Selective Induction of Prostate Carcinomas in F344 Rats Treated with Intraperitoneal Injections of N-Hydroxy-3,2'-dimethyl-4-aminobiphenyl

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Groups of F344 and Wistar rats were given an intraperitoneal injection of N-hydroxy-3,2'-dimethyl-4-aminobiphenyl (N-OH-DMAB) at a dose of 5 mg/kg body weight with a 1-week dietary pretreatment with ethinyl estradiol (EE), and this regimen was repeated 10 times at one-week intervals. Additional groups were given N-OH-DMAB 10 times without the dietary EE pretreatment. The total experimental period was 60 weeks. Carcinomas and atypical hyperplasias of the prostate developed in 8 (42%) and 16 (84%) of 19 F344 rats without the dietary EE treatment and in 1 (6%) and 7 (39%) of 18 rats with the EE diet, respectively. No prostatic tumors were found in Wistar rats, although atypical hyperplasias were observed in 6 of 18 rats with and 4 of 8 rats without the EE supplementation. Tumor yields in other organs were extremely low, resulting in good survival of the animals. A comparison of the results with those obtained for DMAB suggests that intraperitoneal administration of N-OH-DMAB in F344 provides a better induction method for models of prostate carcinogenesis.

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

3,2'-Dimethyl-4-aminobiphenyl (DMAB) is a carcinogen which induces carcinomas of rat prostate when given subcutaneously.[1, 2] However, its action is not specific for the prostate and it induces many types of tumors in a variety of organs.[2-4] This is a major causative factor increasing the animal mortality rate. While it has been shown that the incidence of prostate lesions can be enhanced by synchronization of carcinogen application with a cellular proliferative phase,[5, 6] tumor development in other organs represents a disturbance of the process of prostate carcinogenesis which limits the usefulness of the DMAB model. This situation is presumably the same for other prostate carcinogens, for example with N-methyl-N-nitrosourea[7, 8] and N-nitrosobis(2-oxopropyl)amine.[9]

DMAB can be metabolized to N-hydroxy DMAB (N-OH-DMAB) by cytochrome P-450(s), this event presumably occurring in all tissues, including the prostate, where P-450(s) are present.[10-12] Our recent study[13] revealed that the ventral lobe of the rat prostate possesses O-acetylase activity which can metabolize N-OH-DMAB to the ultimate carcinogen. This metabolic circumstance suggests that DMAB and/or N-OH-DMAB can be metabolized to exert carcinogenicity in the prostate through local final activation. N-OH-DMAB administered via the abdominal cavity may give different tumor responses in various organs from those with DMAB injected subcutaneously, because of differences in distribution and metabolism of the compounds resulting from the different routes of application.

In this experiment, we examined the carcinogenic potential of N-OH-DMAB for the prostate when given intraperitoneally. Since Wistar rats have been demonstrated to have a high activity of O-acetylase in the prostate,[13] F344 and Wistar rats were compared.

MATERIALS AND METHODS

Five-week-old male F344 and Wistar 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*. Ethinyl estradiol (EE) was purchased from Sigma Chemical Co., St. Louis. MO and added to powdered diet at a concentration of 0.75 ppm.[5] N-OH-DMAB was prepared by the method of Hech *et al.*[14] Six-week-old rats of each strain were divided into 2 groups (20 rats each); for the first group, a 1 week-1 week EE diet-basal diet cycle was repeated 10 times and then the animals were given basal diet for the remainder of the 60-week experiment. The rats received a single ip injection of N-OH-DMAB at 5 mg/kg body weight in DMSO 2 days after each change to the basal diet. The second group was given basal diet throughout the experiment and received N-OH-DMAB at the same time as the first group. All the surviving animals killed at the end of experimental week 60, as well

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as rats which died or which had been killed earlier upon becoming moribund, were subjected to routine autopsy. All organs containing 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 the use of Fisher's exact probability test.

RESULTS

While the growth rates of both strains of rats that were not given the EE diet were similar to those expected from background data, the intermittent administration of EE diet significantly suppressed weight gain; the average body weights for the first 20 weeks showed a 14 to 22% reduction in F344 and a 14 to 19% decrease in Wistar rats. The differences in final average body weights between groups with and without EE diet were 4% in F344 rats and 8% in Wistar rats.

The final survival rates of animals in groups with and without the EE diet were 90 and 95% for F344 rats and 90 and 40% for Wistar rats. These values are presented in terms of effective numbers of rats in Table I. Neoplastic lesions of the prostate and seminal vesicles were classified as described previously.[5, 6] Carcinomas and atypical hyperplasias were present in 42 and 84%, respectively, of F344 rats in the group not receiving the EE diet, these values being significantly higher than in the EE-supplemented case (see Table I). There were no significant differences in the incidences of atypical hyperplasias of the seminal vesicles in this strain. No carcinomas of the prostate were observed in Wistar rats. Slightly more hyperplastic lesions in the seminal vesicles of Wistar rats were evident with carcinogen alone than with EE plus carcinogen.

