Tumorigenic Activity of the 4,5- and 9,10-Dihydrodiols of Benzo[j]fluoranthene and Their syn- and anti-Diol Epoxides in Newborn Mice

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ABSTRACT

Benzo[j]fluoranthene (B[j]F), trans-4,5-dihydro-4,5-dihydroxy-B[j]F, and trans-9,10-dihydro-9,10-dihydroxy-B[j]F were evaluated for tumorigenic activity in newborn CD1 mice. These dihydrodiols were assayed at doses of 1.10 and 0.275 µmol/mouse. B[j]F and the syn- and anti-diol epoxides derived from these dihydrodiols were assayed at doses of 1.10, 0.275, and 0.110 µmol/mouse (80 mice/group). trans-4,5-Dihydro-4,5-dihydroxy-B[j]F was more potent than trans-9,10-dihydro-9,10-dihydroxy-B[j]F in inducing pulmonary tumors in both female and male mice. Administration of 1.10 µmol of trans-4,5-dihydro-4,5-dihydroxy-B[j]F resulted in a 90-92% incidence of pulmonary tumors with an average of 3.6 and 4.2 tumors/mouse among female and male mice, respectively. A similar tumorigenic activity was observed for B[j]F in lung. trans-9,10-Dihydro-9,10-dihydroxy-B[j]F was significantly less tumorigenic (P < 0.05), producing a 44 and 64% incidence of pulmonary tumors at a dose of 1.10 µmol with an average of 0.8 and 1.0 tumor/mouse in female and male mice, respectively. A statistically significant (P < 0.001) incidence of hepatic tumors was also produced by 0.110 µmol/mouse administered either B[j]F or trans-4,5-dihydro-4,5-dihydroxy-B[j]F at a dose of 1.10 µmol/mouse. In comparing the tumorigenicity of the diasteromeric diol epoxides derived from both trans-4,5-dihydro-4,5-dihydroxy-B[j]F and trans-9,10-dihydro-9,10-dihydroxy-B[j]F, the anti-diastereomers exhibited greater tumorigenic activity. The most tumorigenic diol epoxide was anti-4,5-dihydroxy-6,6a-epoxy-4,5,6,6a-tetrahydro-B[j]F. At a dose of 0.275 µmol, this diol epoxide induced a 96 and 100% incidence of pulmonary tumors in female and male mice, with an average of 8.6 and 5.0 tumors/mouse, respectively. anti-9,10-Dihydroxy-11,12-epoxy-9,10,11,12-tetrahydro-B[j]F at this dose produced a 56 and 95% incidence of pulmonary tumors in female and male mice with an average of 1.0 and 2.8 tumors/mouse, respectively. syn-4,5-Dihydroxy-6,6a-epoxy-4,5,6,6a-tetrahydro-B[j]F and syn-9,10-dihydroxy-11,12-epoxy-9,10,11,12-tetrahydro-B[j]F at a dose of 0.275 µmol did not induce a significant incidence (P > 0.05) of pulmonary tumors in female or male mice. These data suggest that anti-4,5-dihydroxy-6,6a-epoxy-4,5,6,6a-tetrahydro-B[j]F may be the principal ultimate carcinogenic form of B[j]F responsible for the induction of lung tumors in newborn mice. In contrast to their anti-isomers, syn-4,5-dihydroxy-6,6a-epoxy-4,5,6,6a-tetrahydro-B[j]F and syn-9,10-dihydroxy-11,12-epoxy-9,10,11,12-tetrahydro-B[j]F did not induce a significant incidence of liver tumors in male mice at a total dose of 0.110 µmol, anti-4,5-Dihydroxy-6,6a-epoxy-4,5,6,6a-tetrahydro-B[j]F and anti-9,10-dihydroxy-11,12-epoxy-9,10,11,12-tetrahydro-B[j]F at total doses of 0.110 and 0.275 µmol had comparable tumorigenic activity in the liver of newborn male mice. These results suggest that either one or both of the activation pathways associated with the formation of these electrophilic metabolites could contribute to the hepatocarcinogenic activity observed for B[j]F in newborn male mice.

