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Enhancing Effect of Cadmium on Rat Ventral Prostate Carcinogenesis Induced by 3,2′-Dimethyl-4-aminobiphenyl

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The effects of cadmium given at different stages during 3,2′-dimethyl-4-aminobiphenyl (DMAB)-induced rat prostate carcinogenesis were investigated using male F344 rats. Animals were given 10 subcutaneous injections of 50 mg/kg body weight of DMAB or the corn oil vehicle at two-week intervals. In addition, cadmium was administered at doses of 0, 10, or 30 μmol/kg body weight as single intramuscular injection on the 1st day of the experiment or one day after the last injection of DMAB at week 20. Two further groups were subjected to administration of cadmium at 10 μmol/kg at week 20 and then 5 μmol/kg at week 40, or 10 μmol/kg at week 20 and then 5 μmol/kg at weeks 30, 40 and 50. At the termination, 60 weeks after the beginning of the experiment, the incidences and multiplicity of ventral prostate carcinomas in the groups given cadmium plus DMAB demonstrated a consistent tendency for increase over control values (groups receiving DMAB or cadmium alone). The numbers of carcinomas per rat and per unit area of prostate section were significantly elevated in the two groups given low doses of cadmium after cessation of DMAB administration. Cadmium alone also induced a few prostate carcinomas. The influence on development of prostate tumors did not appear to be a result of the induced severe testicular atrophy because serum testosterone levels were not affected. The results indicate that cadmium and DMAB can act synergistically to cause rat prostate carcinogenesis.

Key words: Rat — Prostate carcinogenesis — Cadmium — DMAB — Combination

Possible links between prostate cancer development and exposure to cadmium in human populations have been reported by several investigators.[1–4] Although Bosland[5] stated in his review that there is no conclusive epidemiological evidence of a causal relationship, cadmium has clearly been shown to have carcinogenic potential for several organs of experimental animals[6–9] including the prostate.[10–13] For example, Waalkes *et al.*[12, 13] reported that subcutaneous or intramuscular injection of cadmium chloride induces ventral prostate tumors in rats after a long latent period. The rat prostate tumors produced by cadmium described in the literature are histologically similar to those induced by the prostate carcinogen, 3,2′-dimethyl-4-aminobiphenyl (DMAB).[14–16] However, the mechanisms by which cadmium induces prostate tumors remain unknown. The present experiment was designed to examine whether DMAB and cadmium could act synergistically in inducing rat ventral prostate carcinomas. Since prostate carcinogenesis associated with cadmium was reported to depend on testicular toxicity, it was given intramuscularly to avoid testicular atrophy as reported by Waalkes *et al.*[13]

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MATERIALS AND METHODS

Chemicals Cadmium chloride ($CdCl_2$), anhydrate, was purchased from Katayama Chemical Industries Co., Ltd., Osaka and DMAB from Matsugaki Pharmaceuticals Co., Osaka (purity >98%). Cadmium was dissolved in sterile saline, and DMAB was suspended in corn oil.

Animals and treatments A total of 230 male 6-week-old F344 rats (Charles River Japan Inc., Kanagawa), weighing approximately 140 g at the beginning of the experiments, were housed in plastic cages on wood chip bedding. They were maintained 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 tap water *ad libitum*. The animals were randomly divided into 13 groups (Fig. 1). The rats in groups 1 to 7 (20 each) received DMAB injection for the first 20 weeks (a subcutaneous injection of DMAB at 50 mg/kg body weight (bw) in 0.5 ml of corn oil every 2 weeks) while groups 8 to 13 (15 each) received the vehicle only. In addition, groups 1 to 6 received cadmium (intramuscular injection in the right thigh) as follows: group 1, a dose of 30 μmol/kg bw, on the 1st day of the experiment; group 2, a dose of 10 μmol/kg bw, on the 1st day of the experiment; group 3, a dose of 30 μmol/kg bw at week 20 (one day

