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Effects of Phenobarbital and Carbazole on Carcinogenesis of the Lung, Thyroid, Kidney, and Bladder of Rats Pretreated with N-Bis(2-hydroxypropyl)nitrosamine

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Studies were made on potential modifying effects of phenobarbital (PB) and carbazole on tumor development induced by N-bis(2-hydroxypropyl)nitrosamine (DHPN), a wide-spectrum carcinogen in rats. Effects on the lung, thyroid, kidney, bladder and liver were investigated. Male F344 rats were given 0.2% DHPN in their drinking water for 1 week and then 0.05% PB or 0.6% carbazole in their diet for 50 weeks. Control animals were treated with either DHPN or PB or carbazole only. Neither PB nor carbazole affected the incidence or histology of lung tumors. However, PB promoted the development of thyroid tumors and preneoplastic lesions of the liver, while carbazole promoted the induction of renal pelvic tumors.

Key words: Phenobarbital — Carbazole — N-Bis(2-hydroxypropyl)nitrosamine — Carcinogenesis — Rat

Phenobarbital (PB) is known to promote hepatocarcinogenesis,[1-4] and has been classified as a weak hepatocarcinogen in rats and mice.[5] It has also been reported to enhance tumorigenesis of the thyroid[6,7] but to inhibit carcinogenesis of the mammary gland[8] and brain[9] in rats. Moreover, Friedman[10] found in an epidemiological survey that use of barbiturates (a mixture of sodium phenobarbital, PB and sodium secobarbital) was associated with an increased incidence of lung cancer in both sexes, and also with cancers of the ovary, thyroid and pancreas. In mice, however, PB was found not to influence carcinogenesis of the lung.[11-14]

Carbazole is widely used industrially as a dye intermediate and for making photographic plates sensitive to UV-light. It is also present in cigarette smoke at 1 μg/cigarette.[15] Its carcinogenicity in the liver and forestomach, but not the lung, of mice of both sexes was recently demonstrated by Tsuda *et al.*[16] 7*H*-Dibenzo[*c*,*g*]carbazole, a derivative of carbazole, was also found to be present in cigarette smoke[17] and its carcinogenicity in mouse skin was demonstrated by Boyland and Brues.[18] The carcinogenicities of several other derivatives of carbazole have also been examined, and nitrogen derivatives have been shown to be carcinogenic in mice.[19] Sellakumar *et al.*[20] reported that tracheal instillation of 7*H*-dibenzo[*c*,*g*]carbazole induced squamous cell carcinoma in the trachea and bronchi of Syrian golden hamsters.

N-Bis(2-hydroxypropyl)nitrosamine (DHPN) is a wide-spectrum carcinogen inducing neoplasias in multiple organs of rats, such as the lung, thyroid, kidney and urinary bladder.[21-23] The practical value of an experimental system using DHPN for two-stage carcinogenesis of these organs has been reported by us[22,24] and others.[25]

In the present experiment, we examined the effects of PB and carbazole on DHPN carcinogenesis in various organs, particularly the lung, of F344 rats.

MATERIALS AND METHODS

One hundred male F344 rats were purchased from Charles River Japan, Kanagawa. They were 6 weeks old and weighed about 120 g at the beginning of experiments. Animals were housed 5 to a plastic cage with hard wood chips for bedding, in an air-conditioned room with a 12 hr-12 hr light-dark cycle, and given food (Oriental MF; Oriental Yeast Co., Tokyo) and water *ad libitum*. N-Bis-(2-hydroxypropyl)nitrosamine (DHPN) was purchasedfrom Nakarai Chemical Co., Kyoto. Phenobarbital was from Iwaki Pharmaceutical Co., Tokyo, and carbazole, technical grade of 96% purity, was from Nihon Jyoryu Kogyo, Ichikawa.

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Five groups of 20 rats each were used. Sixty rats were given 0.2% DHPN in their drinking water for 1 week and then they were divided into three groups. From one week after the end of DHPN administration, these three groups, 1, 2 and 3, were given 0.05% PB, 0.6% carbazole and basal diet, respectively, for 50 weeks. The concentration of carbazole used was the highest one used in a previous long-term carcinogenesis experiment in mice.[16] Groups 4 and 5, which had not been treated with DHPN, were given PB and carbazole in the same way as groups 1 and 2, respectively. The total intake of DHPN per rat was estimated from the total water consumption in each cage, which was measured every 2 days.

All rats were killed at the end of week 52. Their lungs, thyroids, kidneys, liver and urinary bladder and any other organs with gross abnormalities were removed, fixed in 10% buffered formalin and prepared for histological examination. In the case of the lungs, the fixative was used to inflate the air spaces by injection via the trachea. The bladder was also inflated with the fixative through the urethra. Paraffin sections (4 μm thickness) were stained with hematoxylin and eosin. Data were analyzed by the use of Fischer's exact probability test and Student's t-test.