INTRODUCTION

B[j]F\textsuperscript{2} is formed from the incomplete combustion of organic matter. It is a common environmental pollutant which has been detected as a component in automobile exhaust, tobacco smoke, and barbecued foods\textsuperscript{(1)}. The mutagenic activity of B[j]F in Salmonella typhimurium\textsuperscript{(2)} and its tumorigenic activity in mice and rats\textsuperscript{(3-6)} are well established. B[j]F\textsuperscript{2} differs from several of the major carcinogenic PAH prevalent in the environment. B[j]F is a nonaltemant PAH which does not possess within its structure a classical bay-region. Thus, the formation of a classical bay-region diol epoxide as an ultimate carcinogenic metabolite of B[j]F is not feasible. Two principal metabolites, B[j]F-4,5-diol and B[j]F-9,10-diol, have been associated with the genotoxic activity of B[j]F\textsuperscript{(1)} (Fig. 1). Both of these dihydrodiols have been shown to be mutagenic in Salmonella typhimurium when assayed in the presence of a microsomal activation system\textsuperscript{(2, 7)}. Thus, B[j]F has 2 distinct mechanisms by which it could ultimately be converted to a tumorigenic agent\textsuperscript{(1)} (Fig. 1). These dihydrodiols, which have been identified as \textit{in vivo} metabolites in mouse skin\textsuperscript{(8)}, are also active as tumor initiators when applied to mouse skin\textsuperscript{(8, 9)}. B[j]F-4,5-diol, when assayed at a total initiator dose of 1.0 µmol, exhibited greater tumorigenic activity than B[j]F-9,10-diol or B[j]F\textsuperscript{(1)} (Fig. 1). These data suggest that B[j]F-4,5-diol is the more potent tumorigenic metabolite and may contribute to a greater extent to the tumorigenic activity observed with B[j]F on mouse skin. Consistent with these results, recent studies have also implicated the anti-4,5-B[j]F-DE\textsuperscript{(1)} as being the principal electrophilic metabolite associated with DNA adduct formation\textsuperscript{(1). In this paper, we report the results of a bioassay performed with newborn CD1 mice in which we have evaluated the tumorigenic activity of B[j]F, B[j]F-4,5-diol, B[j]F-9,10-diol, and the syn- and anti-diol epoxides derived from these dihydrodiols. These studies provide data on the relative tumorigenic activity of these dihydrodiol metabolites of B[j]F in newborn mice. These data also provide insight into the intrinsic tumorigenic activity of the diol epoxides that represent the ultimate electrophiles associated with the 2 suspect activation pathways responsible for the genotoxic activity of B[j]F\textsuperscript{(1)}.

MATERIALS AND METHODS

Chemicals. B[j]F was obtained from the Commission of the European Communities Bureau of Reference, Brussels, Belgium. The syntheses used for the preparation of B[j]F-4,5-diol, B[j]F-9,10-diol, and the syn- and anti-isomers of B[j]F-4,5-DE and B[j]F-9,10-DE have been reported\textsuperscript{(12)}. The conditions for the HPLC analysis of B[j]F-4,5-diol and B[j]F-9,10-diol have been described previously\textsuperscript{(8)}. The syn- and anti-isomers of B[j]F-4,5-DE and B[j]F-9,10-DE were analyzed on a Hewlett-Packard model 1090 HPLC equipped with a HP-1040 diode array UV detector. These diol epoxides were chromatographed on a 10-µm LiChrosorb Si 60 column (4 x 250 mm) under isocratic conditions using hexane:tetrahydrofuran (3:17) containing 0.5% triethylamine. At a flow rate of 1 ml/min, syn-B[j]F-4,5-DE and syn-B[j]F-9,10-DE eluted at 3.7 and 4.0 min, respectively. Under these conditions, the purity of the diol epoxides was established by HPLC. The diol epoxides were analyzed by HPLC using a semipreparative column (LiChrosorb Si 60, 4 x 250 mm) eluted with hexane:acetone (95:5) at a flow rate of 3 ml/min, and the purity of the diol epoxides was confirmed by HPLC using a semipreparative column (LiChrosorb Si 60, 4 x 250 mm) eluted with hexane:acetone (95:5) at a flow rate of 3 ml/min.
anti-B[\(j\)]F-4,5-DE, anti-B[\(j\)]F-9,10-DE were weaned at 28 days of age and separated by sex. The purity of all compounds was >99% as determined by HPLC. Pups were administered i.p. injections of either DMSO alone or the appropriate compound in DMSO. Each pup received 5, 10, and 20 \(\mu\)l of either DMSO or a solution of each compound in DMSO on days 1, 8, and 15 of life, respectively. The total dose of each compound administered per mouse in this assay is given in Tables 1 and 2. Mice were housed 4/cage in Micro-Isolator cages obtained from Lab Products, Inc., Maywood, NJ. The hardwood bedding used in these studies was Beta-Chip purchased from North Eastern Products, Warrenburg, NY. Mice were given water and NIH-07 diet ad libitum and were kept under controlled conditions with a 12-h light/dark cycle until they were 52 weeks of age.