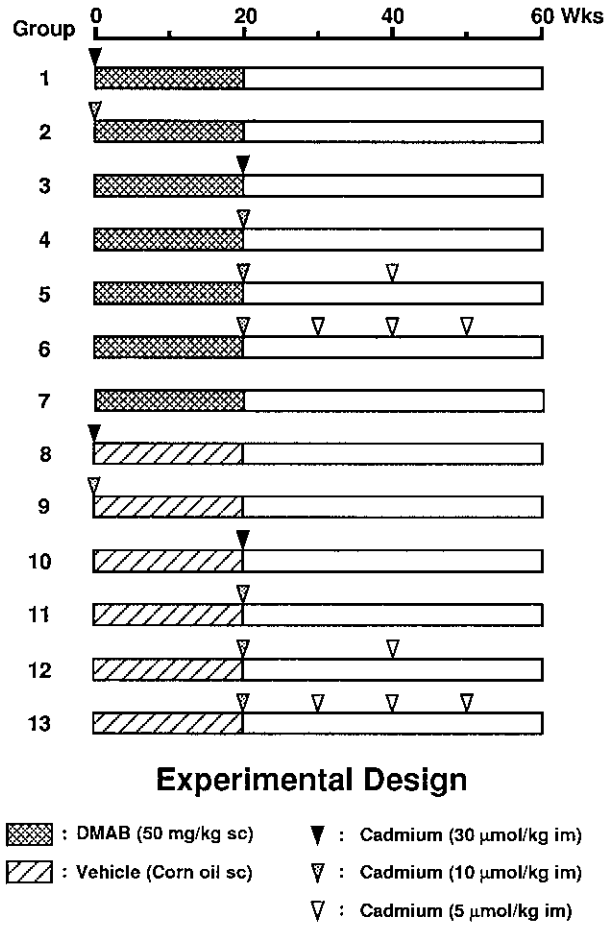

Fig. 1. Experimental design.

after the last injection of DMAB); group 4, a dose of 10 μmol/kg bw at week 20; group 5, doses of 10 μmol/kg bw at week 20 and 5 μmol/kg at week 40; group 6, doses of 10 μmol/kg at week 20 and then 5 μmol/kg at weeks 30, 40 and 50. Groups 8 to 13, which served as the corresponding controls to groups 1 to 6, were given cadmium without DMAB treatment.

All surviving rats were killed at the end of experimental week 60 and subjected to complete autopsy. Rats that died or were killed upon becoming moribund were also autopsied. All organs were examined for gross abnormalities, and slices were taken from all major organs as described previously[14] and fixed in 10% buffered formalin. For tissue preparation of the accessory sex organs, two or three sagittal slices through the ventral prostate, as well as 4 sagittal samples of the dorsolateral prostate including the urethra and 5 transverse samples for each side of the seminal vesicles together with the anterior prostate (coagulating glands) were embedded in paraffin. Single sections (4 μm) were cut from all tissues and stained with hematoxylin and eosin for histological examination. The total area of the ventral prostate was measured using a color video image processor (VIP-21C, Olympus-Ikegami Tsushin Co., Tokyo).

At week 60, blood samples were obtained from the aorta of randomly selected rats in groups 1, 2, 6 and 7 which had been given DMAB and cadmium, and serum testosterone levels were measured by radioimmunoassay.

Differences in body and organ weights and serum levels of testosterone were analyzed by means of Student's t test (two tailed) with the F-test for variability. Incidences of tumors and other histopathological lesions were analyzed by using Fisher's exact probability test (one tailed) and differences in numbers of tumors by using Student's t test (one tailed).

