Tumorigenic Activity of Benzo(e)pyrene Derivatives on Mouse Skin and in Newborn Mice


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ABSTRACT

The tumorigenic activities of benzo(e)pyrene and several of its derivatives were determined in two mouse tumor models. Newborn Swiss-Webster mice were given i.p. injections of 0.4, 0.8, and 1.6 µmol of compound on the first, eighth, and 15th day of life, respectively. When the mice were 62 to 66 weeks old, the experiment was terminated by killing the animals. Benzo(e)pyrene, trans-4,5-dihydroxy-4,5-dihydrobenzo(e)pyrene, and trans-9,10-dihydroxy-9,10-dihydrobenzo(e)pyrene had little or no tumorigenic activity in lung tissue, although trans-9,10-dihydroxy-9,10-dihydrobenzo(e)pyrene did induce a significant number of hepatic tumors. The tumor-initiating activities of benzo(e)pyrene and several of its derivatives were determined on the skin of female CD-1 mice. A single topical application of 1.0 to 6.0 µmol of the test compound was followed 7 days later by twice-weekly applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate for 35 weeks. Control mice and mice treated with 6.0 µmol of benzo(e)pyrene, trans-4,5-dihydroxy-4,5-dihydrobenzo(e)pyrene, trans-9,10-dihydroxy-9,10-dihydrobenzo(e)pyrene, and trans-9,10-dihydroxy-9,10,11,12-tetrahydrobenzo(e)pyrene had a tumor incidence of <20% and had ≤0.25 papillomas/mouse. 9,10-Dihydrobenzo(e)pyrene was the only derivative tested that had significant tumor-initiating activity on mouse skin; an initiating dose of 2.5 µmol gave a 67% tumor incidence and 1.43 papillomas/mouse.

INTRODUCTION

The bay region theory of polycyclic hydrocarbon carcinogenesis was formulated from results of studies on the biological activities of benzo(a)pyrene and its bay-region epoxides and from existing carcinogenicity data for alkyl- and fluoro-substituted benzo(a)anthracenes (5, 6). Quantum mechanical calculations predict that, for a given polycyclic hydrocarbon, epoxides which are part of the bay region on a saturated, nonaromatic double bond in the 11,12-position, which in principle could be oxidized to form a bay-region diol-epoxide. The 9,10-dihydroderivative of B(e)P is a symmetrical compound that also has a bay-region nonaromatic double bond. Thus, this compound is a potential metabolic precursor of a reactive tetrahydrodioxepine. Recent studies on the metabolism of B(e)P 9,10-dihydrodiol and 9,10-H2 B(e)P with rat liver microsomes have shown that a bay-region epoxide was not a detectable metabolite of B(e)P 9,10-dihydrodiol but was formed from 9,10-H2 B(e)P (12). Mutagenicity studies were consistent with these observations in that rat liver microsomes or a reconstituted monooxygenase system purified from these microsomes metabolically activated 9,10-H2 B(e)P, but not B(e)P 9,10-dihydrodiol, to bacterial mutagens (12).

In this study, we have compared the tumorigenicity of B(e)P to that of the 4,5- and 9,10-dihydrodiols of B(e)P, 9,10-H2 B(e)P, and B(e)P H4-9,10-diol. The latter compound was used to serve as a negative control, since it cannot be metabolized to a bay-region epoxide on the benzo ring.

MATERIALS AND METHODS

Chemicals. B(e)P (99% pure) was purchased from Aldrich Chemical Co., Milwaukee, Wis., and had no detectable impurities in its 220-MHz nuclear magnetic resonance spectra. B(e)P 4,5-dihydrodiol, B(e)P 9,10-dihydrodiol, B(e)P H4-9,10-diol, 9,10-H2 B(e)P (8), and B(a)P 7,8-dihydrodiol (3) were synthesized as previously described. TPA was purchased from Dr. Peter Borchert, University of Minnesota Medical School, Minneapolis, Minn.

