Carcinogenicity of Derivatives of 7,12-Dimethylbenz(a)anthracene

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SUMMARY

Eleven derivatives of 7,12-dimethylbenz(a)anthracene were tested for carcinogenicity in Sprague-Dawley female rats by the s.c. injection of 1.0 or 0.1 mg of compound dissolved in 0.1 ml of sesame oil on alternate days for 20 doses. Eight derivatives [7-hydroxymethyl-12-methyl-, 7-formyl-12-methyl-, 7-methoxymethyl-12-methyl-, 7-acetoxy-12-methyl-, 7-benzoxy-12-methyl-, 7-iodomethyl-12-methyl-, 7-bromomethyl-12-methyl-, and 7-chloromethyl-12-methylbenz(a)anthracene] were carcinogenic at the 1-mg dose level, and 5 [benzoxy-12-methyl-, 7-formyl-12-methyl-, 7-bromomethyl-12-methyl-, 7-hydroxy-12-methyl- and 7-iodomethyl-12-methylbenz(a)anthracene] were carcinogenic at the 0.1-mg dose level (200 days of observation). These observations suggest that the formation of 7-hydroxymethyl-12-methylbenz(a)anthracene may be the first step in the chain of events which result in cancer caused by these polycyclic hydrocarbons.

INTRODUCTION

In 1938, Fieser (8) postulated that polycyclic hydrocarbons must be transformed in vivo to products capable of reacting with cellular components in order to induce cancer. Despite considerable experimental data showing binding of these compounds to cellular components, evidence for the existence of a metabolic intermediate capable of inducing cancer was lacking until 1965 when Boyland et al. (3) demonstrated that 7-hydroxy-DMBA, a metabolite of DMBA, induced cancer in rats and mice. Because the metabolite was less carcinogenic than DMBA in their test system, Pataki and Huggins (15) concluded that DMBA exerted its effect directly as a consequence of molecular geometry and not via metabolic transformation. The studies presented in this report were undertaken to adduce evidence for or against these 2 hypotheses. We therefore selected for study compounds which would be expected to be converted to 7-hydroxy-DMBA by hydrolysis in vivo (e.g., esters and halides) and compounds which would not be expected to be converted to 7-hydroxy-DMBA owing to the presence of a ring substituent in position 4 or 5. The expectation that compounds capable of being converted to 7-hydroxy-DMBA would be potent carcinogens was fully realized, whereas no cancers were obtained with compounds bearing substituents in position 4 or 5.

MATERIALS AND METHODS

Chemicals. The compounds tested, except DMBA, were prepared by synthesis in this laboratory. 7-Hydroxymethyl-, 7-iodomethyl-, 7-acetoxy-, 7-methoxymethyl-12-methylbenz(a)anthracene, 4-methoxy-, and 4-hydroxy-7,12-dimethylbenz(a)anthracene were synthesized as previously described (10). 5-Methoxy-7,12-dimethylbenz(a)anthracene was prepared as described by Smith et al. (17). 7-Formyl-12-methyl-, 7-bromomethyl-12-methyl-, and 7-chloromethyl-12-methylbenz(a)anthracene were prepared as described by Pataki et al. (16). DMBA was purchased from Eastman Organic Chemicals, Rochester, N.Y., and purified by chromatography on Florisil. 7-Benzoyloxy-12-methylbenz(a)anthracene was synthesized by dissolving 7-hydroxy-DMBA (600 mg) in pyridine (6 ml), adding benzoyl chloride (3 ml), and heating briefly on a steam bath. The solution was chilled and poured into a mixture of ice water from which an oil then separated. The oil was washed with NaHCO3 (5%) and water, dissolved in benzene, and dried over Na2SO4. Benzene was evaporated under reduced pressure, and the product was crystallized from a mixture of benzene-ethanol, m.p. 153–155°; yield 568 mg.

\[
\text{C}_2\text{H}_2\text{O}_2
\]

Calculated: C 86.14, H 5.36

Found: C 86.38, H 5.52

Except for 7-iodomethyl-12-methylbenz(a)anthracene, which decomposes on melting, each compound was purified by recrystallization until a sharp melting point in agreement with literature values was obtained.

