Jpn. J. Cancer Res. 80, 312–316, April 1989

Effects of Age on Multiple Organ Carcinogenesis Induced by 3,2′-Dimethyl-4-aminobiphenyl in Rats, with Particular Reference to the Prostate

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The effects of age on multi-organ carcinogenesis induced by 3,2′-dimethyl-4-aminobiphenyl (DMAB) in male F344 rats were examined. Groups of 5-, 35-, and 65-week-old animals were given 4 weekly sc injections of DMAB at a dose of 200 or 150 mg/kg body weight. Prostate carcinomas were induced in 8 to 19% of rats treated, no significant differences in the incidence between different ages being observed. Tumors in the small intestine, skin, pancreas and peritoneum, however, developed more frequently in young than in old animals, whereas higher incidences of testis, preputial and mammary gland lesions were found in the 35- and/or 65-week-old groups. Colon and Zymbal gland carcinogenesis did not reveal any age dependence.

Key words: 3,2′-Dimethyl-4-aminobiphenyl — Rat — Carcinogenesis — Age — Prostate

Although a number of chemical carcinogens, including 3,2′-dimethyl-4-aminobiphenyl (DMAB),[1-3] N-methylnitrosourea[4] and N-nitrosobis(2-oxopropyl)-amine,[5] can induce rat prostate carcinomas, as yet no model has proved satisfactory for studying all the aspects. However, it has been shown that with all three carcinogens the tumorigenic potential to the prostate is enhanced if administration is timed to coincide with cell proliferation occurring in the prostate epithelium.[1-5] For example, it was demonstrated in our laboratory that DMAB can induce a high incidence of prostate carcinomas in rats when synchronized with a cyclic dietary administration of ethinyl estradiol.[2,3] The carcinomas induced, however, were all of the *in situ* type and tumor development in other organs frequently resulted in early death.

Development of carcinoma of the prostate in man has an age dependency, being predominantly found in the aged.[6,7] Spontaneous prostate tumors in rats also develop late in life.[8,9] However, many chemical carcinogenesis experiments have indicated that young animals are more sensitive than old ones.[10-14] Whether the available data only reflect the long time period required for neoplasia to develop, or whether other age factors are involved remains, however, unclear. The prostate carcinogen DMAB is an aromatic amine which requires metabolic activation to exert its carcinogenicity,[15] and it is very likely that cell proliferative conditions in target organs and variation in enzymatic potential for activation or detoxification of carcinogens might be age-dependent and therefore might influence the carcinogenic response.

Whether the prostate, like the mammary glands, is particularly sensitive to carcinogen, resulting in the development of carcinomas,[16,17] at a specific age range of the animal remains unknown. Cell proliferating activity and hormonal status, proposed to be important factors determining the high sensitivity in the breast[16,17] might also be involved in the prostate.

The present study was conducted to clarify the effect of age on induction of tumors by DMAB, with particular reference to prostate tumors, in persuance of our long term aim to establish a satisfactory animal model of human prostate cancer.

MATERIALS AND METHODS

Four-week-old male F344 rats were purchased from Charles River Japan Inc., Kanagawa. The animals were housed in plastic cages with hard wood chip bedding, in an air-conditioned room with a 12 h-12 h light-dark cycle, and were given food (Oriental MF; Oriental Yeast Co., Tokyo) and water *ad libitum*. DMAB with a purity of more than 98% was obtained from Matsugaki Pharmaceutical Co., Osaka.

Experiment 1 Animals were divided into 3 groups according to the age at which administration of DMAB was started; 4 weekly sc injections of DMAB at a dose of 200 mg/kg body weight were given beginning at 5 weeks of age in group 1, at 35 weeks of age in group 2, and at 65 weeks of age in group 3, 25 rats each group. However, all 65-week-old rats which received DMAB at this dose died within 1 week of the first injection. All surviving animals in groups 1 and 2 were sacrificed 60 weeks after the first injection of DMAB.

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Experiment 2 Because of the unforeseen death of all animals in group 3 due to DMAB toxicity in Experiment 1, a further study comparing 5-week-old rats and 65-week-old rats (25 and 30 animals, respectively) was performed with the dose reduced to 150 mg/kg body weight. The experiment was terminated 60 weeks after the first administration of DMAB.

At autopsy, all organs were examined for gross abnormalities, and slices were taken from the major organs as described previously[1] and fixed in 10% buffered formalin. Paraffin sections (4 μm) were stained with hematoxylin and eosin for histological examination.

RESULTS

Average body weights at different time points are summarized in Table I. Administration of DMAB at either 200 or 150 mg/kg decreased the body weights of 35- and 65-week-old animals to about 80% of the original values 5 weeks after the first injection. Although the body weights of the 5-week-old group increased during the treatment, the body weight gain was reduced by about 15% as compared to normal untreated age-matched control F344 rats. The mortality rate of the 5-week-old rats treated with 150 mg/kg was similar to that of the 35-week-old rats given 200 mg/kg and less than that of the 5-week-old rats receiving 200 mg/kg. The 65-week-old rats in Experiment 2 had all died by experimental week 46.

