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

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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 PBNA² 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. 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. In Group 4, pure PANA in DMSO, 19 intact mice were given pure PANA by the same route and in the same dose as those in Group 1. In Group 5, 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-

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

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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 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**


**Table 1**

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<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Total dose (mg)</th>
<th>Duration of experiment (days)</th>
<th>Malignant tumors</th>
<th>Lung carcinoma</th>
<th>Kidney carcinoma</th>
<th>Cholangiocarcinoma</th>
<th>Hemangiosarcoma</th>
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a Difference between experimental and control groups, p = 0.01.
b Difference between experimental and control groups, p = 0.05.

**Table 2**

<table>
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<th>Group</th>
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<th>Duration of experiment (days)</th>
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<th>Lung carcinoma</th>
<th>Kidney carcinoma</th>
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a Difference between experimental and control groups, p = 0.01.
b Difference between experimental and control groups, p = 0.05.
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.
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