterone (9).

In this paper, we report the induction of early prostatic carcinomas in rats by repeated treatments with EE and DMAB.

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[3] The abbreviations used are: DMAB, 3,2′-dimethyl-4-aminobiphenyl; EE, ethinyl estradiol.

MATERIALS AND METHODS

Male F344 rats (purchased from Charles River Japan, Inc., Kanagawa, Japan) were 6 weeks old and weighed about 120 g at the beginning of the experiments. Animals 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, Japan) and water *ad libitum*. EE was purchased from Sigma Chemical Co., St. Louis, MO. DMAB was obtained from Matsugaki Pharmaceutical Co., Osaka, Japan. EE was added to the basal powdered diet at a dose of 0.75 ppm. For this diet 15 mg of EE were dissolved in 100 ml of corn oil and then mixed with 20 kg of diet. Diet containing EE is named EE diet in this paper. The animals were divided into 3 groups of 25 rats each. The rats in groups 1 and 3 were given EE diet for ten 3-week periods alternating with 2-week periods of basal diet and then were given basal diet for the rest of the 60-week experiment. The rats in group 1 received a single injection of DMAB at 50 mg/kg body weight in 0.5 ml of corn oil 2 days after each change to the basal diet (*i.e.*, on the third day of feeding basal diet), while at this time group 3 received an injection of vehicle only, because our previous study showed that maximal proliferating response of the epithelium of the ventral prostate that was assessed by [^{3}H]thymidine incorporation using autoradiography occurred on that day (9). As a control group 2 was given the basal diet throughout the experiment and DMAB at the same time as group 1. All animals killed in experimental week 60 were subjected to routine autopsy. All organs were examined for gross abnormalities, and slices were taken from the prostate, including the seminal vesicle and coagulating gland, liver, lung, kidney, urinary bladder, stomach, small and large intestine, Zymbal's gland, preputial gland, and observable abnormal lesions, all being fixed in 10% buffered formalin. Ventral lobes of the prostate and seminal vesicles including coagulating glands were weighed before fixation. For tissue preparation of the accessory sex organs, two sagittal slices of the ventral prostate, a sagittal piece of the dorsolateral prostate including the urethra, and a transverse sample from each side of the seminal vesicle including the coagulating glands were embedded in paraffin. A single section (4 μm) was cut and stained with hematoxylin and eosin.

RESULTS

Table 1 shows the final average weights of the body, ventral lobe of the prostate, and seminal vesicles with coagulating glands. Intermittent administration of EE slightly suppressed growth of the rats, and the final body weight of rats given EE and DMAB was significantly lower than that of rats given DMAB ($P < 0.001$). The average weights of the ventral portion of the prostate and seminal vesicle were similar in the three groups. The relative weight of the seminal vesicle to body weight in group 1, however, was significantly lower than that of group 2 ($P < 0.01$). The incidences of proliferative and neoplastic lesions of the prostate gland and seminal vesicle in the three groups are shown in Table 2.

The histological appearance of the lesions of the prostate gland have been described in our previous paper (4). All carcinomas were microscopic and were located in the ventral lobe, involving one to three acini (Fig. 1). The cells in carcinomas varied in size, and showed nuclear atypism and a cribriform pattern (Fig. 2). Mitoses were frequently seen in the tumor. Multiple lesions of carcinoma were also observed in four rats in group 1 and one in group 2.

Table 1 *Final average body and prostate gland weights of rats treated with EE and/or DMAB*

Group	Treatment	Effective no. of rats	Weight				
				Prostate gland (ventral lobe)		Seminal vesicles	
			Body (g)	g	% of body weight	g	% of body weight
1	EE + DMAB	21	363.0 ± 23.3[a,b]	0.44 ± 0.09	0.12 ± 0.02	1.13 ± 0.15	0.31 ± 0.04[c]
2	DMAB	19	411.3 ± 39.3	0.47 ± 0.08	0.11 ± 0.02	0.09 ± 0.15	0.27 ± 0.03
3	EE	25	394.0 ± 19.4	0.46 ± 0.15	0.11 ± 0.04	1.12 ± 0.16	0.29 ± 0.04

[a] Mean ± SD.
[b] Significantly different from group 2, at $P < 0.001$.
[c] Significantly different from group 2, at $P < 0.01$.