Tumorigenic Study in Newborn Mice. Pregnant mice of the Swiss-Webster BLU:Ha (ICR) strain were obtained from Blue Spruce Farms, Altamont, N. Y., 1 to 14 days before parturition. Within 24 hr of birth, 10 pups in each litter were given i.p. injections of the first dose of compound. Additional injections were given on the eighth and 15th day of life. A total dose of 2.8 µmol of compound was divided into 3 injections of 0.4, 0.8, and 1.6 µmol in 5, 10, and 20 µl of DMSO, respectively.
RESULTS

Since B(e)P is weakly, if at all, carcinogenic in rodent bioassays (2), we planned to give newborn mice a high dose of 5.6 μmol of B(e)P and its 2 metabolically possible trans-dihydrodiols. However, when only 68% of the mice given the first injection of 0.8 μmol of B(e)P 9,10-dihydrodiol survived to the second day of life, the dose of this dihydrodiol had to be reduced. Mice that had received the first injection of 0.8 μmol of B(e)P in 10 μl of DMSO were kept on study and were given additional injections to make their total dose 5.6 μmol of B(e)P in 70 μl of DMSO. Due primarily to toxicity from DMSO itself, the survival rate at weaning in control mice given a total volume of 70 μl of DMSO was 59%, and the survival rate at weaning in mice given 5.6 μmol of B(e)P in 70 μl of DMSO was 52% (Table 1). For a total dose of 2.8 μmol of compound, a total volume of 35 μl of DMSO was sufficient for solution. In mice given a total volume of 35 μl of DMSO, the survival rates at weaning were 96% in control mice and 86, 72, and 90% in mice given a total dose of 2.8 μmol of B(e)P, B(e)P 4,5-dihydrodiol, and B(e)P 9,10-dihydrodiol, respectively (Table 1).

The tumorinitiating activities of B(e)P and its dihydrodiols in newborn mice with the 2 dosage protocols are summarized in Table 1. Since the experiment was not terminated until the mice were 62 weeks old, the incidence of pulmonary tumors in control mice was 2- to 3-fold higher than that observed in several previous experiments where the mice were sacrificed at 20 to 40 weeks of age. At the high doses tested, neither B(e)P nor its dihydrodiols induced more pulmonary tumors than occurred spontaneously in control mice. A few hepatic tumors were observed in male mice, but little difference was observed among the groups except that the incidence of hepatic tumors was significantly higher in mice given B(e)P 9,10-dihydrodiol than in other mice.

The tumor-initiating activities of B(e)P and its derivatives on mouse skin are shown in Table 2. Female CD-1 mice were treated once with the test compound, followed 7 days later by twice-weekly applications of the tumor promoter TPA for 25 weeks. The application of 1.0 to 6.0 μmol of B(e)P and its dihydrodiols gave no increase in tumors with increasing doses. The tumor incidence ranged from 0 to 15% with an average of 3.2 papillomas/mouse. As a positive control for this study, 0.10 μmol of B(a)P 7,8-dihydrodiol was administered. After 25 weeks of promotion, B(a)P 7,8-dihydrodiol induced a 70% tumor incidence with an average of 3.2 papillomas/mouse.
**Table 1**

<table>
<thead>
<tr>
<th>Dose (μmol)</th>
<th>% of mice with tumors</th>
<th>No. of tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>B(e)P</td>
<td>1.0</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0.14</td>
</tr>
<tr>
<td>B(e)P 4,5-dihydrodiol</td>
<td>2.5</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0.17</td>
</tr>
<tr>
<td>B(e)P 9,10-dihydrodiol</td>
<td>1.0</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0.11</td>
</tr>
<tr>
<td>B(e)P H₄-9,10-diol</td>
<td>6.0</td>
<td>0.25</td>
</tr>
<tr>
<td>9,10-H₂ B(e)P</td>
<td>2.5</td>
<td>1.43</td>
</tr>
<tr>
<td>B(e)P 7,8-dihydrodiol</td>
<td>0.10</td>
<td>3.22</td>
</tr>
</tbody>
</table>

