Marked Differences in the Carcinogenic Activity of Optically Pure (+)- and (—)-trans-7,8-Dihydroxy-7,8-dihydrobenzo(a)pyrene in Newborn Mice

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ABSTRACT

Optically pure (+)- and (—)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrenes ([+]- and (—)-BP 7,8-dihydrodiol) were tested for carcinogenicity by giving newborn mice i.p. injections of 20, 40, and 80 nmol of compound on Days 1, 8, and 15 of life. The animals were killed at 17 weeks of age. Control mice had 0.10 pulmonary adenomas per mouse, whereas animals treated with (+)-BP 7,8-dihydrodiol had 0.16 and 9.28 pulmonary adenomas per mouse, respectively. When a 5-fold higher dose was administered according to the above dosage schedule, (+)-BP 7,8-dihydrodiol caused 2.34 pulmonary adenomas per mouse and (—)-BP 7,8-dihydrodiol caused 32.2 pulmonary adenomas per mouse. When 200, 400, and 800 nmol of benzo(a)pyrene or (+)-BP 7,8-dihydrodiol were administered sequentially on Days 1, 8, and 15 of life, 4.13 and 18.5 pulmonary adenomas per mouse, respectively, were observed when the mice were 17 weeks of age. This high dose of (—)-BP 7,8-dihydrodiol killed most of the mice. Administration of (—)-BP 7,8-dihydrodiol caused a high incidence of malignant lymphomas, whereas (+)-BP 7,8-dihydrodiol and benzo(a)pyrene had little or no ability to cause malignant lymphomas.

INTRODUCTION

BP, a widespread environmental pollutant (3), is metabolized with high stereospecificity by enzymes in liver microsomes (5, 21, 23, 28, 29). Microsomes from the livers of 3-methylcholanthrene-pretreated rats metabolize BP to the (—)-enantiomers of BP 4,5-dihydrodiol, BP 7,8-dihydrodiol, and BP 9,10-dihydrodiol to a greater than 10-fold excess relative to their (+)-enantiomers (21-23, 28).

Studies on the carcinogenic activity of racemic BP 7,8-dihydrodiol on mouse skin indicate that this compound has about the same or slightly more activity than does BP as a complete carcinogen (13, 15), and other studies have shown that racemic BP 7,8-dihydrodiol (20) and biosynthetically formed BP 7,8-dihydrodiol (2) are potent tumor initiators on mouse skin. 7,8-Dihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene, which is related to BP 7,8-dihydrodiol but with the double bond removed from the 9,10-position of the molecule, was completely inactive in eliciting tumors (13). These results suggest that BP 7,8-dihydrodiol exerts its carcinogenic effect after metabolism to 1 or more of the reactive BP 7,8-diol-9,10-epoxides. A recent study from our laboratory indicated that the optically pure (—)-enantiomer of BP 7,8-dihydrodiol was 50 to 100% more active than was BP and 5- to 10-fold more active than the (+)-enantiomer of BP 7,8-dihydrodiol when these compounds were tested as initiators of tumors on mouse skin (12). Studies in the newborn mouse revealed that racemic BP 7,8-dihydrodiol and racemic BP 7,8-diol-9,10-epoxide 2 were, respectively, about 15- and 40-fold more active than was BP in causing pulmonary adenomas (7, 8). In the present study, we have tested the optically pure (+)- and (—)-enantiomers of BP 7,8-dihydrodiol for carcinogenicity in newborn mice. The results indicate that the (—)-enantiomer of BP 7,8-dihydrodiol is at least 10 to 20 times more active than is BP or the (+)-enantiomer of BP 7,8-dihydrodiol in causing pulmonary adenomas and malignant lymphomas in newborn mice.

MATERIALS AND METHODS

BP was obtained from the Sigma Chemical Co. (St. Louis, Mo.), and (±)-, (—)-, and (+)-BP 7,8-dihydrodiol were synthesized as previously described (4, 21). All chemicals were of analytical grade purity. The compounds were dissolved in anhydrous DMSO, and they were stored under argon at −90°C. Manipulation of the compounds and injections were done under subdued light.

