[CANCER RESEARCH 44, 3098–3100, July 1984]

Carcinogenicity of *N*-Phenyl-1-naphthylamine and *N*-Phenyl-2-naphthylamine in Mice

Heng-wen Wang,[1] Da Wang, and Ruo-wang Dzeng

Shanghai Institute of Cell Biology, Academia Sinica, 320 Yo-Yang Road, Shanghai, China

ABSTRACT

Technical *N*-phenyl-1-naphthylamine (PANA), which is an optic isomer of *N*-phenyl-2-naphthylamine (PBNA), has been used as a rubber additive without suspicion of its being carcinogenic. When male ICR mice were given repeated s.c. injections of both technical and pure PANA in dimethyl sulfoxide, it resulted in high percentage of malignant tumors similar to that in mice given technical PBNA. PANA had a tendency to induce hemangiosarcoma. Similar injections of PANA and PBNA into male TA-1 mice gave similar results. Previous unilateral nephrectomy enhanced both PANA and PBNA induction of renal hemangiosarcomas. The similar carcinogenic potency of PANA and PBNA suggests other routes of metabolic activation besides dephenylation for both chemicals in mice.

INTRODUCTION

After confirmation of the carcinogenicity of PBNA[2] in animals, its optic isomer, PANA, was tested for its carcinogenicity in comparison with that of PBNA in 2 animal models (7–11). It was found that PANA, also an antioxidant used in the rubber industry, had the same carcinogenic potency as did PBNA in both models.

MATERIALS AND METHODS

Chemicals

Technical PANA and PBNA were products of the Nanking Factory of Chemical Industry.

Chemically pure PANA and PBNA were obtained by fractionated distillation under reduced pressure from the technical products. Chemically pure DMSO was obtained from Shanghai Solvents Co.

Mice

Young adult male ICR mice and TA-1 mice were supplied by Tienjing Medical College.

Methods

ICR mice were used in Experiment 1, while TA-1 mice were used in Experiment 2. In each experiment, several groups were given different treatments as described below.

Experiment 1. The following groups were used. In Group 1, technical PBNA in DMSO, 26 mice were given s.c. injections of 16 mg of technical PBNA in 0.1 ml of DMSO 3 times weekly. In Group 2, technical PANA in DMSO, 30 mice were given technical PANA in DMSO in the same route and dose as those for Group 1. In Group 3, pure PANA in DMSO, 26 mice were given chemically pure PANA in DMSO by the same route and in the same dose as those in Group 1. In Group 4, pure PANA in DMSO (low dosage), 25 mice were given 3 times weekly s.c. injections of 5.3 mg of pure PANA in 0.1 ml DMSO. In Group 5, DMSO controls, 24 mice were given 3 times weekly s.c. injections of 0.1 ml of DMSO.

The sites of injection were changed frequently. Each experimental and control animal received 27 injections and was then kept in the animal house until the end of the tenth experimental month. At that time, all animals were sacrificed and underwent detailed gross examination. Paraffin sections were prepared from Bouin-fixed liver, spleen, kidneys, lungs, stomach, bladder, prostate gland, and seminal vesicles and were stained with hematoxylin and eosin. Detailed histological examination was made for all specimens. Animals found dead or moribund before the end of the experiment were examined in the same way.

Experiment 2. In some groups of TA-1 mice, the left kidney was removed using pentobarbital sodium anesthesia.

Injections were begun 1 week after unilateral nephrectomy. In Group 1, nephrectomy plus pure PANA, 16 unilaterally nephrectomized mice were given twice weekly s.c. injections of 16 mg of pure PANA in 0.1 ml of DMSO. The first 6 injections were given with only 8 mg of PANA. In Group 2, nephrectomy plus technical PANA, 13 unilaterally nephrectomized mice were given technical PANA in the same dose and by the same route as those in Group 1. In Group 3, technical PANA, 19 intact mice were given technical PANA by the same route and in the same dose as those in Group 1. Group 4 contained natural controls (18 mice). In Group 5, technical PBNA, 19 intact mice were given technical PBNA by the same route and in the same dose as those in Group 1. In Group 6, nephrectomy plus pure PBNA, 17 unilaterally nephrectomized mice were given pure PBNA by the same route and in the same dose as those in Group 1.