Results

Administration of DHPN retarded body weight gain: the average body weight after treatment with DHPN for 1 week was 166.7 g, while that of the controls was 205.2 g. The total DHPN intake per rat was estimated as 225.6 mg. Carbazole also reduced body weight gain. The final average body weights in groups 1 to 5 were 415, 378, 428, 441 and 413 g, respectively.

Table I summarizes the incidences and numbers per rat of adenomas and carcinomas of the lung in each group. These lesions were classified as described previously.[21] Adenomas and carcinomas of the lung were observed at high incidences of 95% and 80%, respectively, one year after treatment only with 0.2% DHPN for one week (group 3). The average numbers of adenomas and carcinomas per rat in group 3 were 2.25 and 1.60, respectively. Subsequent treatment with PB or carbazole did not affect the incidence of these tumors or their number per rat, and there was no significant difference in the incidences of lung tumors (adenoma and/or carcinoma) in the three groups. Histologically, almost all the tumors were of the bronchiolo-alveolar cell type. Only 3 of 19 carcinomas in group 1, 3 of 18 in group 2 and 4 of 32 in group 3 were squamous cell carcinomas, the rest being adenocarcinomas. No metastasis of any lung carcinoma was found. Neither PB nor carbazole alone induced any neoplastic lesion of the lung (groups 4 and 5).

The incidences of thyroid tumors are summarized in Table II. Tumors were classified as adenomas and carcinomas. The incidences of carcinomas in rats given DHPN and then PB (group 1) was significantly higher ($P<0.05$) than that in rats given DHPN only (group 3). The numbers of carcinomas per rat in these two groups were also significantly different ($P<0.02$). One of the carcinomas in group 1 metastasized to the lung. The incidence of adenomas in group 1 was slightly higher than that in group 3, but the difference was not statistically significant. Carbazole did not affect the development of thyroid tumors.

Table III shows the incidences of tumors in the kidney and urinary bladder. The kidney tumors were histologically identified as renal cell tumors and nephroblastomas arising from the renal parenchyma, and papillomas and transitional cell carcinomas in the pelvis.

Table I. Incidences and Numbers of Lung Tumors in Groups 1–5

Group	Treatment	Effective No. of rats	Adenomas		Carcinomas		Tumors[a]	
			Incidence (%)	No. per rat	Incidence (%)	No. per rat	Incidence (%)	No. per rat
1	DHPN→PB	19	19(100)	2.84±1.12	13(68)	1.00±0.88	19(100)	3.84±1.38
2	DHPN→Carbazole	19	17 (89)	2.05±1.18	11(58)	0.95±1.03	18 (95)	3.00±1.73
3	DHPN	20	18 (90)	2.25±1.41	16(80)	1.60±1.29	20(100)	3.85±1.90
4	PB	20	0	—	0	—	0	—
5	Carbazole	20	0	—	0	—	0	—

a) Tumors include both adenomas and carcinomas.

Table II. Incidences and Numbers of Thyroid Tumors in Groups 1–3

Group	Treatment	Effective No. of rats	Incidence (%)		No. of tumors[a] per rat
			Adenomas	Carcinomas	
1	DHPN→PB	19	10(53)	19(100)[c]	2.63±1.12 (64)[b,d]
2	DHPN→Carbazole	19	8(42)	15 (79)	1.67±0.79 (32)
3	DHPN	20	7(35)	14 (70)	1.56±1.34 (38)

a) Tumors include both adenomas and carcinomas.
b) Numbers in parentheses are total numbers of tumors.
c) Significantly different from group 3 at $P<0.05$.
d) Significantly different from group 3 at $P<0.02$.

Table III. Incidences of Kidney and Urinary Bladder Tumors in Groups 1–3

Group	Treatment after DHPN	Effective No. of rats	Kidney (%)					Urinary bladder (%)		
			Parenchyma		Pelvis					
			Renal cell tumors	Nephroblastomas	Papillomas	Carcinomas	Tumors[a]	Papillomas	Carcinomas	Tumors[a]
1	PB	19	7(37)	2(11)	2(11)	1(15)	3(16)	0 —	1 (5)	1 (5)
2	Carbazole	19	4(21)	4(21)	5(26)[b]	7(37)	11(58)[b]	4(21)	3(16)	7(37)
3	—	20	6(30)	2(10)	0 —	4(21)	4(21)	2(11)	1 (5)	3(15)

a) Tumors include both papillomas and carcinomas.
b) Significantly different from group 3 at $P<0.05$.

