Comparative Tumorigenicity of 1-Nitropyrene, 1-Nitrosopyrene, and 1-Aminopyrene Administered by Gavage to Sprague-Dawley Rats

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ABSTRACT

The carcinogenic activities of 1-nitropyrene, a mutagenic component of diesel exhaust, and its reduced derivatives 1-nitrosopyrene and 1-aminopyrene were evaluated in male and female Sprague-Dawley rats. Within 24 h of birth, groups of 22-36 rats were treated by gavage with trioctanoin or the appropriate compound in trioctanoin once weekly for 16 weeks. The approximate total doses per rat were as follows: 1-nitropyrene, high dose (800 μmol); 1-nitropyrene, low dose (320 μmol); 1-nitrosopyrene (320 μmol); 1-aminopyrene (320 μmol). The experiment was terminated after 94 weeks. The main site of tumor induction was the mammary glands of female rats. Percentages of incidences of mammary adenocarcinomas in female rats were as follows: 1-nitropyrene, high dose (63%); 1-nitropyrene, low dose (42%); 1-nitrosopyrene (19%); 1-aminopyrene (4%); triocanoin (3%). These incidences were significantly greater than those of controls for the female rats treated with either 1-nitropyrene or 1-nitrosopyrene. Low and generally insignificant incidences of tumors of a variety of other sites were also observed in rats treated with 1-nitropyrene. The induction of mammary tumors by 1-nitropyrene confirms the results of a previous study (Hirase et al., Cancer Res., 44: 1158-1162, 1984). The present results also demonstrate that, under the conditions of this bioassay, 1-nitropyrene was significantly more carcinogenic than either 1-nitrosopyrene or 1-aminopyrene.

INTRODUCTION

Numerous studies have indicated that organic extracts of diesel exhaust, among many other environmental sources, contain nitropolycyclic aromatic hydrocarbons (1-18). In the case of diesel engine emissions it was estimated that the amounts of 1-nitropyrene and dinitropyrenes emitted annually in the United States are >14,000 and 200 kg, respectively (19). It was also reported that 30-40% of the mutagenicity of diesel particulate extract could be attributed to 1-nitropyrene and dinitropyrenes (20). More than 100 nitro compounds have been identified in diesel engine emissions (20); 1-nitropyrene is one of the most abundant (14). Most members of this class of compounds are capable of inducing mutations as shown in various short term in vitro assays, and representative examples have been reported to induce tumors in long term studies using rats, mice, hamsters, and dogs (20). Upon i.p. injection into A/J mice and newborn CD-1 mice, 1-nitropyrene induced lung and liver tumors, respectively (21, 22). In newborn Sprague-Dawley rats, it induced adenocarcinomas in the mammary glands and malignant fibrous histocytomas at the site of injection (23). Although 1-nitropyrene had been reported to cause s.c. tumors in F344 rats, these results were later corrected as negative due to contamination with significant amounts of tumorigenic dinitropyrenes (24, 25). On mouse skin, 1-nitropyrene had no detectable tumor-initiating activity (26, 27).

In order to assess the possible risk associated with human exposure to diesel exhaust, further studies to evaluate the carcinogenic potency of representative nitropolycyclic aromatic hydrocarbons, and in particular 1-nitropyrene, are required. In this study we examined the carcinogenicity of 1-nitropyrene, free of dinitropyrenes, following p.o. administration to Sprague-Dawley rats. We chose to administer the compound p.o. because this route seemed more relevant to human exposure than s.c. injection, and it also avoids the problem of accumulation of the compound at the injection site, as noted in previous studies (23). We also tested 1-nitrosopyrene and 1-aminopyrene, which are formed by nitroreduction of 1-nitropyrene (28-30).