Histopathology. All tissues were examined during necropsy for gross lesions with an illuminated magnifier. The number of tumors at each organ site was recorded and tissue stored in 10% buffered formalin. Only 5% of the mice in the experimental group receiving 0.275 \(\mu\)mol of anti-B[\(j\)]F-4,5-DE survived the initial treatment and were alive at 1 year of age. Because of the limited number of mice within this experimental group, tissue from these animals was not examined microscopically. In view of the low incidence of tumors observed upon necropsy of mice treated at a total dose of 0.110 \(\mu\)mol with either syn-B[\(j\)]F-4,5-DE or syn-B[\(j\)]F-9,10-DE, tissue from mice in these experimental groups was also not submitted for histopathological evaluation. The lungs of all other treated mice were examined microscopically. Alveolar-bronchiolar carcinomas, varying in size and number from several large masses to multiple small masses, were the primary lung tumors observed and were much more common than adenomas (Fig. 2). The nuclei of these cells were large and variable sized, cribiform to vesicular, with 2–4 small nucleoli. These cells were pleomorphic with variable amounts of eosinophilic and finely vacuolated cytoplasm. Pulmonary adenomas, although much less common than carcinomas, were typical type II adenomas and were often located at the pleural surface (13). They were unencapsulated with even or irregular, but well-demarcated, borders. They consisted of a uniform population of cuboidal cells with eosinophilic cytoplasm and a central hyperchromatic nucleus, and formed a solid or trabecular pattern. There was compression of local tissue and little evidence of invasion into the alveolar spaces.

The livers of all male mice and all tissue with suspect lesions or tumors were also submitted for histopathological examination with the exception of the 3 experimental groups previously noted. Alveolar carcinomas, and hepatocellular carcinomas were much less common than adenomas. Carcinomas were much less common than adenomas or foci or adenomas. Lymphoma and some spindle cell sarcomas, most likely hemangiosarcomas, were also present, but were not considered to be compound-related. The significance of differences observed between experimental groups was evaluated using the \(\chi^2\) test.

RESULTS

Tables 1 and 2 summarize the results of the bioassay in newborn mice. As is frequently observed for tumorigenic PAH under these assay conditions, compound-related tumor formation was observed primarily in lung and liver. Alveolar-bronchiolar carcinomas were the primary lung tumors observed (Fig. 2). The incidence and multiplicity of these pulmonary carcinomas are outlined in Table 1. The tumors were locally invasive with little to a moderate amount of compression of the parenchyma. Growth occurred into the airways and along the alveolar walls. Clumps of tumor cells were free in the alveolar spaces, within airway lumens and occasionally within vessels. Within the tumor masses, particularly the larger masses, heterogenous growth patterns were common. Some cells contained eosinophilic granules similar to bronchial epithelial cells, and some tumors appeared to arise directly from bronchial epithelium. These tumors were fairly well differentiated, however, they were distinguished from adenomas by the presence of tumor clumps within alveoli, heterogenous growth patterns, atypical and pleomorphic cellular features, and the presence of mitotic figures. Hepatic tumors, as listed in Table 2, reflect the incidence and multiplicity of the both adenomas and hepatocellular carcinomas observed within each experimental group. The morphology of the adenomas (Fig. 3) was consistent with previous reports in the literature (13). Hepatocarcinomas were characterized by poorly demarcated and invasive growth into the hepatic parenchyma (Fig. 3). These tumors exhibited solid or trabecular growth with highly pleomorphic cells with large and often bizarre nuclei.