Table 2 *Incidence of hyperplastic and neoplastic lesions in the prostate gland and seminal vesicle of rats treated with EE and/or DMAB*

Group	Treatment	Effective No. of rats	No. (%) of rats with lesions in				
			Prostate gland				
			Ventral lobe			Dorsal lobe (atypical hyperplasia)	Seminal vesicle (atypical hyperplasia)
			Hyperplasia	Atypical hyperplasia	Carcinoma		
1	EE + DMAB	21	21 (100)	20 (95.2)[a]	18 (85.7)[a]	2 (9.5)	19 (90.5)[b]
2	DMAB	19	19 (100)	7 (36.8)	1 (5.2)	0	11 (57.8)
3	EE	25	0	0	0	0	0

[a] Significantly different from group 2, at $P < 0.001$.
[b] Significantly different from group 2, at $P < 0.01$.

Table 3 *Tumors in organs other than the prostate of rats treated with EE and/or DMAB*

Group	Effective no. of rats	No. (%) of rats with tumors in						
		Subcutis	Ear duct	Preputial gland	Mammary gland	Small intestine	Large intestine	Testis
1	21	3 (14)	1 (5)	1 (5)	1 (5)	1 (5)	3 (14)	1 (5)
2	19	1 (5)	0	0	0	1 (5)	0	0
3	25	0	0	0	0	0	0	0

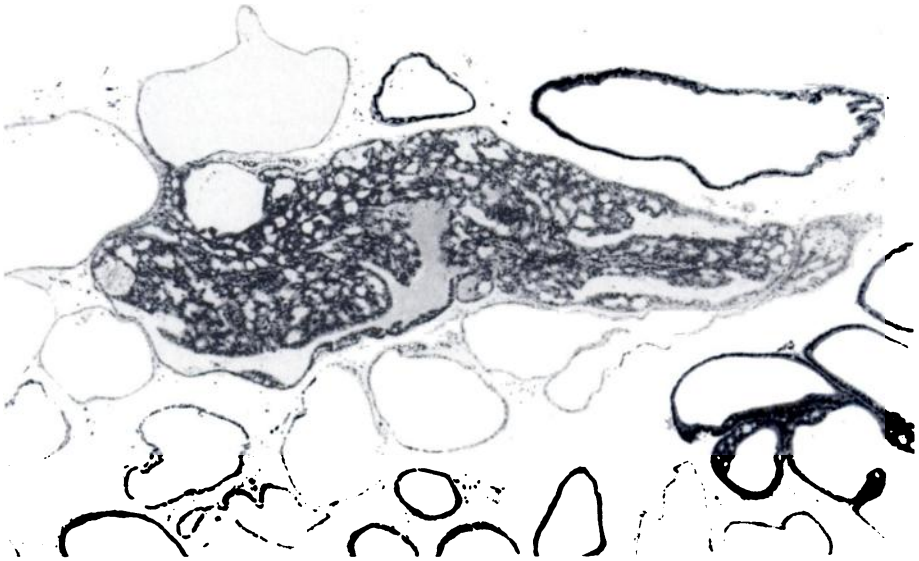

Fig. 1. Carcinoma involving more than two acini (EE plus DMAB). Surrounding acini are dilated and their epithelium is atrophied. Atypical hyperplasia is also seen. H & E, × 40.

Carcinomas of the prostate were found in 18 of 21 rats (85.7%) in group 1 but in only one of 19 rats (5.2%) in group 2. Rats of group 1 also had a high incidence (95.2%) of atypical hyperplasia in the ventral lobe of the prostate (Fig. 3). The incidences of atypical hyperplasia and carcinoma were significantly higher in group 1 than in group 2 by the χ^2 test ($P < 0.001$). Atypical hyperplasias were also frequently seen in the seminal vesicles (Fig. 4) with a significantly higher incidence in group 1 ($P < 0.01$) than in group 2. Atypical hyperplasia of the seminal vesicle was composed of tall columnar cells with elongated but less basophilic nuclei which varied in size. They had an irregular papillary appearance mixed with a cribriform pattern (Fig. 4). There were no substantial pathological lesions in the dorsolateral lobe in any group except for two cases of atypical hyperplasia of the dorsal lobe in group 1. Neither invasive growth of carcinomas into the adjacent organs nor metastases were observed. The epithelium of the ventral lobe in all three groups showed various degrees of atrophy, but no clear atrophy was observed in other parts of the prostate.