RESULTS

Body and organ weights Administration of DMAB suppressed the body weight gain by about 6% at 20 weeks after the start of the study as compared with non-DMAB-treated controls. Cadmium also reduced the body weight gain, but only in group 6 were the final body weights significantly lower than those of the control group 7 (Table I). When groups given the low and high doses of cadmium or 2 and 4 injections were compared, the final body weights were always lower in the high-dose or 4-dose groups. Cadmium-associated decrease in prostate weight was noted in groups 1, 2–6 when compared to the control values. However, such suppression of prostate weight by cadmium was not related to dose or injection frequency except when the agent was given at the beginning of the experiment. There were no clear cadmium-related changes in the weights of ventral prostate and seminal vesicles. The seminal vesicle weights showed a similar tendency to that observed for the prostate.

Survival curves Survival curves for groups 1 to 7 are shown in Fig. 2. Poor survival was observed in group 3 given the larger dose of cadmium at the end of the DMAB administration period. Major causes of death in these animals were pulmonary bleeding and/or development of tumors at the right thigh (cadmium injection site). Group 6 also showed poor survival, but no tumor development at the cadmium injection site was noted; lung bleeding was the cause of death in 6 of 7 animals which died early.

Incidences of prostate tumors Tumorous lesions of the prostate complex were confined to the ventral prostate. Atypical hyperplasias and carcinomas were observed in the ventral prostate, but only atypical hyperplasias were found in the seminal vesicles. The histological characteristics of these lesions have been detailed.[14–16] Atypical

Table I. Final Body and Organ Weights

Group	Treatment[a]	Effective no. of rats[b]	Weights (g, mean±SD)		
			Body	Ventral prostate	Seminal vesicle
1	Cd(H)+DMAB	16	443.0±34.5	0.53±0.10[f]	1.21±0.26[d]
2	Cd(L)+DMAB	16	461.0±27.3[d]	0.68±0.01	1.41±0.21
3	DMAB+Cd(H)	9	433.1±23.4	0.56±0.17[c]	1.30±0.38
4	DMAB+Cd(L)	16	442.4±35.9	0.57±0.14[e]	1.15±0.40[c]
5	DMAB→Cd(L)×2	16	441.3±27.8	0.57±0.13[e]	1.28±0.20
6	DMAB→Cd(L)×4	13	422.1±26.7[c]	0.63±0.07	1.37±0.35
7	DMAB	19	441.2±19.5	0.69±0.11	1.39±0.17
8	Cd(H)+vehicle	15	442.3±22.9	0.48±0.18	1.11±0.36
9	Cd(L)+vehicle	15	461.5±24.3	0.66±0.19	1.27±0.31
10	vehicle+Cd(H)	11	446.5±27.5	0.62±0.17	1.35±0.31
11	vehicle+Cd(L)	14	470.7±28.3	0.61±0.20	1.33±0.21
12	vehicle→Cd(L)×2	14	472.4±31.4	0.63±0.10	1.38±0.21
13	vehicle→Cd(L)×4	14	453.5±19.3	0.70±0.09	1.45±0.18

a) Cd(H); 30 μmol cadmium/kg, Cd(L); 10 μmol/kg. (The last dose of Cd in groups 5 and 12 and the last 3 doses in groups 6 and 13 were 5 μmol/kg.)
b) Numbers of animals surviving for 60 weeks.
Significantly different from the group 7 value at *c*) $P<0.05$, *d*) $P<0.02$, *e*) $P<0.01$ and *f*) $P<0.001$.