Bladder tumors were papillomas and transitional cell carcinomas. There were no significant differences in the incidences of either renal cell tumors or nephroblastomas in the three DHPN-treated groups. However, the incidence of papilloma of the renal pelvis was significantly higher ($P<0.05$) in group 2 (DHPN-carbazole) than in group 3 (DHPN only). There was also a significant difference in overall incidence of pelvic tumors (papillomas plus carcinomas) in these two groups. Treatment with DHPN and carbazole (group 2) also induced a slightly higher incidence of tumors of the urinary bladder (papillomas or carcinomas) than treatment with DHPN alone (group 3).

Liver lesions, liver cell foci and hyperplastic nodules were also observed (Table IV). The incidence of liver cell foci in rats given DHPN and then PB (group 1) was significantly higher than that in rats given DHPN alone (group 3) or PB alone (group 4). There was a significant difference between the incidences of hyperplastic nodules in groups 1 and 4, but not between those in groups 1 and 3. Carbazole did not affect the development of these lesions.

Table IV. Incidences of Preneoplastic Lesions of the Liver in Groups 1–5

Group	Treatment	Effective No. of rats	Foci	Hyperplastic nodules
1	DHPN→PB	19	19(100)[a]	4(48)[b]
2	DHPN→Carbazole	19	11 (58)	0 —
3	DHPN	20	5 (25)	1 (5)
4	PB	20	4 (20)	0 —
5	Carbazole	20	9 (45)	1 (5)

a) Significantly different from groups 1 and 4 at $P<0.001$.
b) Significantly different from groups 4 at $P<0.05$.

Discussion

As shown in previous studies,[21–24] in this study DHPN induced tumors in multiple organs of F344 rats. Under the present exper-

imental conditions, the incidence of tumors was highest in the lung and less in the thyroid, kidney and bladder. The incidences were similar to those observed in our previous study in which the same strain of rats was also given the same dose of DHPN for 1 week.[21] In the present study, the incidences of lung adenomas and carcinomas in the group given DHPN only were rather too high to allow assessment of the promoting potential of PB and carbazole, but quantitative analyses showed that neither PB nor carbazole had any promoting or inhibitory effect on lung tumorigenesis in rats initiated with DHPN. PB and carbazole also did not affect the histological types of tumors of the lung. This absence of effect of PB on the development of lung tumors in rats is consistent with reports that PB did not affect the incidence of spontaneous lung tumors,[11] or lung tumors induced by dimethylnitrosamine[12] or N-ethylnitrosourea[13, 14] in mice. These experimental data do not support the epidemiological evidence for the promoting effect of barbiturates on the development of human lung cancer. Friedman[10] proposed the possible involvement of aryl hydrocarbon hydroxylase as a cause of the association between barbiturate use and lung cancer. This enzyme, which can activate polycyclic hydrocarbons to active carcinogens, was found to be more readily inducible in many patients with lung cancer than in persons without this disease.[26] PB has been shown to induce this enzyme in many mammalian organs including the lung of hamsters.[27] An experiment in which PB is given simultaneously with a carcinogen that is metabolized by aryl hydrocarbon hydroxylase would be necessary to test this mechanism.

In other organs, PB promoted the development of thyroid tumors. This result is consistent with previous results on rats initiated with N-nitrosomethylurea[6] or DHPN.[25] The reason why PB promotes tumorigenesis in the thyroid is unknown, but has been suggested to be because of increased release of TSH from the pituitary resulting from increased metabolism of thyroid hormones in PB-treated liver.[28]

Neither PB nor carbazole affected induction of tumors in the renal parenchyma, but carbazole increased the incidence of transitional cell tumors in the renal pelvis significantly and the incidence of urinary bladder tumors slightly, but not significantly. These two findings suggest that carbazole has a promoting effect on the transitional epithelium. This promoting potential might be clearer in a system with the bladder carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine as the initiator.[29]

Although carbazole was found to be carcinogenic to mouse liver,[16] it showed no promoting effect in rat liver. DHPN seemed to have low initiating activity in rat liver, because it induced preneoplastic lesions, such as foci and hyperplastic nodules, but not hepatocellular carcinomas,[21, 22, 24, 25] and in rat liver PB and estrogen seem to have little, if any, promoting activity.[24, 25] In a short-term experiment with N-diethylnitrosamine(DEN) as the initiator, carbazole at a dose of 0.02% increased the number, but not the area of placental glutathione S-transferase-positive foci in rat liver (unpublished data). Therefore, at a higher dose, carbazole would probably have a promoting effects on rat liver carcinogenesis initiated with DEN.

The present experiment confirmed that organ specificities of the modifying potentials of chemicals can be examined by using a wide-spectrum carcinogen as the initiator. Good examples of this technique have already been reported.[6, 22, 24, 30-32]

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