Administration of 1.10 \(\mu\)mol B[j]F induced a 92 and 100% incidence of pulmonary tumors in female and male mice, respectively. While female mice at this dose did not develop a significant incidence of hepatic tumors, 56% of the males developed liver tumors with an average of 2.2 tumors/mouse (Table 2). B[j]F administered at a total dose of 0.275 \(\mu\)mol produced an elevated incidence of pulmonary tumors in both female and male mice (44–50% incidence) and a significant increase (\(P < 0.005\)) in hepatic tumors among males (38% incidence) relative to controls. The tumorigenic activity of B[j]F-4,5-diol closely paralleled the response observed with B[j]F, producing a similar incidence of pulmonary and hepatic tumors. This was evident in the average number of tumors induced in both organ sites. In contrast, B[j]F-9,10-diol at a dose of 1.10 \(\mu\)mol produced a much lower incidence as well as fewer pulmonary tumors than either B[j]-F-4,5-diol or B[j]F. Among the male mice administered 1.10 \(\mu\)mol B[j]F-9,10-diol, however, there was an 82% incidence of hepatic tumors with an average of 2.8 tumors/mouse. B[j]F-9,10-diol was as active, if not more active, than B[j]F-4,5-diol or B[j]F in producing liver tumors in newborn male mice.

Fig. 1. Metabolic activation pathways associated with B[j]F-4,5-diol and B[j]F-9,10-diol.
Table 1 Lung tumor induction in newborn mice administered B(1)F and B(1)F metabolites

CD1 mice were given i.p. injections of either DMSO or compound in DMSO on the 1st, 8th, and 15th days of life. Mice were weaned at 28 days of age, separated by sex, and sacrificed at 52 weeks of age.

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<th>Compound</th>
<th>Total dose (μmol)</th>
<th>Total no. of pups treated</th>
<th>Effective no. of mice (sex)</th>
<th>% of mice with tumors</th>
<th>Pulmonary tumors</th>
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<sup>a</sup> The effective number of mice refers to the number of mice alive at 52 weeks of age. An initial experimental group of 80 mice was separated into subgroups consisting of males and females.

<sup>b</sup> Unless specified otherwise, all data reflect histologically confirmed alveolar-bronchiolar carcinomas.

<sup>c</sup> Data reflect gross necropsy results only.

<sup>d</sup> p < 0.05.

<sup>e</sup> p < 0.01.

<sup>f</sup> p < 0.001.

<sup>g</sup> p < 0.005.

Differences in the intrinsic tumorigenic activity of the syn- and anti-diol epoxides derived from both B(1)F-4,5-diol and B(1)F-9,10-diol were clearly discernable in this bioassay. The anti-diasteromer of each diol epoxide exhibited greater tumorigenic activity than the syn-isomer. Each of these diol epoxides was administered at doses of 1.10, 0.275, and 0.110 μmol. At a 1.10-μmol dose, syn-B(1)F-4,5-DE induced an 41 and 65% incidence of pulmonary tumors among female and male mice, respectively. Extensive toxicity was associated with the administration of 1.10-μmol of anti-B(1)F-4,5-DE, with only 5% of the treated animals surviving until 52 weeks of age. Based upon the response observed at dose levels of 0.275 and 0.110 μmol, it is evident that anti-B(1)F-4,5-DE is much more potent as a tumorigenic agent than B(1)F or syn-B(1)F-4,5-DE. The incidence of pulmonary tumors in female and male mice administered anti-B(1)F-4,5-DE at a dose of 0.275 μmol was 96 and 100%, with an average of 8.6 and 5.0 tumors/animal, respectively (Table 1). A high incidence of tumors was also observed in mice administered anti-B(1)F-4,5-DE. At a dose of 0.110 μmol, 89 and 85% of the female and male mice developed pulmonary tumors with an average of 2.1 and 2.9 tumors/animal, respectively. The greater potency of anti-B(1)F-4,5-DE relative to its syn-isomer was also apparent in the incidence of hepatic tumors in male mice. At a dose of 0.275 μmol, anti-B(1)F-4,5-DE produced an 83% incidence of hepatic tumors in male mice with an average of 4.2 tumors/mouse. There was also a 33% incidence of hepatocarcinoma within this experimental group. In contrast, syn-B(1)F-4,5-DE induced only a 29% incidence of hepatic tumors in male mice with an average of 0.4 tumors/mouse. In addition, syn-B(1)F-4,5-DE was also considerably less active in inducing pulmonary tumors than B(1)F or its corresponding dihydrodiol precursor at equivalent doses.