All experimental mice received a total dosage of 328 mg of PANA or 304 mg of PBNA, after which they were kept without any particular treatment in the animal house until the end of the ninth experimental month. Then all animals were sacrificed and examined as in Experiment 1.

RESULTS

Experiment 1. The essential results are summarized in Table 1. Malignant tumors at multiple body sites were sometimes observed. From Table 1, it may be seen that both technical and pure PANA were as carcinogenic to ICR mice as was technical PBNA with slightly different organotropism. While both chemicals induced lung carcinomas (Fig. 1), under the present experimental conditions PANA seemed to have a higher affinity to the kidney and hemovascular system (Fig. 2) than did PBNA.

Experiment 2. From Table 2, it may be seen that technical PANA was carcinogenic to intact TA-1 mice after repeated s.c. injections and showed an affinity to the blood vessels of the kidney. Previous unilateral nephrectomy greatly promoted the susceptibility of the remaining kidney to induction of hemangiosarcomas by technical PANA (Fig. 3). Pure PANA seemed to have practically the same carcinogenic potency and organotropism as did the technical product. Fig. 4 shows a hemangiosarcoma of the kidney. Unilateral nephrectomy also greatly pro-

[1] To whom requests for reprints should be addressed.

[2] The abbreviations used are: PBNA, *N*-phenyl-2-naphthylamine; PANA, *N*-phenyl-1-naphthylamine; DMSO, dimethyl sulfoxide.

Received May 13, 1983; accepted March 30, 1984.

Table 1

Carcinogenicity of repeated injections of PANA and PBNA into ICR mice

Group	No. of mice	Total dose (mg)	Duration of experiment (days)	Malignant tumors		Lung carcinoma		Kidney carcinoma		Cholangiocarcinoma		Hemangiosarcoma								Lymphoma	
												Total		Lung		Kidney		Liver			
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Technical PBNA	26	432	289	9[a]	34.6	6[b]	23.1	1	3.8	0		1	3.8	1	3.8	0		0		1	3.8
Technical PANA	30	432	295	14[a]	46.6	5[b]	16.6	1	3.3	2	6.6	5[b]	16.6	3	10	1	3.3	1	3.3	1	3.3
Pure PANA	23	432	291	12[a]	52.2	3	13	3	13	0		4[b]	17.4	0		4[b]	17.4	0		2	8.6
Pure PANA	25	135	290	11[a]	44	6[b]	24	1	4	0		4[b]	16	3	12	1	4	0		0	
DMSO control	24	0	296	0		0		0		0		0		0		0		0		0	

[a] Difference between experimental and control groups, $p = 0.01$.
[b] Difference between experimental and control groups, $p = 0.05$.

Table 2

Carcinogenicity of repeated injection of PANA and PBNA into intact and unilaterally nephrectomized TA-1 mice

Group	No. of mice	Nephrectomy	Total dose (mg)	Duration (days)	Malignant tumors		Hemangiosarcoma of kidney		Lung carcinoma		Kidney carcinoma		Seminal vesicle carcinoma		Pleural mesothelioma	
					No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Pure PBNA	16	+	328	273	12[a]	75	12[a]	75	1	6.3	0		0		0	
2. Technical PANA	13	+	328	262	13[a]	100	13[a]	100	1	7.6	1	7.6	1	7.6	0	
3. Technical PANA	19	–	328	273	7[b]	36.8	7[b]	36.8	0		0		0		0	
4. Control	18	–	0	273	0		0		0		0		0		0	
5. Pure PBNA	17	+	304	254	15[a]	88.2	8[a]	47.1	8[a]	47.1	3	17.6	1	5.8	2	11.7
6. Technical PBNA	21	+	304	256	19[a]	90.5	16[a]	76.2	9[a]	42.8	0		1	4.7	3	14.3
7. Technical PBNA	19	–	304	255	4	21.1	0		4	21.1	0		0		0	

[a] Difference between experimental and control groups, $p = 0.01$.
[b] Difference between experimental and control groups, $p = 0.05$.

moted the susceptibility of the remaining kidney to the induction of hemangiosarcomas as well as the development of lung carcinomas by repeated injections of PBNA in DMSO.

