Evidence for Bay Region Activation of Chrysene 1,2-Dihydrodiol to an Ultimate Carcinogen


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ABSTRACT

The tumor-initiating activities of chrysene and the three metabolically possible trans-dihydrodiols at the 1,2-, 3,4-, and 5,6-positions of chrysene were determined on the skin of female CD-1 mice. A single topical application of 0.4, 1.25, or 4.0 µmol of each compound was followed 7 days later by twice-weekly applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate for 25 weeks. The most potent tumor initiator was chrysene 1,2-dihydrodiol, which had approximately twice the tumorigenic activity of the parent hydrocarbon chrysene at all doses tested. Chrysene 3,4-dihydrodiol and chrysene 5,6-dihydrodiol had no significant tumorigenic activity. 1,2-Dihydroxy-1,2,3,4-tetrahydrochrysene, a compound related to chrysene 1,2-dihydrodiol but with the conjugated nonaromatic double bond removed from the 3,4-position of the molecule, had less than 25% of the tumorigenic activity of chrysene 1,2-dihydrodiol. These results indicate that chrysene 1,2-dihydrodiol is a proximate carcinogenic metabolite of chrysene and that a chrysene 1,2-diol-3,4-epoxide, in which the epoxide group forms part of the bay region in the molecule, is a likely candidate as an ultimate carcinogenic metabolite of chrysene.

INTRODUCTION

The bay region theory of polycyclic hydrocarbon carcinogenicity predicts that a chrysene 1,2-diol-3,4-epoxide, if enzymatically formed, would be an ultimate carcinogenic metabolite of chrysene (2-5). Perturbational molecular orbital calculations (5) indicate that electronic properties of the π-electron system are responsible for the unusually high chemical reactivity of bay region diol epoxides (3, 10). There is now direct evidence from tumorigenicity studies that bay region diol epoxides of benzo(a)pyrene (6, 7, 12-14) and benzo(a)anthracene (11, 22, 23) are ultimate carcinogenic metabolites. Indirect evidence, based on mutagenesis and DNA binding studies, has recently suggested that bay region diol epoxides of 7-methylbenzo(a)anthracene (15, 20), 7,12-dimethylbenzo(a)anthracene (17, 21), 3-methylcholanthrene (9, 19, 21), dibenzo(a)anthracene (24), and chrysene (25) are ultimate carcinogenic metabolites of their respective parent hydrocarbons. Although mutagenesis and DNA binding experiments have proved valuable in studies aimed at the identification of ultimate carcinogenic metabolites of polycyclic hydrocarbons, conclusions based on these experiments are only suggestive and in a few cases have been shown to be incorrect. Since not all mutagens are carcinogens and since covalent binding of chemicals need not be accompanied by tumor formation, the identification of proximate and ultimate carcinogenic metabolites can come only from carcinogenicity studies (cf. Ref. 16). In our continuing attempt to identify ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons, we have tested chrysene and its 3 metabolically possible dihydrodiols for tumorigenic activity in CD-1 mice. Wood et al. (24) previously reported that metabolic activation of chrysene 1,2-dihydrodiol by liver microsomes or a purified and reconstituted monooxygenase system resulted in the formation of a metabolite(s) that was 20 times more mutagenic to strain TA 100 of Salmonella typhimurium than were the metabolites formed from chrysene or chrysene 3,4- and 5,6-dihydrodiol. When the double bond in the 3,4-position of chrysene 1,2-dihydrodiol was saturated, the resulting tetrahydrodiol (H4-chrysene 1,2-diol) could not be metabolically activated to mutagenic metabolites, suggesting that a chrysene 1,2-diol-3,4-epoxide was the active mutagenic metabolite formed from chrysene 1,2-dihydrodiol (24). In the present study, we present tumorigenicity data indicating that chrysene 1,2-dihydrodiol is a proximate carcinogenic metabolite of chrysene. These data support the concept of bay region activation of chrysene to an ultimate carcinogenic metabolite.

MATERIALS AND METHODS

Chemicals. Chrysene was purchased from Aldrich Chemical Company (Milwaukee, Wis.) and was purified by several recrystallizations from benzene to obtain material with a melting point of 256°. Analytically pure samples of racemic chrysene 1,2-, 3,4-, and 5,6-dihydrodiol and H4-chrysene 1,2-diol were obtained by unequivocal chemical synthesis.
as described (8). 12-O-Tetradecanoylphorbol-13-acetate was purchased from the Department of Laboratory Medicine and Pathology at the University of Minnesota.