The greater potency observed for the anti-isomer of B(1)F-4,5-DE was also observed for the diasteromeric epoxide derivatives of B(1)F-9,10-diol. An increase in toxicity was associated with the administration of anti-B(1)F-9,10-diol at a dose of 0.110 μmol, 89 and 85% of the female and male mice developed pulmonary tumors with an average of 2.1 and 2.9 tumors/animal, respectively. The greater potency of anti-B(1)F-9,10-DE relative to its syn-isomer was also apparent in the incidence of hepatic tumors in male mice. At a dose of 0.275 μmol, anti-B(1)F-9,10-DE produced an 83% incidence of hepatic tumors in male mice with an average of 4.2 tumors/mouse. There was also a 33% incidence of hepatocarcinoma within this experimental group. In contrast, syn-B(1)F-9,10-DE induced only a 29% incidence of hepatic tumors in male mice with an average of 0.4 tumors/mouse. In addition, syn-B(1)F-9,10-DE was also considerably less active in inducing pulmonary tumors than B(1)F or its corresponding dihydrodiol precursor at equivalent doses.
Table 2  Liver tumor induction in newborn mice administered B[jlF and B[jlF metabolites

CD1 mice were given i.p. injections of either DMSO or compound in DMSO on the 1st, 8th, and 15th days of life. Mice were weaned at 28 days of age, separated by sex, and sacrificed at 52 weeks of age.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total dose (μmol)</th>
<th>Effective no. of mice (sex)</th>
<th>% tumor-bearing mice</th>
<th>No. of tumors/mouse</th>
<th>% of mice with carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>B[jlF</td>
<td>0.110</td>
<td>29 (F)</td>
<td>6.8</td>
<td>0.07°</td>
<td>ND</td>
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<tr>
<td></td>
<td>0.275</td>
<td>32 (F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>1.10</td>
<td>38 (F)</td>
<td>2.6</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>B[jlF-4,5-diol</td>
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<td>34 (F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.10</td>
<td>38 (F)</td>
<td>2.6</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>B[jlF-9,10-diol</td>
<td>0.275</td>
<td>22 (F)</td>
<td>4.5</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.10</td>
<td>39 (F)</td>
<td>7.9</td>
<td>0.21</td>
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<tr>
<td>Anti-B[jlF-4,5-DE</td>
<td>0.110</td>
<td>36 (F)</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.275</td>
<td>33 (M)</td>
<td>42.4°</td>
<td>0.79</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>1.10</td>
<td>1 (F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Syn-B[jlF-4,5-DE</td>
<td>0.110</td>
<td>26 (F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.275</td>
<td>34 (M)</td>
<td>23.5°</td>
<td>0.35</td>
<td>ND</td>
</tr>
<tr>
<td>Anti-B[jlF-9,10-DE</td>
<td>0.110</td>
<td>33 (M)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.275</td>
<td>34 (F)</td>
<td>8.8</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.10</td>
<td>39 (M)</td>
<td>77.5°</td>
<td>2.82</td>
<td>15.6</td>
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<tr>
<td>Syn-B[jlF-9,10-DE</td>
<td>0.110</td>
<td>28 (F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.275</td>
<td>35 (M)</td>
<td>25.7°</td>
<td>0.40°</td>
<td>ND</td>
</tr>
<tr>
<td>DMSO</td>
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<td>33 (F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33 (M)</td>
<td>9.1</td>
<td>0.18</td>
<td>0</td>
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</tbody>
</table>

° The effective number of mice refers to the number of mice alive at 52 weeks of age. An initial experimental group of 80 mice was separated into subgroups consisting of males and females.

b Unless specified otherwise, all data reflect histologically confirmed adenomas and hepatocellular carcinomas.

c Data reflect gross necropsy results only.

° P > 0.05.

° P < 0.005.

° P < 0.001.

° P < 0.05.