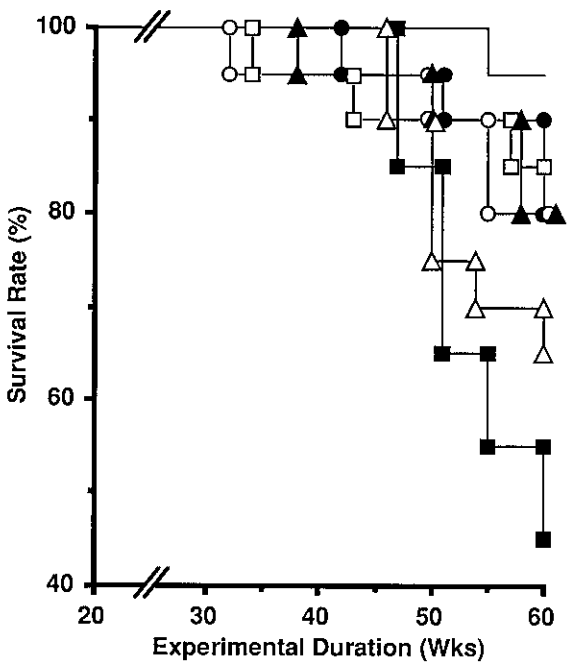

Fig. 2. Survival curves for rats treated with DMAB and cadmium. ●, group 1: Cd(30)+DMAB; ○, group 2: Cd(10)+DMAB; ■, group 3: DMAB+Cd(30); □, group 4: DMAB+Cd(10); ▲, group 5: DMAB→Cd(×2); △, group 6: DMAB→Cd(×4); ——, group 7: DMAB

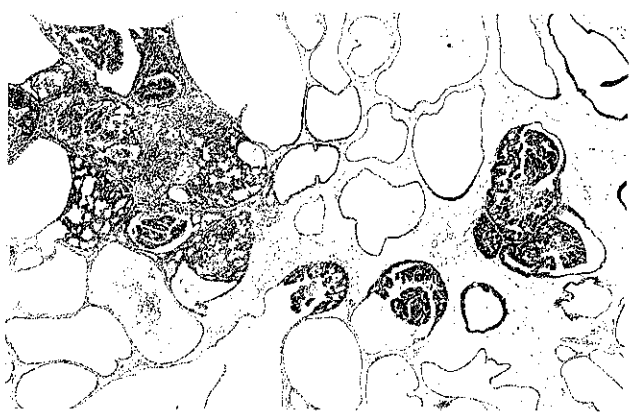

Fig. 3. Overview illustrating ventral prostate carcinomas in a rat given a high dose of cadmium and DMAB. Multiple tumor development is evident. H & E, ×40.

hyperplasia refers to small proliferative lesions with slight cellular atypia in the lining epithelium of the acinus. Carcinomas are large proliferative lesions occupying acinar luminal spaces, and often involving several neighboring acini. Carcinomas of the ventral prostate were all microscopic and often multifocal (Fig. 3). Tumor cells were columnar with round to oval hyperchromatic and pleomorphic nuclei, forming cribriform microglandular and sometimes papillary patterns (Figs. 4 and 5). Loss of cellular and/or nuclear polarity was a commom feature. Mitoses were frequently observed. These histological findings are characteristic of carcinomas. The yields of these lesions are summarized in Table II. The incidence of atypical hyperplasia ranged from 87.5 to 100% in the ventral prostate and from 75 to

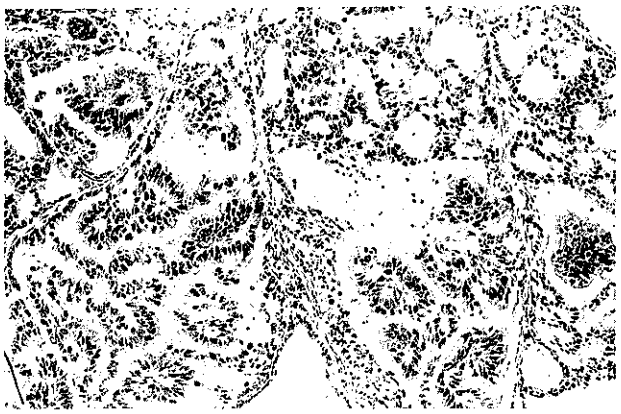

Fig. 4. Portion of Fig. 3 at higher magnification. Note the prominent papillary pattern, loss of nuclear and cellular polarity and increased mitotic figures. H & E, ×200.