MATERIALS AND METHODS

Chemicals. Triocanoin was obtained from Fluka Chemical Corp., Ronkonkoma, NY, and was distilled before use (220-230°C at 0.5-0.6 mm). Commercial 1-nitropyrene (Aldrich Chemical Co., Inc., Milwaukee, WI) was purified by column chromatography on silica gel with elution by 10% benzene in hexane. Its purity was assessed by gas chromatography with electron capture detection. A Hewlett-Packard Model 5890A gas chromatograph equipped with a 60Ni electron capture detector and a DB-5 capillary column (60 m, 0.25 μm, J & W Scientific, Inc., Rancho Cordova, CA) were used for this analysis. The oven temperature was set at 315°C, the detector temperature at 325°C, and the injection port temperature at 295°C. Helium was the carrier gas (flow rate, 1 ml/min). The make-up gas consisted of 5% methane and the split ratio was 20:1. Standard samples of dinitropyrenes were prepared as described in the literature (31). 1-Nitropyrene was eluted at 16.9 min, 1,3-dinitropyrene at 24.6 min, 1,6-dinitropyrene at 26.7 min, and 1,8-dinitropyrene at 28.2 min. The purity of 1-nitropyrene was >99.9%; no dinitropyrenes were detected.

1-Aminopyrene was purchased from Aldrich Chemical Co. and purified by column chromatography on silica gel with elution by 10% ethyl acetate in benzene. Its purity was 99.9% based on high performance liquid chromatographic analysis with a Vydac C18-10 μ reverse phase column (4.6 mm x 25 cm; Separations Group, Hesperia, CA) eluted with a linear gradient from 0% CH3OH-H2O to 100% CH3OH-H2O in 10 min at a flow of 1.0 ml/min. It was eluted at 83 min.

1-Nitrosopyrene was prepared as described previously (29). A solution of 1-aminopyrene (1.08 g, 4.9 mmol) in 300 ml of CH2Cl2 was cooled to 0°C, and m-chloroperbenzoic acid (1.7 g, 10 mmol) in 100 ml of CH2Cl2 was added dropwise under an atmosphere of N2. After addition of the peracid, stirring was continued for 45 min. The reaction mixture was washed with 1 N NaOH, H2O, 6 N HCl, and H2O. The crude product in CH2Cl2 was purified by column chromatography on silica gel with elution by hexane to give 1-nitrosopyrene (400 mg, 1.7 mmol, 35%). This reaction was repeated several times in order to obtain ample material for the bioassay. Its mass spectrum and proton nuclear magnetic resonance spectrum were consistent with those previously reported and its purity was >99.9% according to high performance liquid chromatographic analysis as described above for 1-aminopyrene.

It was eluted at 104.5 min.

Tumorigenicity Experiment. Thirty-six pregnant Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Kingston, NY. Preliminary experiments were carried out with the use of 5 newborn rats per group. Each animal was treated by gavage with the appropriate compound in trioctanoin at day 1 (within 24 h of birth), day 8, and day 15 of life. Doses were as follows: 1-nitropyrene, high dose (250 μmol/kg body weight; 40.5 μmol/ml trioctanoin); 1-nitropyrene, low dose (100 μmol/kg body weight; 16.2 μmol/ml trioctanoin);

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2 To whom requests for reprints should be addressed.
RESULTS

Weight curves are shown in Fig. 1. There was no significant weight loss in any of the treated groups. Survival data are summarized in Fig. 2 and Table 1. Survival times were significantly shorter ($P < 0.01$) in the rats treated with 1-nitropyrene than in controls. Survival in the other groups was not affected by treatment.

The incidence of palpable mammary tumors in female rats is shown in Fig. 3. The first palpable mammary tumors were observed at 20 and 24 weeks of age in the groups treated with the higher and lower doses of 1-nitropyrene, respectively. In the case of 1-nitrosopyrene, 1-aminopyrene, and in the control groups, the first palpable mammary tumors were observed at 48 weeks of age. The incidences of palpable mammary tumors in female rats administered the higher and lower doses of 1-nitropyrene were significantly higher than in controls at 36 and 56 weeks, respectively. The palpable mammary tumor incidence in female rats was also significantly higher than in controls at 64 weeks and at termination in both groups treated with 1-nitropyrene ($P < 0.0001$). At termination, palpable mammary tumors per female rat were as follows: 1-nitropyrene, high dose (3.5 ± 2.2); 1-nitropyrene, low dose (2.6 ± 2.1); 1-nitrosopyrene (1.9 ± 1.7); 1-aminopyrene (1.5 ± 1.5); and triocetanoin (0.8 ± 0.8). These values were significantly higher than those in controls ($P < 0.005$) in all treated groups. The incidence of palpable mammary tumors in treated male rats was not significantly different from that in control rats.