**Tration of 1.10 μmol of anti-B[jlF-9,10-DE (53% survival 1 year after dose administration as compared to 79% for syn-B[jlF-9,10-DE). For this pair of isomers, a sufficient number of animals survived the 1.10-μmol dose to allow an assessment of the tumorigenic potency of both the syn- and anti-diol epoxides. Administration of 1.10 μmol of anti-B[jlF-9,10-DE resulted in a 100% incidence of pulmonary tumors, with an average of 15.1 to 11.1 tumors/mouse for female and male mice, respectively. In contrast to these results, a 1.10-μmol dose of syn-B[jlF-9,10-DE induced a 68 and 55% inciden of pulmonary tumors with an average of 0.88 and 0.78 tumor/mouse among female and male mice, respectively. The greater tumorigenic potency of anti-isomer of B[jlF-9,10-DE is also evident in comparing the incidence of hepatic tumors observed in male mice (Table 2). At a dose of 1.10 μmol, the incidence of hepatic tumors among male mice treated with anti-B[jlF-9,10-DE was 96% with an average of 5.5 tumors/mouse. This is in contrast to male mice treated with syn-B[jlF-9,10-DE, which at the same dose produced a 68% incidence of liver tumors with an average of 2.2 tumors/animal. The tumorigenic activity of syn-B[jlF-9,10-DE in the liver of male mice was comparable to that observed with B[jlF. Syn-B[jlF-9,10-DE was also considerably less active than B[jlF in inducing lung tumors in either female or male mice.

**DISCUSSION**

A previous bioassay demonstrated that B[jlF was tumorigenic in newborn mice when assayed at a dose of 1.10 μmol/mouse (3). In the present study, B[jlF was assayed at doses of 0.110, 0.275, and 1.10 μmol/mouse. The results of this bioassay provide clear insight into the dose-response relationship associated with the tumorigenic activity of B[jlF in lung and liver of newborn mice. Unlike the previous bioassay, which was maintained for 35 weeks, the present study was terminated when the mice were 52 weeks of age. This difference is likely re-
B[βJF DIGOLS AND DIOL EPOXIDES IN NEWBORN MICE

The tumorigenic responses obtained for B[βJF and B[βJF-4,5-diol at dose levels of 1.10 and 0.275 μmol demonstrate similar potency with regard to the induction of lung tumors in male and female newborn mice. At a total dose of 1.10 μmol, mice administered B[βJF-4,5-diol on average developed a greater number of lung tumors than mice treated with B[βJF. B[βJF-9,10-diol, in contrast, was significantly less potant (P < 0.001) in inducing lung tumors than B[βJF. These data suggest that between these 2 genotoxic metabolites of B[βJF, the 4,5-dihydrodiol is more likely to be responsible for the induction of lung tumors. These data correlate with results of bioassays performed on mouse skin. B[βJF-4,5-diol is more tumorigenic than B[βJF-9,10-diol as a tumor initiator on mouse skin and forms significantly more adducts with DNA (8, 9, 11). The similar tumorigenic potency of B[βJF and B[βJF-4,5-diol in the induction of lung tumors, however, suggests that further metabolic activation of this dihydrodiol may be a critical factor which influences tumorigenic activity in newborn mice.

Liver tumor induction occurred to a significant extent only among male newborn mice in the experimental groups treated with B[βJF or derivatives of B[βJF. The sex specificity associated with the induction of hepatic tumors in male newborn mice can be seen in a number of previously reported newborn mouse bioassays using polycyclic aromatic hydrocarbons (14–19). This increased sensitivity for liver tumor induction among male newborn mice has also been observed in mice administered N-nitrosodiethylamine and quinoline (20–22). There are data which suggest that this difference is hormonally linked (23, 24). In contrast to the distinct differences in tumorigenic activity observed in lung, B[βJF, B[βJF-4,5-diol, and B[βJF-9,10-diol exhibited similar tumorigenic potency in liver of male newborn mice. Both metabolic dihydrodios, which represent divergent pathways of activation (Fig. 1), may be associated with the tumorigenic response observed for B[βJF at this organ site.