Incidence data for lesions of the prostate and seminal vesicles are summarized in Table II. Atypical hyperplasias and carcinomas of the ventral prostate, and atypical hyperplasias of the dorsal prostate and seminal vesicles were observed but their incidences were low and no significant differences between groups, except for the incidence of atypical hyperplasia of seminal vesicles between the 2 groups in the Experiments 1 and 2, were observed. The differences in incidences between the 2 doses, 200 and 150 mg of DMAB, in the 5-week-old rats were also not significant.

The tumorigenic responses in the small intestine, on the other hand, did differ with age (Table III). In both of the experiments, the incidences and numbers of tumors of the small intestine in the 5-week-old rats were higher than in older rats. No such variation was evident with regard to colon lesions.

Tumor development in other organs, including the Zymbal glands, preputial glands, mammary glands, skin, peritoneum and testes, is summarized in Table IV. At least in Experiment 1, the incidences of tumors of the

Table I. Average Body Weights (g) of Rats Given DMAB

Experimental week	Experiment 1[a]		Experiment 2	
	Groups[b] 1	2	1	2
1	116	410	88	465
2	117	404	111	415
3	138	375	137	398
4	161	357	165	379
10	256	372	284	371
20	318	403	335	387
30	361	411	375	377
40	376	420	391	376
50	368	419	403	—
60	362	451	406	—

a) Data on 65-week-old rats are not available because all these animals died within the first experimental week.
b) Groups 1 and 2 in Experiment 1 represent 5- and 35-week-old rats treated with 200 mg/kg DMAB, and those in Experiment 2 represent 5- and 65-week-old rats treated with 150 mg/kg DMAB, respectively.

Table II. Incidences (%) of Atypical Hyperplasias and Carcinomas of the Prostate of Rats Treated with DMAB at Various Ages

Age at first DMAB injection (weeks)	Dose of DMAB (mg/kg)	Effective No. of rats	Prostate			Seminal vesicles
			Ventral		Dorsal	
			Atypical hyperplasia	Carcinoma	Atypical hyperplasia	Atypical hyperplasia
Experiment 1						
5	200	25[a]	12 (48.0)	2 (8.0)	0	12 (48.0)
35	200	21[a]	7 (33.3)	4 (19.0)	2 (9.5)	1 (4.8)
Experiment 2						
5	150	24[a]	18 (75.0)	3 (12.5)	0	10 (41.7)
65	150	22[b]	10 (45.5)	3 (13.6)	0	1 (4.5)

a), *b*) Effective numbers represent the numbers of rats which survived for longer than 38 weeks (*a*) and for longer than 16 weeks (*b*) after starting the experiment.

Table III. Incidences and Frequency of Tumors in the Small Intestine and Colon of Rats Treated with DMAB at Various Ages[a]

Age at first DMAB injection (weeks)	Dose of DMAB (mg/kg)	Effective No. of rats[b]	Small intestine		Colon	
			Incidence (%)	No./rat[c]	Incidence (%)	No./rat[c]
Experiment 1						
5	200	25*	11 (44.0)	0.68±0.95	15 (60.0)	1.40±1.44
35	200	21*	3 (14.3)[d]	0.18±0.50[d]	18 (85.7)	2.10±1.80
Experiment 2						
5	150	24*	7 (29.2)	0.33±0.56	13 (54.2)	0.83±0.96
65	150	22**	1 (4.5)[d]	0.05±0.21[d]	12 (54.5)	1.18±1.50

a) Tumors were all adenomas or adenocarcinomas.
b) Effective numbers represent numbers of rats which survived for 38 weeks or longer (*), or for longer than 16 weeks (**).
c) Mean±SD.
d) Significantly different from the corresponding 5-week-old group at $P<0.05$.

Table IV. Incidences of Tumors in the Zymbal Glands, Preputial Glands, Mammary Glands, Skin, Peritoneum and Testis of Rats Treated with DMAB at Various Ages

Age at first DMAB injection (weeks)	Effective No. of rats[a]	No. (%) of rats with tumors in the					
		Zymbal glands[b]	Preputial glands[c]	Mammary glands[d]	Skin[e]	Peritoneum[f]	Testis[g]
Experiment 1							
5	25	4 (16.0)	4 (16.0)	4 (16.0)	8 (32.0)	6 (24.0)	0 —
35	21	1 (4.8)	11 (52.4)*	13 (61.9)**	1 (4.8)*	0 —*	14 (66.7)***
Experiment 2							
5	24	1 (4.2)	6 (25.0)	5 (20.8)	5 (20.8)	5 (20.8)	2 (8.3)
65	22	4 (18.2)	2 (9.1)	7 (31.8)	4 (18.2)	0 —*	21 (95.5)***

a) Number of rats as in Table III.
b) Adenomas and carcinomas.
c) Adenomas and adenocarcinomas.
d) Fibroadenomas.
e) Sebaceous adenomas and adenocarcinomas and malignant fibrous histiocytomas.
f) Mesotheliomas.
g) Interstitial cell tumors.
Significantly different from the corresponding 5-week-old group at * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Table V. Incidences of Pancreatic Tumors