**DISCUSSION**

B(e)P and its 2 metabolically possible trans-dihydrodiols had little or no tumorigenic activity in newborn mice or on mouse skin (Tables 1 and 2). We conclude that in these tumor models the compounds have low intrinsic activity and that metabolism of the compounds yields few or no tumorigenic products. 9,10-H₂ B(e)P, a synthetic derivative of B(e)P and a known metabolic precursor of the bay-region tetrahydroepoxide, was active as a tumor initiator on mouse skin (Table 2). The results of this tumorigenicity study are consistent with previous results from mutagenicity studies. When tested for metabolic activation with rat liver microsomes, B(e)P, B(e)P 9,10-dihydrodiol, and B(e)P H₄-9,10-diol had little or no mutagenic activity and B(e)P 4,5-dihydrodiol (K-region dihydrodiol) had some mutagenic activity towards strains TA98 and TA100 of Salmonella typhimurium (12). However, 9,10-H₂ B(e)P was metabolically activated to potent mutagenic products, and B(e)P H₄-9,10-epoxide had high intrinsic mutagenic activity in both bacterial and mammalian cells (12).

Previous studies with rat liver microsomes have shown that relatively little, if any, B(e)P 9,10-dihydrodiol was formed metabolically from B(e)P and that B(e)P 9,10-dihydrodiol was metabolized primarily to 4,5,9,10-tetrahydroxy-4,5,9,10-tetrahydrobenzo(e)pyrene along with phenolic derivatives of the dihydrodiol rather than to a bay-region diol-epoxide (12). In a study of the metabolism of 9,10-H₂ B(e)P by rat liver microsomes, several products were identified; one product was B(e)P H₄-9,10-diol (see Chart 1 legend for nomenclature), which would be expected to be formed by spontaneous and...

enzymatic hydration of the bay-region tetrahydroepoxide, B(e)P H$_2$-9,10-epoxide. These results indicate that 9,10-H$_2$ B(e)P was metabolized to the bay-region tetrahydroepoxide and that this metabolite was an ultimate mutagenic product (12).

From quantum mechanical calculations associated with the bay region theory (6, 7), high chemical reactivities are predicted for the bay-region epoxides of B(e)P, dibenzo-(a,h)anthracene, benzo(a)anthracene, and benzo(a)pyrene (AE$_{exc}/eta = 0.714, 0.738, 0.766$, and 0.794, respectively). In each of these cases except for B(e)P, bay-region diol-epoxides are either known to have or are thought to have high biological activity (1). Although B(e)P shows little or no carcinogenic activity (2), its bay-region tetrahydroepoxide does have the predicted high intrinsic mutagenic activity (0.4 to 1.8 times that of the corresponding 9,10-epoxy-9,10,11,12-tetrahydrobenzo(a)pyrene in strains TA98 and TA100 of S. typhimurium and in Chinese hamster V79 cells) and appears to be an ultimate reactive metabolite which mediates the mutagenic and tumorigenic activity of 9,10-H$_2$ B(e)P (12). Because of the difficulty in synthesizing the reactive bay-region diol-epoxides of B(e)P in a pure state (8), these compounds were unavailable for the present tumorigenicity studies.

Recent isolation of the diastereomerically pure benzo(e)pyrene 9,10-diol-11,12-epoxides in which the epoxide is cis or trans to the benzylic 9-hydroxyl group (13), however, has led to the striking observation that these diol-epoxides, unlike B(e)P H$_2$-9,10-epoxide, have very low mutagenic activity compared to the diastereomeric benzo(a)pyrene 7,8-diol-9,10-epoxides in which the epoxide is cis or trans to the 7-hydroxyl group. The very weak mutagenicity of the B(e)P 9,10-diol-11,12-epoxides and their probable low tumorigenicity may be related to the fact that these diol-epoxides are structurally rigid and have their hydroxyl groups locked in a diaxial conformation (12, 13). It is important to note that these are the first examples of such conformationally rigid diol-epoxides.

Thus, several factors may contribute to the very low tumorigenic activity of B(e)P. If newborn mice and mouse skin metabolize B(e)P and B(e)P 9,10-dihydriodiol in a manner analogous to rat liver microsomes, then very little, if any, bay-region diol-epoxides are formed. Based on their low mutagenicity, these diol-epoxides are anticipated to have only weak tumorigenicity. Nonetheless, these diol-epoxides may be responsible for the hepatic tumors caused by the 9,10-dihydriodiol in the newborn mouse.

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