Pregnant Swiss-Webster BLU:Ha (ICR) mice were obtained from Blue Spruce Farms (Altamont, N. Y.). The mice delivered their babies 2 to 5 days after arrival in our laboratory. Each mother was left with 10 of the healthiest newborns. BP and (±)-, (+)-, and (—)-BP 7,8-dihydrodiol were administered sequentially to the babies at 20, 40, and 80 nmol of benzo(a)pyrene on Days 1, 8, and 15 of life.
were dissolved in anhydrous DMSO at a final concentration of 200 nmol/5 μl. For (+)- and (−)-BP 7,8-dihydrodiol, additional solutions were prepared at final concentrations of 100 and 20 nmol/5 μl. The newborn mice, within 24 hr after birth and on the 8th and 15th day of life, were given i.p. injections of 5, 10, and 20 μl, respectively, of solutions containing the compound. Control mice received injections of DMSO. The mice were weaned at 25 days of age, fed Purina laboratory chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum, and kept in plastic cages with corn cob bedding. Animals that died or were sacrificed when the experiment was terminated were autopsied. All tissues that appeared abnormal were fixed in formalin and were examined histologically. Pulmonary adenomas were counted macroscopically in animals sacrificed at 17 weeks of age, and the pulmonary adenomas were confirmed by histopathological examination. Malignant lymphomas were determined by histological examination of abnormal tissues.

**RESULTS**

Control mice and mice treated with 140 nmol of (+)-BP 7,8-dihydrodiol or (−)-BP 7,8-dihydrodiol during the first 2 weeks of life had 0.10, 0.16, and 9.28 pulmonary adenomas per animal, respectively, at 17 weeks of age (Table 1). These results indicate that the 140-nmol dose of the (−)-enantiomer of BP 7,8-dihydrodiol caused lung adenocarcinomas.

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total dose (nmol)</th>
<th>No. of mice injected on Day 1</th>
<th>Sex</th>
<th>Weaning</th>
<th>No. of mice alive at 12 wk</th>
<th>No. of pulmonary adenomas</th>
<th>% of mice with pulmonary adenomas</th>
<th>No. of mice injected</th>
<th>% of mice with malignant lymphoma</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td>60</td>
<td>M</td>
<td>27</td>
<td>27</td>
<td>27</td>
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<td>15</td>
<td>0.15</td>
<td>0</td>
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<td>M</td>
<td>22</td>
<td>22</td>
<td>21</td>
<td>13</td>
<td>100</td>
<td>51.2</td>
<td>13b</td>
</tr>
<tr>
<td>(±)-BP 7,8-dihydrodiol</td>
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<td>M</td>
<td>22</td>
<td>19</td>
<td>13</td>
<td>100</td>
<td>665</td>
<td>51.2</td>
<td>13b</td>
</tr>
<tr>
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<td>1400</td>
<td>M</td>
<td>22</td>
<td>21</td>
<td>21</td>
<td>4</td>
<td>20</td>
<td>0.20</td>
<td>0</td>
</tr>
<tr>
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<td>700</td>
<td>M</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>10</td>
<td>56</td>
<td>3.00</td>
<td>0</td>
</tr>
<tr>
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<td>M</td>
<td>29</td>
<td>29</td>
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<td>595</td>
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<td>20</td>
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<td>30.8</td>
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<td>M</td>
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<td>1</td>
<td>1</td>
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<td>M</td>
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<td>2</td>
<td>2</td>
<td>100</td>
<td>5.00</td>
<td>4a</td>
</tr>
</tbody>
</table>