**Animals.** Female CD-1 mice (7 to 8 weeks old) were purchased from Charles River Mouse Farms, North Wilmington, Mass. Mice were anesthetized with ether and shaved on the dorsal surface with electric clippers 2 days before treatment. Due to the poor solubility of chrysene dihydrodiols in acetone, all compounds were dissolved in freshly distilled tetrahydrofuran containing 5% anhydrous dimethyl sulfoxide and were applied in 200 µl of the solvent to the backs of the mice (30 mice/treatment group). Control mice received solvent alone, and all mice received twice-weekly applications of 12-O-tetradecanoylphorbol-13-acetate (16 nmol/200 µl acetone) beginning 7 days after treatment with the chrysene derivatives or solvent. Formation of

![Chart 1. Skin tumor-initiating activity of chrysene, chrysene 1,2-dihydrodiol, and H4-chrysene 1,2-diol. Thirty female CD-1 mice received a single topical application of the compounds (4.0 µmol) in 200 µl of tetrahydrofuran:dimethyl sulfoxide (95:5). Seven days after initiation with the hydrocarbon, the mice received twice-weekly applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (16 nmol/application) in 200 µl of acetone for 25 weeks. A, percentage of mice with tumors; B, tumors observed per mouse during 25 weeks of promotion.](chart.png)
shown in Chart 1. The first appearance of tumors caused by tumor development after initiation with 4.0 μmol of chrysene, chrysene 1,2-dihydrodiol was observed at 10 weeks of promotion. The onset of tumor development did not occur until 16 weeks of promotion when mice were initiated with H4-chrysene 1,2-diol again demonstrating the weaker tumorigenic activity of this compound compared to chrysene and chrysene 1,2-dihydrodiol.

RESULTS

The tumor-initiating activities of chrysene and its 3 metabolically possible trans-dihydrodiols on mouse skin are shown in Table 1. Female CD-1 mice were treated once with the hydrocarbon followed by twice-weekly applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate commencing 7 days after initiation. After 25 weeks of promotion, a single dose of 0.4, 1.25, or 4.0 μmol of chrysene resulted in a 25 to 52% tumor incidence and 0.32, 0.97, and 1.45 papillomas/mouse, respectively. The same doses of chrysene 1,2-dihydrodiol induced tumors in 39 to 79% of the mice and produced a 2-fold higher incidence of papillomas per mouse than did the parent hydrocarbon, chrysene. Chrysene 3,4- and 5,6-dihydrodiol had no significant tumorigenic activity. At the single dose tested (4.0 μmol), H4-chrysene 1,2-diol caused a 23% tumor incidence with 0.43 papilloma/mouse. This compound had approximately 25 to 35% and 10 to 20% of the tumorigenic activity of chrysene and chrysene 1,2-dihydrodiol, respectively, when the data were expressed as either percentage of mice with tumors or papillomas per mouse. The time course of tumor development after initiation with 4.0 μmol of chrysene, chrysene 1,2-dihydrodiol, and H4-chrysene 1,2-diol is shown in Chart 1. The first appearance of tumors caused by chrysene or chrysene 1,2-dihydrodiol was observed at 10 weeks of promotion.

Table 1

Tumorigenicity of chrysene and chrysene dihydrodiols on mouse skin

<table>
<thead>
<tr>
<th>Hydrocarbon</th>
<th>Dose (μmol)</th>
<th>% of mice with tumors</th>
<th>Tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.4</td>
<td>25</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>43</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>52</td>
<td>1.45</td>
</tr>
<tr>
<td>Chrysene 1,2-</td>
<td>0.4</td>
<td>39</td>
<td>0.71</td>
</tr>
<tr>
<td>dihydrodiol</td>
<td>1.25</td>
<td>60</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>79</td>
<td>3.38</td>
</tr>
<tr>
<td>Chrysene 3,4-</td>
<td>0.4</td>
<td>13</td>
<td>0.13</td>
</tr>
<tr>
<td>dihydrodiol</td>
<td>1.25</td>
<td>13</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>Chrysene 5,6-</td>
<td>0.4</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>dihydrodiol</td>
<td>1.25</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>H4-chrysene 1,2-</td>
<td>4.0</td>
<td>23</td>
<td>0.43</td>
</tr>
</tbody>
</table>

diethyl sulfoxide (95:5). Seven days after application of each compound, the animals were treated with 12-O-tetradecanoylphorbol-13-acetate (16 nmol/200 μl acetone) twice weekly for 25 weeks. Control mice received the solvent followed by twice-weekly application of 12-O-tetradecanoylphorbol-13-acetate. Each treatment group originally consisted of 30 mice, and at least 27 mice in each group were alive at 25 weeks of promotion.

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