DISCUSSION

The carcinogenicity of both technical and pure PANA to 2 mouse strains raises serious doubts as to their safety when used as rubber antioxidants. Epidemiological and other studies are needed to clarify their action in humans. Good ventilation and dust removal seem to be necessary in the workshop where compounding of the rubber additives and mixing and milling of rubber take place.

Previously, PBNA was suspected to be carcinogenic since metabolic studies seemed to indicate its dephenylation *in vivo* (2–6). The tumor spectra in rats and mice repeatedly given PBNA in our experiments suggested other routes of metabolic activation besides dephenylation for its carcinogenicity. That PANA is as carcinogenic as is PBNA despite different organotropisms as shown in our present experiments also suggests other routes of metabolic activation of both compounds. Anderson *et al.* (1) reported absence of dephenylation in their *in vitro* metabolic studies of PBNA in 7 animal species. Further *in vivo* metabolic studies would be of interest.

Unilateral nephrectomy seemed to facilitate the development of renal hemangiosarcomas by both PANA and PBNA, while the latter compound seemed to induce mainly lung cancers in intact mice. The hemangiosarcomas induced were diffuse and easy to diagnose. Whether this operation may help to develop a short-term *in vivo* carcinogenicity test awaits further experimentation.

ACKNOWLEDGMENTS

We thank Professor Xue Xuan-xian for the preparation of pure PBNA and PANA; Chen Jian's technical assistance is much appreciated.

REFERENCES

1. Anderson, M. M., Mitchum, R. K., and Beland, F. S. Hepatic microsomal metabolism and micromolecular binding of the antioxidant *N*-phenyl-2-naphthylamine. Xenobiotica, *12:* 31–43, 1982.
2. Batten, P. L., and Hathway, D. E. Dephenylation of *N*-phenyl-2-naphthylamine in dogs and its possible oncogenic implications. Br. J. Cancer, *35:* 342–346, 1977.
3. International Agency for Research on Cancer. IARC Monographs on Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 16, pp. 331–333. Lyon, France: International Agency for Research on Cancer, 1978.
4. International Agency for Research on Cancer. IARC Monographs on Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 28, Appendix 2, pp. 367–373. Lyon, France: International Agency for Research on Cancer, 1982.
5. Kummer, R., and Tordoir, W. F. Phenyl-beta-naphthylamine (PBNA), another carcinogenic agent? Tijdschr. Soc. Geneeskd., *53:* 415–419, 1975.
6. Moore, R. M., Jr., Wolf, B. S., Stein, H. P., Thomas, A. W., and Franklin, J. F. Metabolic precursors of a known human carcinogen. Science (Wash. DC), *195:* 344, 1977.
7. Wang, D., and Wang, H-w. The carcinogenicity of refined antioxidant D in Wistar rats. Tumor (Shanghai), *1:* 242–243, 1981.
8. Wang, H-w., Dzeng, R-w., and Wang, D. The carcinogenicity of *N*-phenyl-2-naphthylamine on ICR mice. Acta Biol. Exp. Sin., *15:* 199–207, 1982.
9. Wang, H-w., Shen, R-j., and Dzeng, R-w. The carcinogenic activity of antioxidant D on C57/BL mice. Acta Biol. Exp. Sin., *14:* 130–137, 1981.
10. Wang, H-w., Wang, D., Tang, P-x., Shen, R-j., and Zeng, R-w. The carcinogenicity of antioxidant D on albino rats. Acta Biol. Exp. Sin., *14:* 77–83, 1981.
11. You, X-j., and Yao, Y.-f. Experimental study of inhalation carcinogenesis of *N*-phenyl-2-naphthylamine aerosol on C57/BL mice. Acta Biol. Exp. Sin., *14:* 139–143, 1981.