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. All carcinomas of the prostate were microscopic, multiple development being observed in 2 F344 rats given EE plus carcinogen. The incidences of tumors in organs other than the prostate and seminal vesicles were very low (see Table II) except for those of pancreatic acinar nodules and/or adenomas found in Wistar rats. Lesions in the pancreas were classified into foci, nodules and

Table I. Incidences of Carcinomas and Atypical Hyperplasias in the Prostate and Seminal Vesicles

| Groups | No. of rats | Ventral prostate | | Seminal vesicles |
		Carcinoma	Atypical hyperplasia	Atypical hyperplasia
F344				
1. N-OH-DMAB+EE	18	1 (6)	7 (39)	8 (44)
2. N-OH-DMAB only	19	8 (42)[a]	16 (84)[b]	6 (32)
Wistar				
3. N-OH-DMAB+EE	18	0	6 (33)	11 (61)
4. N-OH-DMAB only	8	0	4 (50)	7 (88)

a) Significant difference ($P<0.05$) from Group 1.
b) Significant difference ($P<0.01$) from Group 1.

Table II. Incidences (%) of Tumors in Organs Other than the Prostate and Seminal Vesicles

Groups	No. of rats	Small intestine[a]	Large intestine[b]	Pancreas[c]	Preputial gland[d]	Subcutis[e]	Liver[f]	Kidney[g]	Peritoneum[h]
F344									
1. N-OH-DMAB+EE	18	0	0	1 (6)	3 (17)	0	1 (6)	0	1 (6)
2. N-OH-DMAB only	19	0	1 (5)	0	1 (5)	1 (5)	2 (11)	0	3 (16)
Wistar									
3. N-OH-DMAB+EE	18	1 (6)	1 (6)	11 (61)	0	0	0	1 (6)	1 (6)
4. N-OH-DMAB only	8	0	1 (13)	7 (88)	0	0	0	0	2 (25)

a) Neurinoma. b) Adenocarcinoma. c) Acinar cell nodule and adenoma. d) Adenocarcinoma. e) Malignant neurinoma.
f) Hyperplastic nodule. g) Renal cell adenoma. h) Mesothelioma.

adenomas according to their size as described in our recent paper.[15]

DISCUSSION

The present data clearly showed that N-OH-DMAB is an effective induction agent for prostate carcinomas in F344 rats. The dose of N-OH-DMAB applied, 5 mg/kg body wt., per injection was one-tenth of that normally used for induction of prostate lesions in rats with DMAB.[2, 5, 6] An earlier toxicity study demonstrated 20 mg of N-OH-DMAB/kg body wt. to be the maximum tolerable dose for F344 rats when given intraperitoneally, because at a level of 30 mg all animals died within 2 weeks (unpublished data). Considering that the maximum tolerable dose of DMAB given subcutaneously in this strain is about 200 mg or less/kg body wt.,[16] the dose of 5 mg of N-OH-DMAB would be approximately comparable to 50 mg of DMAB, the reason for its choice.

It is therefore noteworthy that tumor development in organs other than the prostate, particularly in the skin/subcutis, preputial and Zymbal glands and colon, was very limited, resulting in a good survival rate. In contrast, recent work with DMAB in which the carcinogen was administered subcutaneously to F344 rats using the same schedule as that in the present study (once every week, 10 times) resulted in a final survival rate of only 49% with development of prostate carcinomas in 55% of animals which survived until the end of the experiment.[17] Although the incidence of prostate carcinomas was comparable to that in the present data, tumor incidences in other organs were high, 82% of the animals developing tumors in the skin/subcutis, for example. DMAB when injected into the subcutis is absorbed relatively slowly and becomes distributed throughout the body via the vein-heart-artery route. N-OH-DMAB and other metabolites which are produced from DMAB in organs such as the liver can then be transported to the various tissues. It is likely therefore that with this carcinogen and route of administration, interactions between carcinogen and tissues will take place throughout the whole body. On the other hand, a large part of N-OH-DMAB injected into the abdominal cavity will be immediately transported to the liver, where several metabolic steps take place. Some carcinogen is activated, giving rise to binding to macromolecules in the liver cells. The majority, however, may become detoxified, thereby reducing the possible exposure of other organs. This may explain why tumor yield in organs other than the prostate was much lower in the present experiment than expected from earlier work.

It is unclear why the Wistar rats used in the present study demonstrated a lower tumor response in the prostate than did the F344 strain, despite high O-acetylation activity. However, our recent studies with DMAB revealed that Wistar rats have a very low survival rats and low incidences of neoplastic lesions of the prostate, suggesting that not only O-acetylation but also other factors (such as detoxification or repair of DNA lesions) are involved in tumor induction.

The lower tumorigenic response in groups with EE diet than in groups without EE diet presumably, at least in part, represents differences in total cumulative dose of N-OH-DMAB. In our previous work[5] tumor yield in the prostate was significantly increased by synchronization of DMAB administration with the peak of DNA synthesis in prostate epithelial cells. In this case the increase in DNA synthesis was evoked by a cyclic treatment with 3 weeks of EE diet followed by 2 weeks on basal diet. While there was also a slight elevation of DNA synthesis associated with the shortened cycle used in the present experiment (unpublished data), a suppressive rather than an enhancing effect was observed. The reasons are unclear and further investigations will be directed at assessing effects on carcinogen metabolism and interaction with cell turnover. The distinct strain differences in tumor yields in the prostate observed earlier[17] similarly require investigation.

In conclusion, in view of the low tumorigenic response in target organs other than the prostate and the associated increase in survival rate, intraperitoneal injection of N-OH-DMAB in F344 rats is a superior model to subcutaneous injection of DMAB for induction of prostate carcinomas.

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