Age at first DMAB injection (weeks)	Effective No. of rats[a]	Acinus				Islets Adenoma
		Focus	Nodule	Adenoma	Carcinoma	
Experiment 1						
5	19	19 (100)	16 (84)	1 (5)	0 —	5 (26)
35	17	12 (71)*	1 (6)*	1 (6)	1 (6)	2 (12)
Experiment 2						
5	19	19 (100)	14 (74)	3 (16)	0 —	1 (5)
65	19	13 (68)**	4 (21)**	0 —	0 —	2 (11)

a) Effective numbers represent the numbers of rats available for histological evaluation.
Significantly different from the corresponding 5-week-old group at * $P<0.05$ and ** $P<0.01$.

preputial glands and mammary glands in the 35-week-old group were significantly higher than in the 5-week-old group, while opposite tendencies were apparent for the skin and peritoneum.

Pancreatic acinar tumors were classified as foci, nodules, adenomas and carcinomas (Table V), the first 3 types of lesions being divided according to their size; less than 1 mm in diameter, 1 to 3 mm, and 3 mm and larger, respectively. In both Experiments 1 and 2, acinar foci and nodules developed more frequently in the 5-week-old groups. There was no inter-group difference with regard to the yield of islet adenomas.

DISCUSSION

The first step of activation of DMAB is believed to be N-hydroxylation and thereafter several pathways have been proposed[15, 18] which yield DMAB metabolite(s) with general toxic effects as well as carcinogenicity. In the present study, the dose of 200 mg/kg of DMAB was lethal to the 65-week-old ("old") rats but not to the 5- ("young") or 35-week-old ("young adult") rats, indicating that there was a clear difference in the toxicity of DMAB between young and old rats. The difference might be attributed to elevated levels of activating enzyme(s) and/or lowered capability of detoxifying enzyme(s). The survival rate of the old rats was very poor and necessitated termination of the experiment earlier than scheduled even when the dose of DMAB was reduced to 150 mg/kg. Reduction of the dose of DMAB to 150 mg/kg, however, did not result in significantly decreased incidence of any type of tumors in comparison with young rats given the 200 mg/kg dose. No significant differences in the incidences of atypical hyperplasia and carcinomas of the prostate between groups were apparent. Nor was any trend perceived suggesting that the incidences in old rats would have increased if the animals had lived as long as scheduled. Thus there was no "advantageous" or "disadvantageous" age for carcinogen administration in terms of prostate carcinoma induction. The low incidences of prostate carcinomas in the present study further indicate that the 4 weekly administrations of DMAB under the described conditions were not sufficient to give suitable tumor yields.

The results of the present experiment indicate that young rats are more susceptible than either young adult or old rats with regard to tumorigenesis in the small intestine but not in the colon. Anisimov earlier reported that dimethylhydrazine-induction of colon tumors was more efficient in young than in old animals (see ref. 11), suggesting a correlation with the age-associated decrease of stem cell proliferation in colon crypts. However, the present data suggest that other factors such as enzymatic activation may be more directly involved than proliferative activity because of the different tumorigenic response observed between the small intestine and colon. The age-related increase evident for tumors of the preputial gland, mammary gland and testis also requires explanation. The increase of testicular tumors was clearly associated with age rather than DMAB carcinogenicity because the incidences were comparable to those reported for spontaneously developing lesions.[19] The other 2 types of tumors, however, were presumably related to carcinogen administration because the yields were clearly higher than those normally encountered spontaneously in male F344 rats, these being 4.9 to 9.4% for preputial gland adenomas and carcinomas and 0 to 8% for mammary fibroadenomas.[19, 20]

Development of pancreatic acinar cell tumors in rats treated with DMAB has recently been demonstrated in our laboratory.[3] The higher susceptibility demonstrated for young animals is similar to that observed in azaserine- or N-nitrosobis(2-oxopropyl)amine-treated rats.[14, 21]

In a series of rat tumor induction experiments with DMAB, we found that carcinomas of the preputial glands, Zymbal glands, subcutis, colon and mesotheliomas were major contributing causes of death. Lowering the incidences of these tumors is essential if a more efficient induction of prostate carcinomas is to be achieved, because this goal will require that the animals survive longer.

In conclusion, the present data clearly showed that the effects of age on DMAB carcinogenesis depend on the target organ. The experimental model should be useful for future elucidation of the various underlying mechanisms.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and from the Ministry of Health and Welfare and a Grant-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control.

(Received December 28, 1988/Accepted February 14, 1989)

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