**Table 1 notes:**
- a Observed in 5 animals that died between weaning and 17 weeks of age and in 8 animals that were killed at 17 weeks of age.
- b Observed in 4 animals that died between weaning and 17 weeks of age and in 4 animals that were killed at 17 weeks of age.
- c Observed in 1 animal that died between weaning and 17 weeks of age and in 1 animal that was killed at 17 weeks of age.
- d Observed in 2 animals that died between weaning and 17 weeks of age and in 13 animals that were killed at 17 weeks of age.
- e Observed in 6 animals that died between weaning and 17 weeks of age and in 5 animals that were killed at 17 weeks of age.
- f Observed in 1 animal that died before termination of the study.
- g Observed in 3 animals that died between weaning and 17 weeks of age and in 1 animal that was killed at 17 weeks of age.
tomer of BP 7,8-dihydrodiol caused about 150-fold more pulmonary adenomas than did an equimolar dose of the (+)-enantiomer after correcting for the small number of pulmonary adenomas in control mice. When a total dose of 700 nmol of hydrocarbon was administered, (-)-BP 7,8-dihydrodiol caused about 15-fold more pulmonary adenomas than did (+)-BP 7,8-dihydrodiol (32.2 versus 2.34 pulmonary adenomas per mouse). When 1400 nmol of BP or (+)-BP 7,8-dihydrodiol were administered, 4.13 and 18.5 pulmonary adenomas per mouse, respectively, were observed, indicating that (+)-BP 7,8-dihydrodiol was about 4- to 5-fold more active than was BP at the 1 high dose studied. Mice treated with 1400 nmol of racemic BP 7,8-dihydrodiol had about 15-fold more pulmonary adenomas than did mice treated with this same dose of BP, whereas administration of this dose of (-)-BP 7,8-dihydrodiol was highly toxic, and most of the animals died. Histological examination of tissues of mice treated with 1400 nmol of (-)-BP 7,8-dihydrodiol revealed malignant lymphoma in a few animals, but the cause of death in most of these animals is unknown. The results described in Table 1 indicate that (-)-BP 7,8-dihydrodiol and racemic BP 7,8-dihydrodiol were highly active in producing malignant lymphoma, whereas (+)-BP 7,8-dihydrodiol and BP had little or no ability to induce malignant lymphoma.

DISCUSSION

Comparison of the carcinogenic activities of the optically pure (+)- and (-)-enantiomers of BP 7,8-dihydrodiol in newborn mice revealed that the (-)-enantiomer of BP 7,8-dihydrodiol was much more carcinogenic than the (+)-enantiomer, but the magnitude of the difference was dependent on the dose of hydrocarbon administered. Treatment of newborn mice with 140 nmol of (-)-BP 7,8-dihydrodiol caused about 150-fold more pulmonary adenomas than did an equimolar dose of the (+)-enantiomer, whereas treatment of the mice with 700 nmol of (-)-BP 7,8-dihydrodiol caused about 15-fold more pulmonary adenomas than did an equimolar dose of (+)-BP 7,8-dihydrodiol. In addition, (-)-BP 7,8-dihydrodiol was strongly active in causing malignant lymphoma while the (+)-enantiomer had little or no activity. Earlier studies showed that the (-)-enantiomer of BP 7,8-dihydrodiol was more potent than was BP or the (+)-enantiomer of BP 7,8-dihydrodiol as an initiator of tumors on mouse skin and that the (+)-enantiomer was less active than BP (12). In the present study (+)-BP 7,8-dihydrodiol was 4- to 5-fold more active than BP in causing pulmonary adenomas in the newborn mouse. It is clear from our data that the relative carcinogenic activities of BP and the (+)- and (-)-enantiomers of BP 7,8-dihydrodiol depend on the dose of compound and the animal model used. In all of our studies, however, (-)-BP 7,8-dihydrodiol was much more carcinogenic than (+)-BP 7,8-dihydrodiol. It is interesting to note that the more tumorigenic (-)-enantiomer of BP 7,8-dihydrodiol has [7R,8R] absolute stereochemistry in light of the recent observation that the more tumorigenic (-)-enantiomer of benz(a)anthracene 3,4-dihydrodiol also has similar [3R,4R] absolute stereochemistry (11).