The incidences of tumors in organs other than the prostate were low, ranging from 5 to 14%, and included tumors of the subcutis, Zymbal's glands, preputial glands, and intestine, but

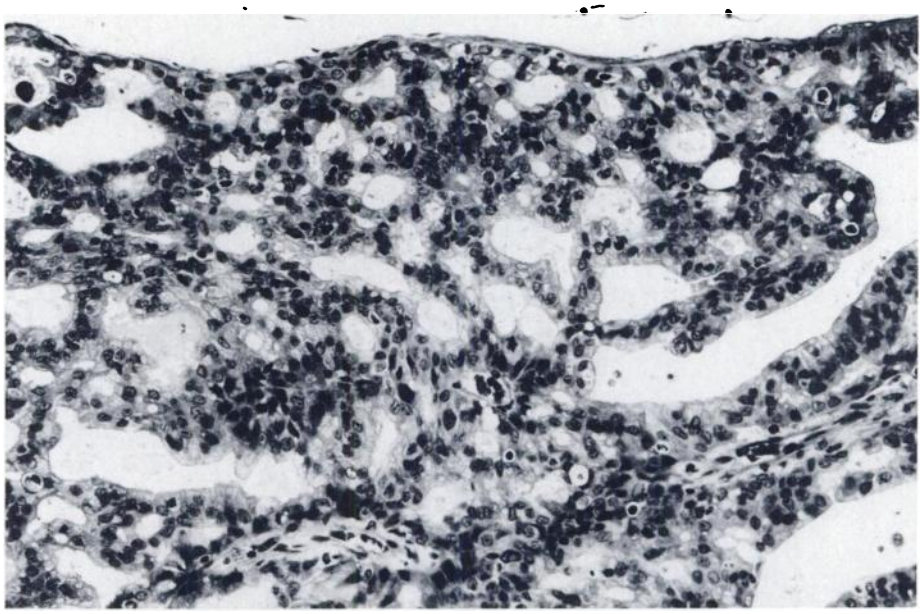

Fig. 2. Portion of Fig. 1 at higher magnification. A cribriform pattern of cells is prominent. Note nuclear atypism and mitoses. H & E, × 200.

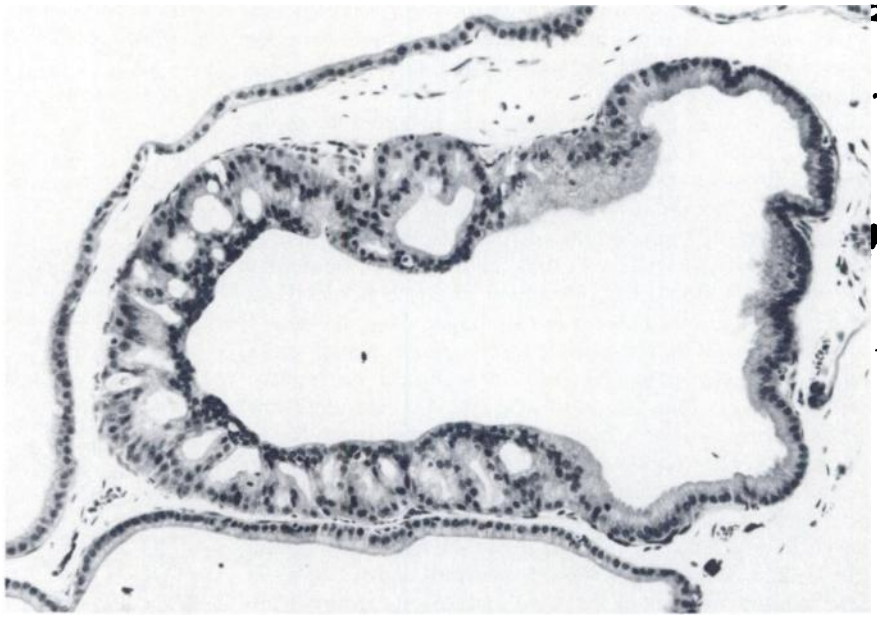

Fig. 3. Atypical hyperplasia of the ventral portion of the prostate gland (EE plus DMAB). Papillary proliferation of atypical epithelium. H & E, × 200.

there were no clear differences in the incidences of these tumors in groups 1 and 2 (Table 3).

DISCUSSION

In the present experiment, we observed 85.7% incidence of carcinoma of the prostate in rats treated intermittently with EE and given DMAB after each treatment with EE. All the carcinomas induced were microscopic and were diagnosed as carcinomas *in situ*. This is the first time that a high incidence of carcinoma *in situ* has been induced in the prostate gland of rats by a relatively simple procedure. DMAB was given during periods of cell proliferation of the prostate gland induced by stimulation with testicular androgen after incomplete atrophy of the prostate gland by 3-week periods of treatment with EE repeated 10 times.

