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Dose Dependence of N-Hydroxy-3,2′-dimethyl-4-aminobiphenyl-induced Rat Prostate Carcinogenesis

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Groups of F344 rats were administered biweekly intraperitoneal injections of N-hydroxy-3,2′-dimethyl-4-aminobiphenyl (N-OH-DMAB) at a dose of 5, 10 or 20 mg/kg body weight or DMAB, the parent compound, at a dose of 25 mg/kg body weight, for a total of 10 times. Prostate carcinomas in the ventral lobe developed in a N-OH-DMAB dose-dependent manner (0, 17.6 and 66.7%, respectively) with limited tumor yields in other organs. Although intraperitoneal administration of DMAB was similarly found to induce prostate tumors, it also caused severe chemical peritonitis, which resulted in a high mortality. The present data confirmed that intraperitoneal administration of N-OH-DMAB provides a relatively specific induction method for models of prostate carcinogenesis.

Key words: N-Hydroxy-3,2′-dimethyl-4-aminobiphenyl — Rat — Prostate — Carcinogenesis — Dose response

N-Hydroxy-3,2′-dimethyl-4-aminobiphenyl (N-OH-DMAB), a proximate carcinogen which is metabolized from DMAB by cytochrome P-450(s), selectively induces rat ventral prostate carcinomas when given intraabdominally.[1] The parent compound, DMAB, is generally applied subcutaneously and exerts carcinogenic potential in a variety of organs,[2–4] which is a disadvantage in investigations aimed at elucidating aspects of prostate neoplasia. The finding that N-OH-DMAB given intraperitoneally only induced small numbers of non-prostatic lesions,[1] indicated that the route of administration might play an important role in determining response to aminobiphenyl carcinogens. This might be due to resultant differences in chemical distribution and tissue metabolization (activation and/or detoxification).[1]

In the present experiment, aimed at optimizing our model of prostate cancer, we investigated the dose-dependence of rat prostate carcinoma induction by N-OH-DMAB. To compare the carcinogenic potential of N-OH-DMAB with that of DMAB, intraperitoneal administration of DMAB was also examined.

MATERIALS AND METHODS

Five-week-old male F344 rats were purchased from Charles River Japan Inc., Kanagawa. The animals were maintained 5 to a plastic cage on wood chip bedding 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 water *ad libitum*. N-OH-DMAB was prepared in NADO Institute, Ltd. (Amagasaki), according to the method of Hech *et al.*[5] The experiment was started when the animals were 6 weeks old. Rats were divided into 5 groups (20 animals each for Groups 1 to 4 and 15 for Group 5) and those of Groups 1 to 3 were given N-OH-DMAB intraperitoneally every other week for 20 weeks (a total of 10 injections) each time at a dose of 5, 10 or 20 mg/kg body weight, respectively, while Group 4 was given DMAB in the same manner at a dose of 25 mg/kg body weight. The reason why DMAB was given intraperitoneally instead of subcutaneously was to compare the carcinogenic potential of the two carcinogens on the basis of the same administration route. Both carcinogens were dissolved in dimethyl sulfoxide (DMSO). Selection of the doses of N-OH-DMAB was based on the data from our previous experiment.[1] An earlier toxicity study demonstrated 20 mg of N-OH-DMAB/kg body weight to be the maximum tolerable dose for F344 rats when given intraperitoneally, because at the level of 30 mg all animals died within 2 weeks (unpublished data). The dose of DMAB originally selected was 50 mg/kg, but since a satellite short-term experiment revealed this dose to be too high for intraperitoneal injection, 25 mg was used. Group 5 received the vehicle. After treatment with carcinogen, animals were maintained without any further chemical administration until week 60, when all surviving animals were killed and subjected to autopsy. All organs including observable abnormal lesions were fixed in 10% buffered formalin and processed for routine histological examination. Paraffin sections were stained with hematoxylin and eosin. Differences in tumor incidences were analyzed statistically by using Fisher's exact probability test and trends in incidences were examined by the Cochran-Armitage test.

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RESULTS

While growth rates of rats in all carcinogen-treated groups were similar to that of the controls, many rats given intraperitoneal injection of DMAB suffered early mortality. The major cause of death was considered to be ileus due to chemically induced peritonitis. Intraperitoneal administration of carcinogens dissolved in DMSO is a source of irritation which results in the development of inflammation. No significant intergroup differences in final average body, liver and prostate weights were evident (Table I).

Neoplastic lesions of the prostate and seminal vesicles were classified as described previously.[5, 6] All carcinomas and atypical hyperplasias of the prostate were located in the ventral lobe, and no neoplastic changes were evident in either the lateral or dorsal lobes. Carcinomas and atypical hyperplasias of the ventral prostate in groups given N-OH-DMAB induced dose-dependently ($P<0.01$); the incidences of carcinoma were 0 for 5 mg, 17.6% for 10 mg and 66.7% for 20 mg, this latter being significantly higher than that of the vehicle control (Table II). One case of seminal vesicle carcinoma was observed in a rat given the 20 mg dose of N-OH-DMAB. The incidences of atypical hyperplasias of the seminal vesicles in rats given N-OH-DMAB also showed dose-dependent increase ($P<0.001$). Carcinomas of the ventral prostate were all adenocarcinomas demonstrating cribriform patterns, and no invasive growth was evident, in line with previous reports.[1-3] The malignant lesion in the seminal vesicles was an invasive adenocarcinoma accompanied with abundant fibrous connective tissue.[6]

Data for tumor development in organs other than the accessory sex organs are summarized in Table III. The most common tumors were mesotheliomas of the peritoneal cavity followed by colon adenomas or carcinomas, both of which developed in an N-OH-DMAB dose-dependent manner in the peritoneal cavity ($P<0.01$ and $P<0.05$, respectively). Early lesions were localized on the surfaces of the testes. Other types of tumor were only sporadically observed.