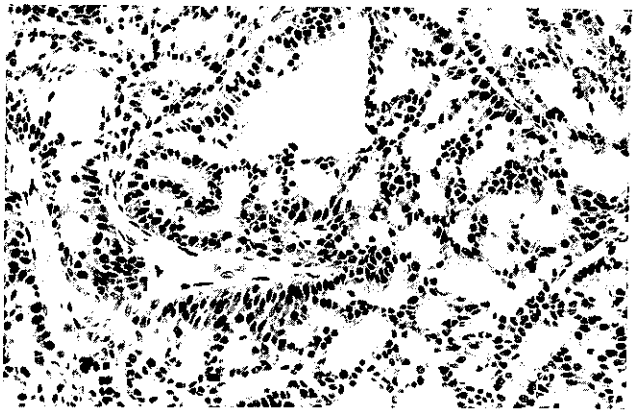

Fig. 5. Ventral prostate carcinoma. A cribriform pattern of cells is apparent. H & E, ×200.

Table II. Incidences and Numbers of Atypical Hyperplasias and Carcinomas of the Ventral Prostate of Rats Given DMAB and Cadmium

Group	Treatment[a]	No. of rats[b]	Ventral prostate				Seminal vesicles
			Atypical hyperplasia (%)	Carcinomas			Atypical hyperplasia (%)
				No. rats (%)	No. per rat[c] (total no.)	No. per tissue[c, d]	
1	Cd(H)+DMAB	16	16 (100)	11 (68.8)	1.00±0.89 (16)	0.62±0.62	13 (81.3)
2	Cd(L)+DMAB	16	14 (87.5)	12 (75.0)	1.19±0.91 (19)	0.64±0.55	14 (87.5)
3	DMAB+Cd(H)	8	7 (87.5)	4 (50.0)	1.00±1.41 (8)	0.73±0.95	6 (75.0)
4	DMAB+Cd(L)	16	15 (93.8)	13 (81.3)[e]	1.56±1.37 (25)[e]	0.90±0.73[e]	16 (100)
5	DMAB→Cd(L)×2	16	14 (87.5)	12 (75.0)	1.94±1.69 (31)[g]	1.21±1.14[f]	16 (100)
6	DMAB→Cd(L)×4	13	12 (92.3)	8 (61.5)	0.85±0.80 (10)	0.51±0.58	11 (84.6)
7	DMAB	19	17 (89.5)	9 (47.4)	0.68±0.89 (13)	0.50±0.58	18 (94.7)

a) Cd(H); 30 μmol cadmium/kg, Cd(L); 10 μmol/kg. (The last dose of Cd in group 5 and the last 3 doses in group 6 were 5 μmol/kg.)
b) Numbers of animals surviving for 60 weeks. One rat in group 3 was excluded because a full histological evaluation could not be performed.
c) Mean±SD.
d) The numbers are expressed per unit ventral prostate section (100 mm^2).
Significantly different from the group 7 value at *e*) $P<0.05$, *f*) $P<0.025$ and *g*) $P<0.001$.

Table III. Incidences of Atypical Hyperplasias and Carcinomas of the Ventral Prostate of Rats Given Cadmium Alone

Group	Treatment[a]	Effective No. of rats[b]	Ventral prostate		Seminal vesicles
			Atypical hyperplasia (%)	Carcinomas (%)	Atypical hyperplasia (%)
8	Cd(H)	15	0	0	1 (6.7)
9	Cd(L)	15	1 (6.7)	1 (6.7)	1 (6.7)
10	Cd(H)	11	0	0	1 (9.1)
11	Cd(L)	14	1 (7.1)	1 (7.1)	1 (7.1)
12	Cd(L)×2	12	0	0	0 –
13	Cd(L)×4	14	2 (14.3)	0	2 (14.3)

a) Cd(H); 30 μmol cadmium/kg, Cd(L); 10 μmol/kg. (The last dose of Cd in groups 12 and the last 3 doses in group 13 were 5 μmol/kg.)
b) Numbers of animals surviving for 60 weeks.