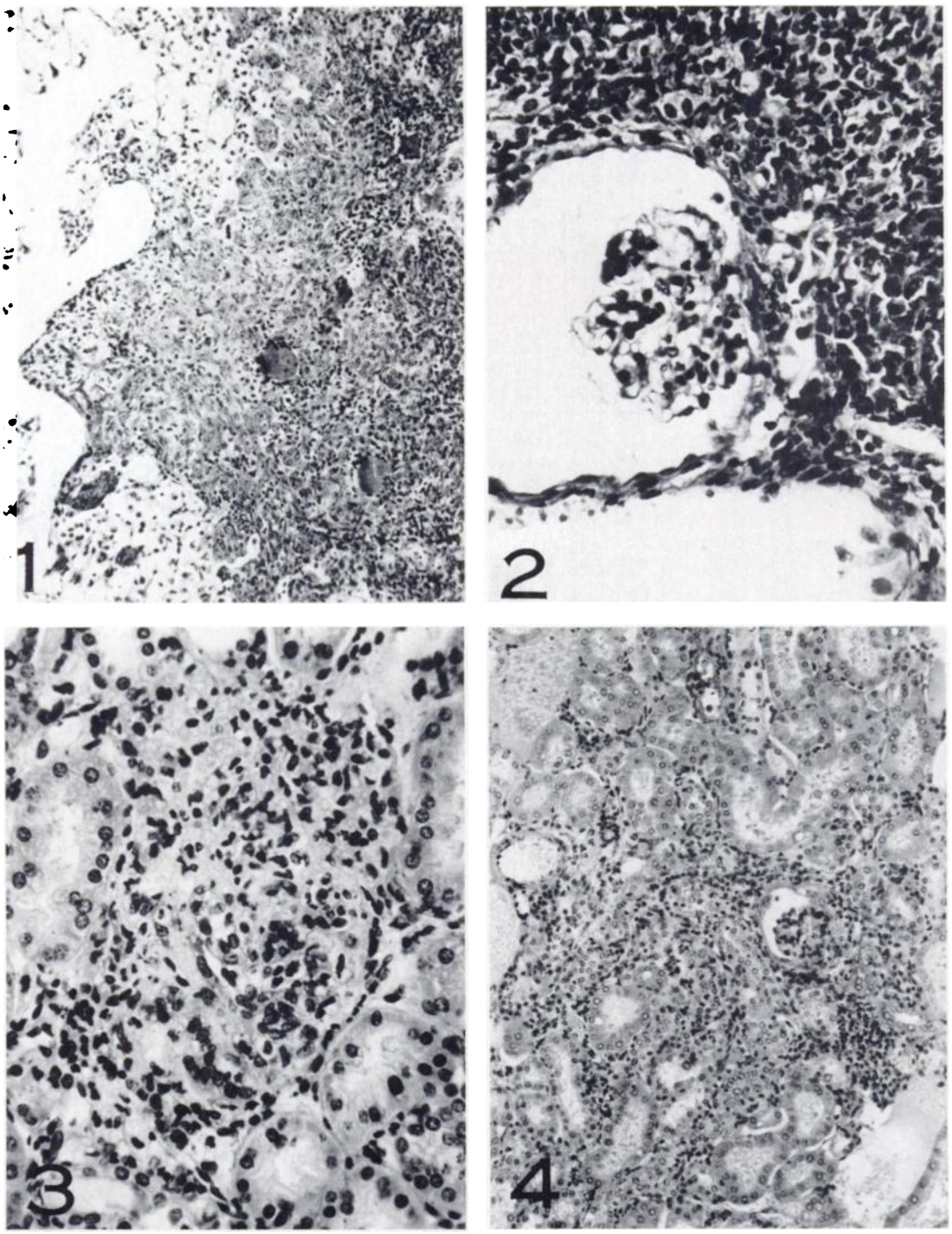

Fig. 1. Alveolar cell carcinoma of the lung in an ICR mouse given s.c. injections of technical PANA in DMSO. H & E, × 100.
Fig. 2. Hemangiosarcoma of the kidney in an ICR mouse given s.c. injections of pure PANA in DMSO. H & E, × 240.
Fig. 3. Hemangiosarcoma of the kidney in a unilaterally nephrectomized TA-1 mouse given s.c. injections of technical PANA in DMSO. H & E, × 240.
Fig. 4. Hemangiosarcoma of the kidney in a unilaterally nephrectomized TA-1 mouse given s.c. injections of pure PANA in DMSO. H & E, × 100.

Carcinogenicity of *N*-Phenyl-1-naphthylamine and *N*-Phenyl-2-naphthylamine in Mice

Heng-wen Wang, Da Wang and Ruo-Wang Dzeng

Cancer Res 1984;44:3098-3100.