DISCUSSION

No apparent long-term suppression of body or prostate weights by N-OH-DMAB at the doses given was ap-

Table I. Mean Body and Organ Weights of Rats Given N-OH-DMAB or DMAB

Groups	Effective No. of rats[a]	Body	Liver	Prostate	
				Ventral	Dorsolateral
1. N-OH-DMAB 5 mg	11	438.2±48.6[b]	13.3±1.3	0.41±0.08	0.61±0.10
2. N-OH-DMAB 10 mg	17	432.3±49.2	13.6±1.3	0.36±0.08	0.61±0.09
3. N-OH-DMAB 20 mg	12	446.2±27.1	13.6±1.4	0.37±0.08	0.69±0.08
4. DMAB 25 mg	4	413.9±83.1	13.3±2.4	0.33±0.10	0.58±0.11
5. DMSO	13	449.8±30.5	12.8±1.1	0.41±0.09	0.63±0.09

a) This includes all animals that survived for 60 weeks.
b) Mean (g)±SD.

Table II. Incidences of Carcinomas and Atypical Hyperplasias in the Prostate and Seminal Vesicles of Rats Given N-OH-DMAB or DMAB

Groups	Effective No. of rats	Ventral prostate		Seminal vesicles	
		Carcinoma	Atypical hyperplasia	Carcinoma	Atypical hyperplasia
1. N-OH-DMAB 5 mg	11	0	5 (45.5)[a]	0	7 (63.4)[b]
2. N-OH-DMAB 10 mg	17	3 (17.6)	10 (58.8)[b]	0	11 (64.7)[b]
3. N-OH-DMAB 20 mg	12	8 (66.7)[b]	12 (100)[b]	1 (8.3)	12 (100)[b]
4. DMAB 25 mg	4	1 (25.0)	2 (50.0)[c]	0	4 (100)[b]
5. DMSO	13	0	0	0	0

a) Significant difference ($P<0.01$) from Group 5.
b) Significant difference ($P<0.001$) from Group 5.
c) Significant difference ($P<0.05$) from Group 5.

Table III. Incidences (%) of Tumors in Organs Other than the Prostate

Groups	Effective No. of rats	No. (%) of rats with tumors in the						
		Peritoneum[a]	Small intestine[b]	Colon[b]	Subcutis[c]	Ear duct[d]	Tongue[e]	Preputial glands[f]
1. N-OH-DMAB 5 mg	11	3 (27.3)	0	1 (9.9)	0	0	0	0
2. N-OH-DMAB 10 mg	17	6 (35.3)	2 (11.8)	1 (5.9)	0	0	1 (5.9)	1 (5.9)
3. N-OH-DMAB 20 mg	12	8 (66.7)	2 (16.7)	5 (41.7)	0	0	0	0
4. DMAB 25 mg	4	2 (50.0)	1 (25.0)	1 (25.0)	2 (50.0)	1 (25.0)	1 (25.0)	0
5. DMSO	13	0	0	0	0	0	0	0

a) Mesotheliomas. *b*) Adenomas or adenocarcinomas. *c*) Malignant neurinomas.
d) Adenoma. *e*) Squamous cell carcinomas. *f*) Cystic adenocarcinoma.

parent although the body weights of animals receiving DMAB were slightly decreased, suggesting that administration into the intra-abdominal cavity instead of the subcutis is more toxic.

The present data confirmed our previous finding that N-OH-DMAB is a particularly effective agent for induction of prostate carcinomas in F344 rats. Tumorigenesis in response to N-OH-DMAB in the ventral prostate showed a clear dose-related increase. Although in the previous study, the 5 mg dose of N-OH-DMAB using the same protocol induced a 42% incidence of ventral prostate carcinomas, no malignant lesions were found at this level in the present experiment. The reason for this inconsistency is unknown.

In all our experiments performed in the past, DMAB was given subcutaneously and no data on the carcinogenicity of DMAB administered intraperitoneally have been available. The present study revealed that DMAB can also induce prostate carcinomas as well as other types of tumors when administered by the intra-abdominal route. However, DMAB proved more irritant to the peritoneum than N-OH-DMAB, resulting in a higher mortality due to peritonitis. This finding indicates that injection into the abdominal cavity is not the most suitable route of administration of DMAB.

In the present study with N-OH-DMAB, tumor development in organs other than the prostate, particularly in the skin/subcutis, preputial and Zymbal glands and colon, was also very limited, as compared to our previous data for subcutaneous administration of DMAB. However, the finding of relatively high incidences of mesothelioma and colon tumors in the group repeatedly given the 20 mg dose of N-OH-DMAB indicates that the optimum level of carcinogen exposure might be less than 10 mg/kg at one administration.

While the present results do suggest that intraperitoneal administration of N-OH-DMAB may provide a better induction method for models of prostate carcinogenesis, it is clear that an even more selective induction of prostate carcinomas would be optimal. Synchronized administration of carcinogen with prostatic cell proliferation may be a possible approach to achieving this purpose, as demonstrated by our earlier studies.[3,7] Furthermore, prevention of chemical peritonitis should also be targeted in establishing a superior model.

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