Tumor induction by these 2 dihydrodios is likely to be influenced by the extent to which they are converted to their diol epoxides. Evaluation of the relative tumorigenic activity of the syn- and anti-diol epoxide derivatives of each of these dihydrodios allows for an assessment of the intrinsic tumorigenic potential of the suspect electrophilic metabolites. Major differences in tumorigenic potency were observed between the syn- and anti-diol epoxides derived from B[βJF-4,5-diol. With respect to tumorigenic activity in lung,

Fig. 2. A, bronchoalveolar carcinoma (anti-B[βJF-DE, 0.275 μmol) characterized by a large poorly defined mass infiltrating along alveolar septa and extending into alveolar spaces. × 25, hematoxylin and eosin. B, higher magnification showing heterogeneous alveolar pattern with pleomorphism and mitosis. Clumps of neoplastic cells extend into alveolar lumens. × 220, hematoxylin and eosin.

Fig. 3. A, hepatic adenoma (anti-B[βJF-DE, 0.275 μmol). × 50, hematoxylin and eosin. B, hepatocellular adenocarcinoma. Irregular trabacula extend and invade throughout the parenchyma. Hepatocytes are pleomorphic and atypical with mitosis present. × 200, hematoxylin and eosin.
syn-B[j]F-4,5-DE was significantly less active (P < 0.001) than B[j]F. Anti-B[j]F-4,5-DE, in contrast, was exceptionally potent as a tumorigenic agent in lung relative to B[j]F and the other dihydrodiols and diol epoxides evaluated in this bioassay. Only at a 4-fold higher dose could syn-B[j]F-4,5-DE or B[j]F induce a tumorigenic response in the liver of male mice comparable to that induced by anti-B[j]F-4,5-DE at a dose of 0.275 μmol. These data clearly indicate that anti-B[j]F-4,5-DE is more potent than the syn-diastereomer in the induction of lung tumors in mice of both sexes as well as liver tumors in males.

Increased tumorigenic activity was also associated with anti-B[j]F-9,10-DE as compared to syn-B[j]F-9,10-DE (P < 0.001). Anti-B[j]F-9,10-DE was not only more active in inducing lung tumors than its syn isomer, but also more potent than B[j]F. This is in contrast to syn-B[j]F-4,5-DE, which was significantly less active than B[j]F (P < 0.001) in inducing lung tumors in both male and female mice. A similar trend in tumorigenic activity in the liver of male mice was also observed for newborn mice administered syn- and anti-B[j]F-9,10-DE. Relative to B[j]F, syn-B[j]F-9,10-DE was not more tumorigenic. In contrast, anti-B[j]F-9,10-DE was more tumorigenic than B[j]F in livers of male mice. This is evident from the observed tumor incidence at doses of 0.275 and 0.110 μmol. Syn-B[j]F-9,10-DE at equivalent dose levels does not produce an increased tumorigenic response over B[j]F in either the lung or liver. These data suggest that syn-B[j]F-9,10-DE is not a major contributor to the tumorigenic activity of B[j]F in newborn mice.

Both of the anti-diol epoxides exhibited more tumorigenic activity than their respective dihydrodiol precursors. These data indicate that for the 2 activation pathways proposed for B[j]F (Fig. 1), the extent to which these metabolic dihydrodiols are further transformed to their diol epoxides is likely a critical factor influencing tumor induction in newborn mice. Based upon the greater tumorigenic activity of anti-B[j]F-4,5-DE in lung, this metabolite of B[j]F could be primarily responsible for tumor induction at this organ site. These data are consistent with the significantly greater tumorigenic activity of B[j]F-4,5-diol as compared to B[j]F-9,10-diol in lungs of newborn mice (P < 0.005). Recently, studies on the types and levels of DNA adducts formed in vivo in mouse skin have also indicated that anti-B[j]F-4,5-DE accounts for more than 86% of DNA adducts formed from B[j]F (10, 11). Differences in liver tumor induction between anti-B[j]F-4,5-DE and anti-B[j]F-9,10-DE are not as apparent. The acute toxicity associated with administration of 1.10 μmol of anti-B[j]F-4,5-DE precludes a direct comparison of its potency as a liver tumorigen with anti-B[j]F-9,10-DE at this dose. However, the incidence of liver tumors among the male mice treated with 0.275 and 0.110 μmol of either anti-B[j]F-4,5-DE or anti-B[j]F-9,10-DE is comparable.