A similar experimental protocol, in which rats were castrated at 6 weeks old and then given methyltestosterone in combination with DMAB intermittently was recently found not to be effective for inducing prostatic carcinomas.[4] DMAB requires metabolic activation to exert its carcinogenicity (10), as shown by Söderkvist *et al.* (11) who found that rat ventral prostate possessed enzymes which could convert promutagenic compounds to ultimate mutagenic metabolites. Thus, the epithelium of the prostate is thought to contain an enzyme(s) that activates DMAB or its metabolite(s). Unlike EE treatment, castration for 3 weeks induced severe atrophy of the prostate gland, which could lead to loss of this activating enzyme(s). This may be one reason why intermittent treatment with EE diet was effective for induction of prostatic carcinomas by DMAB whereas castration and then administration of methyltestosterone in combination with DMAB was not. The reason for the preferential induction of tumors in the ventral lobe of the prostate by DMAB is not known. A difference in activity of the enzyme(s) in the lobes, and/or the higher cell proliferative responses of the ventral lobe to testosterone for restoration of glands other than the dorsolateral lobes (9) could be one of the reasons. The

[4] T. Shirai and N. Ito, unpublished data.

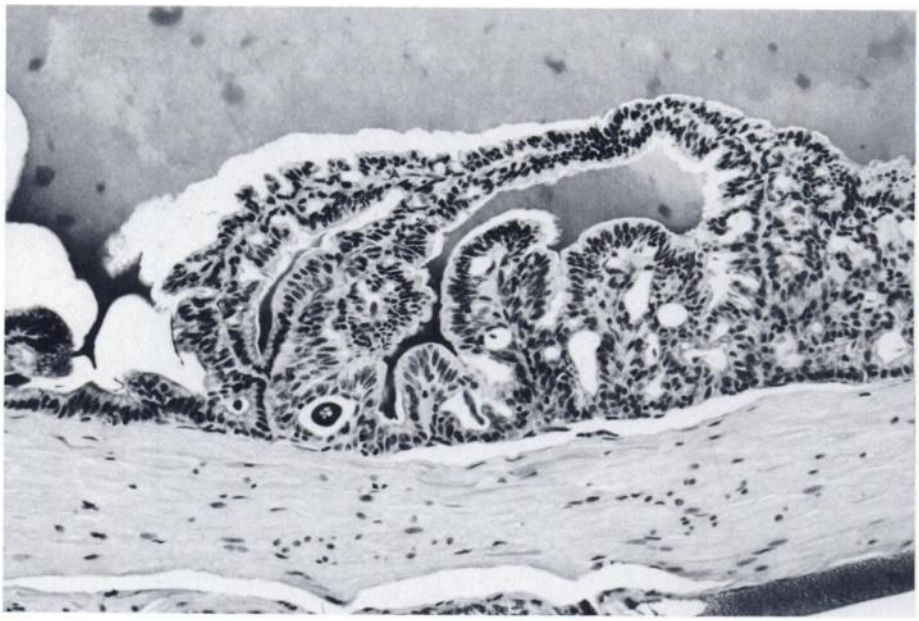

Fig. 4. Atypical hyperplasia of the seminal vesicle (EE plus DMAB). H & E, × 200.

hyperplastic and *in situ* lesions found in the ventral prostate in this study, however, are morphologically comparable to those developed in the dorsolateral prostate (5) and as found by other investigators (3, 4, 6).

In previous studies (3, 4), the total dose of DMAB ranged from 840 to 1000 mg/kg body weight/rat, and the highest incidence of prostatic carcinoma was 38.6% (3). The fact that in the present experiment the total intake of DMAB per rat was only 500 mg/kg body weight but the incidence of prostate carcinomas was 87.5% indicates that alternate treatment with DMAB with normal and EE diet was a very effective method for inducing prostatic carcinoma in rats. Thus, the present data show that cell proliferation during carcinogen exposure plays an important role for enhancement of prostate carcinogenesis. Another advantage of the present method was that the incidence of tumors in organs other than the prostate was much lower than those observed previously (4).

In recent autopsies on human materials the incidence of latent prostatic carcinoma was found to be about 21 to 37% (12, 13), and no clear difference was found in the incidences in Japan and the United States, even though prostatic cancer is more common in aged subjects in the USA (14). Some factors such as diet and hormones have been suggested to influence the growth and progression of early carcinomas to invasive carcinomas (15, 16). The present method perhaps provides a system in which factors that cause progression of *in situ* lesions to clinical, invasive carcinoma in rats can be studied.

More advanced lesions, such as invasive carcinomas, may develop if the dose of DMAB is increased and/or if the experimental period is extended. Further experiments on these possibilities are in progress.

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