Table IV. Weights and Lesions of the Testes

Group	Treatment[a]	No. of rats[b]	Testicular weight (g)[c]	No. (%) of rats with	
				Severe atrophy	Leydig cell tumors
1	Cd(H)+DMAB	16	1.83±1.11[d,f]	9 (56.3)[e]	9 (56.3)
2	Cd(L)+DMAB	16	2.79±0.66	3 (18.8)	3 (18.8)
3	DMAB+Cd(H)	9	1.52±0.68[d]	7 (77.8)[e]	5 (55.6)
4	DMAB+Cd(L)	16	1.93±0.64[d]	14 (87.5)[e]	12 (75.0)[e]
5	DMAB→Cd(L)×2	16	1.30±0.39[d]	16 (100)[d]	12 (75.0)[e,h]
6	DMAB→Cd(L)×4	13	1.65±0.45[d,g]	11 (84.6)[e]	8 (61.5)[i]
7	DMAB	19	3.04±0.44	2 (10.5)	5 (26.3)
8	Cd(H)+vehicle	15	1.32±1.02[f]	11 (73.3)	10 (66.7)
9	Cd(L)+vehicle	15	2.54±0.95	4 (26.7)	4 (26.7)
10	vehicle+Cd(H)	11	1.54±0.57[g]	10 (90.9)	9 (81.2)
11	vehicle+Cd(L)	14	2.07±0.58	11 (78.6)	7 (50.0)
12	vehicle→Cd(L)×2	14	1.85±0.62	11 (78.6)	3 (21.4)
13	vehicle→Cd(L)×4	14	1.86±0.58	11 (78.6)	2 (14.3)

a) Cd(H); 30 μmol cadmium/kg, Cd(L); 10 μmol/kg. (The last dose of Cd in groups 5 and 12 and the last 3 doses in groups 6 and 13 were 5 μmol/kg.)
b) Numbers of animals surviving for 60 weeks.
c) The weights represent the sum of both testicles (mean±SD).
d) Significantly different from the group 7 values at $P<0.001$.
e) Significantly different from the group 7 values at $P<0.01$.
f) Significantly different from each corresponding group (group 2 or 9) given the low dose of cadmium at $P<0.01$.
g) Significantly different from each corresponding group (group 5 or 11) given the low dose of cadmium at $P<0.05$.
h) Significantly different from group 12 at $P<0.01$.
i) Significantly different from group 13 at $P<0.05$.

100% in the seminal vesicle. Carcinoma incidence in the ventral prostate ranged from 47.4 to 81.3%, and the incidence values in the experimental groups given DMAB and cadmium were all higher than in the control value, but only the difference between groups 4 and 7 was statistically significant ($P<0.05$). The numbers of carcinomas per rat in the cadmium plus DMAB groups were also higher than in the DMAB control; the differences between groups 4 and 5 and group 7 were significant. Statistical evaluation of the numbers of carcinoma per unit area of ventral prostate section also revealed that the values in groups 4 and 5 were significantly higher than that of group 7. When the tumor yields were compared between high and low doses of cadmium, i.e., groups 1 vs. 2, 3 vs. 4, and 5 vs. 6, the incidences and numbers were always higher, albeit not significantly, in the lower cadmium dose groups. Prostate tumors were also present in groups given cadmium alone but the incidences were very low (Table III). No observable histological lesions were present in the dorso-lateral and anterior lobes of the prostate in any group.

Testicular atrophy With the exception of group 2, testicular weights of animals in groups given DMAB and cadmium were all significantly lower than the values for rats given DMAB alone. A high dose or large total dose of cadmium reduced the testicular weight to roughly 60% of the control value (Table IV). Histological examination revealed atrophied testes with loss of the normal seminiferous tubular structures, and most tubules contained mineralized materials (Fig. 6). There were, however, some cases without any atrophy. Leydig cell tumors frequently occurred in such severely atrophied testes.