The diol epoxides derived from B[j]F-4,5-diol and B[j]F-9,10-diol differ from bay-region diol epoxides, which have frequently been identified as the principal metabolites ultimately responsible for the carcinogetic activity of alternate PAH. Several studies have implicated the involvement of (±)-anti-7,8-dihydro-7,8-dihydroxy-9,10-epoxy-B[a]P as the ultimate carcinogetic metabolite of B[a]P (15, 25). Bay-region diol epoxides have been implicated as the principal activation pathway for several other alternate PAH such as chrysene, phenanthrene, benz[a]anthracene, dibenz[a,h]pyrene, and dibenzo-[a,l]pyrene (14, 16–19). The anti- and syn-diol epoxides of B[j]F-4,5-diol are chemically distinct from bay-region diol epoxides of these alternate PAH. The oxirane of B[j]F-4,5-DE is comprised of a trisubstituted olefin with one of its carbon atoms associated with a ring junction. The increased substitution associated with the dibenzyl carbon atom, which is part of the epoxide of either anti- or syn-B[j]F-4,5-DE, makes comparison of its reactivity with that observed with bay-region diol epoxides difficult to predict. There are also significant differences in the preferred conformations associated with bay-region diol epoxides relative to that of either anti- or syn-B[j]F-4,5-DE. Computer modeling of the bay-region diol epoxide of B[a]P has indicated the benzylic carbon of the oxirane is almost planar to the aromatic ring system. In contrast, the benzylic carbon of the oxirane of either anti- or syn-B[j]F-4,5-DE deviates significantly from the planarity of their aromatic ring systems (7). The diol epoxides derived from B[j]F-9,10-diol are also structurally different from those which are associated with alternate PAH. These diol epoxides are within a 4-sided bay-region that most closely resembles the fjord region of alternate PAH such as benzol[c]phenanthrene, benzo[c]chrysene, and benzo[g]chrysene. In contrast to the diol epoxides within the fjord region of these alternate PAH, however, a 5-membered ring in the case of anti- and syn-B[j]F-9,10-DE forms part of the region within which the epoxide moiety resides. This has been previously referred to as a pseudo bay-region (26). In contrast to the anti- and syn-isomers of B[j]F-4,5-DE, as well as diol epoxides within the fjord region of B[c]P, the anti- and syn-B[j]F-9,10-diol have similar planarity to that observed with the bay-region diol epoxides of alternate PAH such as B[a]P (7).

The bioassay of these chemically distinct diols and dihydrodiol epoxides has provided further insight into the genotoxicity of B[j]F metabolites and their potential role in producing a tumorigenic response in newborn mice. Metabolic activation of B[j]F via the formation of B[j]F-4,5-diol and ultimately anti-B[j]F-4,5-DE appears to be the major mechanism by which B[j]F induces lung tumors in newborn mice as well as tumors in mouse skin. The principal activation pathway by which B[j]F induces liver tumors in newborn male mice is not readily apparent from the bioassay results summarized in Table 2. As mentioned previously, B[j]F, B[j]F-4,5-diol, and B[j]F-9,10-diol had similar potency with regard to the induction of liver tumors in male mice. In comparing the ability of the syn- and anti-isomers of each of the epoxides derived from these dihydrodiols to induce liver tumors in male mice, it is evident that the anti-diastereomers are at least 4 times more potent. As was observed with both respective dihydrodiols, there is little difference in the ability of either anti-4,5- or anti-9,10-B[j]F-DE to induce liver tumors. In the absence of additional data, it is not possible to determine the extent to which each of these specific metabolic activation pathways is ultimately involved with liver tumor induction by B[j]F in male newborn mice. Studies are in progress to assess the contribution of each of these potential activation pathways to the tumorigenic activity observed in liver as well as other organ sites. The extent of involvement of these 2 potential pathways of activation pathways is also being assessed in other species.

REFERENCES


Tumorigenic Activity of the 4,5- and 9,10-Dihydrodiols of Benzo[jj]fluoranthene and Their \textit{syn}- and \textit{anti}-Diol Epoxides in Newborn Mice

Edmond J. LaVoie, Zhen-Min He, Yun Wu, et al.


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