Upon histological examination, the mammary tumors were classified into adenomas, adenocarcinomas, fibromas, spindle cell sarcomas, and myxomas (Table 1). 1-Nitropyrene at both doses (groups 2 and 4) induced significant incidences of adenocarcinomas as compared to 1-nitrosopyrene (group 6), 1-aminopyrene (group 8), and controls (group 10). 1-Nitrosopyrene also induced a significant number of adenocarcinomas compared to controls.

DISCUSSION

Our results demonstrate that 1-nitropyrene, a major mutagenic component in diesel engine emissions, induces mammary tumors in female Sprague-Dawley rats. These results are consistent with those reported previously by Hirose et al. (23), who administered 1-nitropyrene to newborn Sprague-Dawley rats by s.c. injection at a dose of 100 μmol/kg body weight/week for 8 weeks, and terminated the experiment after 62 weeks. They observed mammary tumors in 47% of the females and mammary adenocarcinomas in 31%. It is difficult to estimate the amounts of 1-nitropyrene responsible for mammary tumor induction following s.c. injection because the compound accumulates at the injection site (23). In this study we administered 1-nitropyrene by gavage to avoid this problem. We also used higher doses and maintained the animals for longer times than those reported previously. These factors may be responsible for
Carcinogenic Activities of 1-Nitropyrene and Derivatives

Weeks

Fig. 2. Percentage of survival of Sprague-Dawley rats treated with 1-nitropyrene (D, high dose; •, low dose); 1-nitrosopyrene (A); 1-aminopyrene (Δ); and trioctanoin (O). A, males; B, females; bar, period of carcinogen treatment.

the somewhat higher incidences (42–63%) of mammary adenocarcinomas found in our study.

Aromatic amines and polycyclic aromatic hydrocarbons have been shown to induce mammary tumors in female Sprague-Dawley rats. The classical hydrocarbon used in this model system is 7,12-dimethylbenz(a)anthracene (33) due to its high potency as a mammary carcinogen; however, it is not known to be present in our environment. Benzo(a)pyrene and 4-aminobiphenyl have been shown to induce mammary tumors in female Sprague-Dawley rats and both compounds are environmental pollutants (34, 35). However, the role of these chemicals, if any, in the etiology of human breast cancer is not known. Nevertheless, it is interesting to compare the potency of 1-nitropyrene, benzo(a)pyrene, and 4-aminobiphenyl as mammary carcinogens.

Dietary administration of 4-aminobiphenyl at a dose of 1.62-mmol/kg diet to female Sprague-Dawley rats for 12 months (approximate total dose of 9–12 mmol/rat) induced mammary tumors in 66% of the animals (35). In the present study the incidence of mammary adenocarcinomas induced by 1-nitropyrene (total dose/rat, 0.8 mmol) was 63%. Other studies demonstrated that 4-aminobiphenyl and some of its derivatives administered s.c., i.p., or in the diet to various strains of rats at doses either comparable to or higher than that used for 1-nitropyrene in the present study, produced mammary tumor incidences ranging from 30 to 90% (36–39). Intragastric administration of benzo(a)pyrene at a total dose of 0.19 mmol to female Lew/Mai rats induced mammary tumors in 67% of the animals (40). Based on our results and those reported previously, it can be estimated that 1-nitropyrene, benzo(a)pyrene, and 4-aminobiphenyl have comparable potency as mammary carcinogens in rats. A comparative bioassay of these environmental agents in female Sprague-Dawley rats would be of interest.