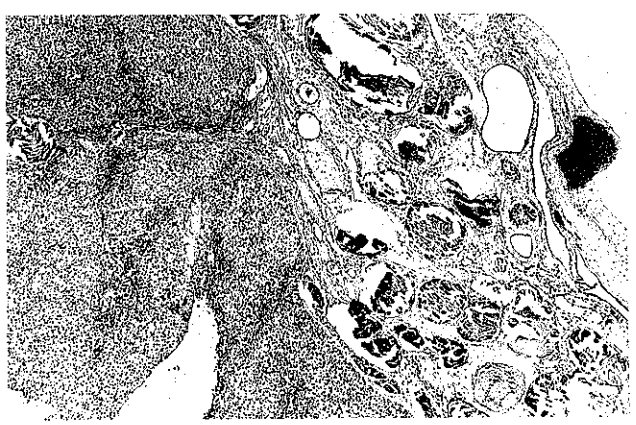

Fig. 6. Testicular atrophy and a Leydig cell tumor in a rat given a high dose of cadmium and DMAB. Note complete destruction of the seminiferous tubules. H & E, ×100.

Table V. Serum Testosterone Levels in Rats Given DMAB and Cadmium

Group	Treatment	No. of samples	Testosterone level (ng/ml)[a]
1	Cd(H)+DMAB	4	0.88±0.41
2	Cd(L)+DMAB	4	0.80±0.22
6	DMAB→Cd(×4)	4	0.53±0.30
7	DMAB	4	0.78±0.51

Only selected groups were subjected to measurement of testosterone levels.
a) Mean±SD.

Serum levels of testosterone Measurement of serum testosterone levels in selected animals did not reveal any statistically significant differences between rats given DMAB plus cadmium and those given DMAB alone (Table V).

Lesions in other organs Tumor development was observed in a variety of organs including the intestines, preputial gland, lung and subcutis. The incidences were all very low and there were no significant differences among the experimental groups. Cadmium-related tumors were often present at the right thigh (i.e., the injection site). Among a total of 32 injection-site tumors, 28 were histologically malignant fibrous histiocytoma, 1 was a rhabdomyosarcoma and 3 were fibromas. Including the animals which died early, the numbers of rats bearing these tumors were 2, 1, 9, 4, 3, 0 and 0 for groups 1 to 7 (20 animals each), respectively, and 4, 1, 2, 0, 0, and 0 for groups 8 to 13 (15 each), respectively. The value in group 3 is significantly higher than that in group 7 at $P<0.01$.

DISCUSSION

Cadmium chloride has been shown to induce ventral prostate tumors in Wistar rats after a single s.c. or i.m. injection in 2-year experiments.[12, 13] Direct administration of cadmium into rat ventral prostate also proved carcinogenic at the injection site.[11] The data from long-term experiments with a single administration suggest that the carcinogenicity of cadmium for the rat prostate is, however, relatively weak, because of the low tumor incidences and long latency.[12, 13] In the present 60-week experiment, cadmium itself induced only a small number of prostate tumors, including preneoplastic atypical hyperplasias (groups 8 to 13). Since neither atypical hyperplasias nor tumors in the prostate were detected in non-treated control F344 rats (a total of 43 animals) in our previous 60-week experiments,[14, 15] the finding of the development of prostate lesions in groups given cadmium alone nevertheless supports cadmium's carcinogenic potential. Repeated DMAB application has been found to be a good approach for investigation of mechanisms of prostate carcinogenesis,[16–21] the primary target being the ventral lobe. In the present experiment, combined treatment with DMAB and cadmium resulted in higher incidences and larger numbers of prostate carcinomas than in the control group receiving DMAB alone. This finding further demonstrates that cadmium can enhance DMAB rat prostate carcinogenesis.