The highly stereospecific metabolism of BP by monoxygenase systems and epoxide hydrase described during the past 3 years (5, 21-23, 28-30) probably plays a critical role in the carcinogenicity of BP. The stereochemical course of metabolism of the (+)- and (-)-enantiomers of BP 7,8-dihydrodiol to their diol-epoxides is shown in Chart 1. Liver microsomal enzymes from 3-methylcholanthrene-treated rats metabolize BP to the (-)-enantiomers of BP 4,5-dihydrodiol, BP 7,8-dihydrodiol, and BP 9,10-dihydrodiol to a greater than 10-fold excess relative to the (+)-enantiomers (21-23, 28, 29). Liver microsomes from 3-methylcholanthrene-treated rats metabolize (+)-BP 7,8-dihydrodiol to (+)-BP 7,8-diol-9,10-epoxide 2 and (-)-BP 7,8-diol-9,10-epoxide 1 in a 6:1 ratio, while (+)-BP 7,8-dihydrodiol is converted to (+)-BP 7,8-diol-9,10-epoxide 1 and (-)-BP 7,8-diol-9,10-epoxide 2 in a 22:1 ratio (21). Similar high stereospecificity in the metabolism of (-)-BP 7,8-dihydrodiol to its 2 optically active BP 7,8-diol-9,10-epoxides by rat liver microsomes was observed by Yang et al. (30). Liver microsomes from untreated or phenobarbital-pretreated rats are less stereospecific in the metabolism of the optically pure BP 7,8-dihydrodiol enantiomers to the BP 7,8-diol-9,10-epoxides than are liver microsomes from 3-methylcholanthrene-treated rats (21).

The very high carcinogenic activity of (-)-BP 7,8-dihydrodiol relative to the (+)-BP 7,8-dihydrodiol and BP on mouse skin (12) and in the newborn mouse suggests that (+)-BP 7,8-diol-9,10-epoxide 2 is the primary ultimate carcinogenic metabolite of BP. Preliminary results in the newborn mouse indicate that (+)-BP 7,8-diol-9,10-epoxide 2 has much more tumorigenic activity than do the other 3 optically pure enantiomers of BP 7,8-diol-9,10-epoxide shown in Chart 1. Racemic BP 7,8-diol-9,10-epoxides 1 and 2 are highly mutagenic (5, 18, 25, 27), and studies with the 4 optically pure enantiomers of the BP 7,8-diol-9,10-epoxides indicate sub-
stantial differences in their mutagenic activities. In Chinese hamster V-79 cells, (+)-BP 7,8-diol-9,10-epoxide 2 was 6 to 18 times more mutagenic than were the other 3 isomers (26). Although only BP 7,8-diol-9,10-epoxide 2 was found bound to DNA when BP was metabolized by bovine bronchial explants (6, 24), studies with cultured BHK 21/C13 cells (9), cultured hamster embryo cells (1), and BHK 21/ C13, as well as secondary mouse embryo fibroblast cells (19), provided chromatographic evidence for the binding of both BP 7,8-diol-9,10-epoxides 1 and 2 to DNA after exposure of the cells to BP. In the studies in which bovine explants were exposed to BP, evidence for the binding of (+)-BP 7,8-diol-9,10-epoxide 2 was provided (17). Both (+)-BP 7,8-diol-9,10-epoxide 2 and (+)-BP 7,8-diol- 9,10-epoxide 1 were found bound to skin DNA, RNA, and protein of skin after topical application of BP to the backs of C57BL/6J mice (10, 16). The relative amounts of the 2 diol-epoxides bound depended on whether binding to DNA, RNA, or protein was measured, but in all cases the amount of (+)-BP 7,8-diol-9,10-epoxide 2 that was bound was greater than the amount of (+)-BP 7,8-diol-9,10-epoxide 1. The studies described above, coupled with investigations showing high carcinogenic activity of benzo(a)pyrene 7,8-oxide on mouse skin and in the newborn mouse, indicate that BP undergoes metabolism to benzo(a)pyrene 7,8-oxide and then to (+)-BP 7,8-dihydriodiol, both of which are proximate carcinogens, and that (+)-BP 7,8-dihydriodiol is metabolized to (+)-BP 7,8-diol-9,10-epoxide 2, which is the major ultimate carcinogenic metabolite of BP.

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REFERENCES


Although benzo(a)pyrene 7,8-oxide is a strong carcinogen on mouse skin and in newborn mice, it is less active than BP in both animal models (Ref. 14; P. G. Wislocki, J. Kapitulnik, W. Levin, H. Yagi, D. M. Jerina, and A. H. Conney. Mutagenicity of Benzo[a]pyrene 4,5-oxide, 7,8-, 9-10, and 11,12- Oxidizes in Newborn Mice, Cancer Letters, in press, 1978.)
Effects of (+)- and (−)-BP 7,8-dihydrodiols in Newborn Mice


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