In order to gain insight into the role of nitroreduction in 1-nitropyrene tumorigenesis, we compared its activity with that of its partially reduced derivative, 1-nitrosopyrene. It had been shown by Wislocki et al. (22) that 1-nitrosopyrene induced more hepatic tumors than 1-nitropyrene in newborn mice. Based on these results the investigators suggested that nitroreduction may be the limiting step in the activation of 1-nitropyrene in newborn mice. In the present study, however, 1-nitrosopyrene was less tumorigenic than 1-nitropyrene. It is possible that 1-nitrosopyrene is fully reduced to 1-aminopyrene either in the gastrointestinal tract or in the liver before it reaches the mammary glands. Based on our results we cannot exclude a role for the nitroreduction pathway in rat mammary tumor induction by 1-nitropyrene. However, nitroreduction of ring-

Table 1 Mammary tumors induced by 1-nitropyrene, 1-nitrosopyrene, and 1-aminopyrene in Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>Dose (µmol/kg body wt/wk)</th>
<th>Sex</th>
<th>No. of animals</th>
<th>Mean survival (wk ± SD)</th>
<th>No. of animals with mammary tumors</th>
<th>Adenomas</th>
<th>Adenocarcinomas</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-Nitropyrene</td>
<td>250</td>
<td>M</td>
<td>36</td>
<td>72 ± 18</td>
<td>10 to 14</td>
<td>1 fibrroma</td>
<td>1 sarcoma</td>
<td>1 myxoma</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>1-Nitropyrene</td>
<td>250</td>
<td>F</td>
<td>24</td>
<td>72 ± 16</td>
<td>7 to 15</td>
<td>1 fibroma</td>
<td>1 sarcoma</td>
<td>1 myxoma</td>
<td>23</td>
</tr>
<tr>
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<td>100</td>
<td>M</td>
<td>25</td>
<td>77 ± 23</td>
<td>2 to 14</td>
<td>1 fibroma</td>
<td>1 sarcoma</td>
<td>1 myxoma</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>1-Nitropyrene</td>
<td>100</td>
<td>F</td>
<td>33</td>
<td>75 ± 19</td>
<td>10 to 14</td>
<td>1 fibrroma</td>
<td>1 sarcoma</td>
<td>1 myxoma</td>
<td>27</td>
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<tr>
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<td>100</td>
<td>M</td>
<td>29</td>
<td>80 ± 15</td>
<td>0 to 1</td>
<td>1 fibrroma</td>
<td>1 sarcoma</td>
<td>1 myxoma</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1-Nitrosopyrene</td>
<td>100</td>
<td>F</td>
<td>26</td>
<td>86 ± 11</td>
<td>7 to 5</td>
<td>1 sarcoma</td>
<td>1 myxoma</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>Aminopyrene</td>
<td>100</td>
<td>M</td>
<td>23</td>
<td>86 ± 12</td>
<td>1 to 1</td>
<td>1 sarcoma</td>
<td>3 fibromas</td>
<td>1 sarcoma</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>Aminopyrene</td>
<td>100</td>
<td>F</td>
<td>27</td>
<td>89 ± 7</td>
<td>16 to 1</td>
<td>1 sarcoma</td>
<td>3 fibromas</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>Trioctanoin</td>
<td>100</td>
<td>M</td>
<td>22</td>
<td>86 ± 12</td>
<td>1 to 0</td>
<td>1 sarcoma</td>
<td>3 fibromas</td>
<td>1</td>
<td>15</td>
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<tr>
<td>10</td>
<td>Trioctanoin</td>
<td>100</td>
<td>F</td>
<td>31</td>
<td>85 ± 16</td>
<td>14 to 1</td>
<td>1 sarcoma</td>
<td>3 fibromas</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

* The means were significantly less than in controls (P < 0.01), only in the groups administered 1-nitropyrene (groups 1–4).

** Significantly different from group 10, P < 0.01.

† Significantly different from groups 6 and 8, P < 0.01.

‡ Significantly different from group 10, P < 0.05.
Many primary aromatic amines are capable of inducing tumors when they are chronically administered to experimental animals. In this study, 1-aminopyrene failed to induce significant incidences of tumors. It structurally resembles 1-naphthylamine to dogs, which led to cancer under chronic conditions with the 2-isomer, gave rise to detectable amounts of tumors. It is one of the few aromatic amines in rodents. Administration of 1- and 2-nitropyrene to dogs, which led to cancer under chronic conditions with the 2-isomer, gave rise to detectable amounts of tumors. It is one of the few aromatic amines in rodents.

**REFERENCES**


CARCINOGENIC ACTIVITIES OF 1-NITROPYRENE AND DERIVATIVES


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