There is conflicting evidence regarding cadmium genotoxicity in terms of mutations, chromosomal aberrations or sister chromatid exchanges.[22] However, the demonstration of genotoxicity in some *in vivo* and *in vitro* systems[22] and the *in vitro* transformation of prostate epithelial cells[23] indicate that cadmium interacts directly and/or indirectly with the DNA of target cells. Therefore, the enhancing effect of cadmium on prostate carcinogenesis might be based on syncarcinogenics rather than on promotion. This question requires further study, especially in view of the fact that significant enhancement was only found when cadmium was administered after DMAB treatment. The tumor yields after combined treatment with both agents exceeded the sums of the individual treatment yields, clearly indicating that they worked in a synergistic fashion.

Cadmium is toxic to rodent testes, and its administration eventually results in testicular atrophy and development of Leydig cell tumors.[11, 12, 24] Based on the data of a dose-response study, Waalkes *et al.*[12] stated that cadmium induces prostate tumors only at doses well below those causing marked degeneration of the testes. In their experiment, a single i.m. injection of cadmium at a dose of 30 μmol/kg bw did not induce testicular atrophy, but did cause prostate tumors in 42% of rats. Based on this finding, the dose of 30 μmol/kg bw was selected as the high dose in the present experiment. However, as the results show, even the lower dose of 10 μmol/kg bw induced marked atrophy of the testes of F344 rats when given at week 20. Since Waalkes *et al.*[12, 13] used Wistar rats, strain difference may be a factor in explaining this discrepancy. More pronounced suppression of body weights and higher incidences of testicular atrophy in groups 3 and 4 as compared with groups 1 and 2 are probably attributable to the differences in dosage of cadmium because of the heavier weights in the former groups and/or the higher susceptibility to cadmium toxicity of older animals. It is possible that the DMAB treatment increased the sensitivity to cadmium.

The degree of testicular atrophy or the incidence of Leydig cell tumors, which reflect testicular toxicity of cadmium, did not negatively correlate with the prostate tumor yields. From the data shown in Table V, it appears that serum testosterone levels are unaffected by cadmium administration even when testicular atrophy is apparent.

This conclusion is supported by the finding that there was no consistent suppression of prostate or seminal vesicle weights among the cadmium groups. Leydig cell tumors, which were frequently observed in rats associated with marked testicular atrophy in the present experiment, are considered to be non-functioning in terms of androgen production.[25,26] The development of substantial numbers of prostate tumors even in the presence of severe testicular atrophy (groups 1, 3–6) also implies that circulating androgen levels were not greatly affected. This is in line with the lack of differences in the measured serum testosterone levels among groups (Table V). This finding of such disagreement between testicular atrophy and androgen levels suggests that cadmium toxicity was exerted in spermatogonia rather than the Leydig cells. The reasons for the greater tumor yields and incidences in the groups given DMAB plus the lower dose of cadmium as compared with groups receiving DMAB plus the higher dose are unclear. Circulating androgen levels could not explain this difference in the tumor response. The data of Waalkes *et al.*[12] similarly indicated a dose-independence for induction. In their experiment, prostatic tumor yields were not elevated at higher doses even though there was no testicular degeneration. Their data seem to be in agreement with the present experimental results. Thus, the carcinogenic character of cadmium for the prostate does not appear to be the same as those of well-known chemical carcinogens which exert a clear dose-related carcinogenicity. This specific feature of cadmium might be due to a balance between its carcinogenicity and its toxicity. Further investigation is needed to clarify this point.

The finding of only a few prostatic carcinomas in animals receiving cadmium alone (groups 8–13) was probably due to the short experimental period compared to the 2-year period of their study.

Cadmium was also reported to induce pancreatic adenomas in Wistar rats.[12,13] However, no pancreatic tumors were noted in the present study, with or without DMAB, which was also recently shown to be tumorigenic in the pancreas.[27]

In summary, while the mechanisms underlying prostate tumorigenesis of cadmium remain unknown, this metal can clearly act synergistically with other carcinogens to cause development of prostate carcinomas. The present findings raise the possibility that occupational cadmium-related prostate carcinomas in man may have resulted in part from combined exposure with other unknown carcinogen(s).

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