Animal Experiments. Female rats were purchased from Sprague-Dawley Farms, Inc., Madison, Wis., and housed in wire cages in a constant temperature animal room with an alternating light-dark cycle of 12 hr. For tumor induction, each compound was administered as a 1- or 0.1-mg dose in 0.1 ml of sesame oil by s.c. injection in the back on alternate days for 20 doses. For the 1-mg doses, animals 50 days old were used, and for the 0.1-mg doses, animals 30 days old were used. Rats bearing tumors were killed 30 days after the appearance of the first palpable tumor. The remainder were sacrificed after 200 days of observation. All tumors were fixed in 10% neutral formalin, embedded in paraffin, sectioned at a thickness of 5 μ and stained with hematoxylin and eosin.

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2The abbreviations used are: 7-hydroxy-DMBA, 7-hydroxy-12-methylbenz(a)anthracene; DMBA, 7,12-dimethylbenz(a)anthracene.

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Table 1
Cancer incidence and tumor induction time in Sprague-Dawley female rats given a 1-mg dose of each compound in 0.1 ml of sesame oil by s.c. injection on alternate days for 20 doses beginning at 50 days of age

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Benz(a)anthracene derivative</th>
<th>Mammary cancer (Tumor induction time (days))</th>
<th>Sarcoma at site of injection (Tumor induction time (days))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>7,12-Dimethyl-</td>
<td>7/19a</td>
<td>19/19a (Mean: 95; Range: 79-107)</td>
</tr>
<tr>
<td>15</td>
<td>7-Hydroxymethyl-12-methyl-</td>
<td>4/14b</td>
<td>13/14a (Mean: 97; Range: 79-111)</td>
</tr>
<tr>
<td>5</td>
<td>7-Formyl-12-methyl-</td>
<td>4/5</td>
<td>4/5 (Mean: 105; Range: 101-113)</td>
</tr>
<tr>
<td>5</td>
<td>7-Methoxy-12-methyl-</td>
<td>0/5</td>
<td>2/5 (Mean: 105-160)</td>
</tr>
<tr>
<td>15</td>
<td>7-Acetoxy-12-methyl-</td>
<td>0/14</td>
<td>14/14a (Mean: 69; Range: 66-87)</td>
</tr>
<tr>
<td>5</td>
<td>7-Benzoyloxymethyl-12-methyl-</td>
<td>0/5</td>
<td>5/5 (Mean: 87; Range: 83-92)</td>
</tr>
<tr>
<td>5</td>
<td>7-Iodomethyl-12-methyl-</td>
<td>0/5</td>
<td>5/5 (Mean: 55; Range: 50-60)</td>
</tr>
<tr>
<td>5</td>
<td>7-Bromomethyl-12-methyl-</td>
<td>0/5</td>
<td>3/5 (Mean: 87; Range: 81-91)</td>
</tr>
<tr>
<td>10</td>
<td>7-Chloromethyl-12-methyl-</td>
<td>0/9</td>
<td>9/9 (Mean: 151; Range: 120-176)</td>
</tr>
<tr>
<td>5</td>
<td>4-Hydroxy-7,12-dimethyl-</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>5</td>
<td>4-Methoxy-7,12-dimethyl-</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>5</td>
<td>Sesame oil</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

a Two rats had tumor of the ear duct.

RESULTS

The number of rats bearing cancer, the site of the cancer, and tumor induction time (the number of days elapsing between initial injection of compound and detection of the first palpable tumor) are summarized in Tables 1 and 2. All derivatives tested except 4-hydroxy- and 4-methoxy-7,12-dimethylbenz(a)anthracene induced sarcoma at the site of injection at the higher (1 mg, 20 doses) dose level, and 3 [DMBA, 7-hydroxy-DMBA, and 7-formyl-12-methylbenz(a)anthracene] induced tumors at other sites. All compounds tested at the lower 0.1-mg, 20-doses dose level induced sarcoma at the site of injection except 4-hydroxy-, 4-methoxy-, and 5-methoxy-DMBA and 7-methoxyethyl-12-methylbenz(a)anthracene. At the higher dose level (1 mg, 20-doses) 7,12-dimethyl-, 7-hydroxy-12-methyl-, 7-formyl-12-methyl-, 7-acetoxy-12-methyl-, and 7-chloromethyl-12-methylbenz(a)anthracene were equipotent. At the lower dose, 7-benzyloxymethyl-12-methylbenz(a)anthracene and DMBA appear to be equipotent and 7-formyl-12-methylbenz(a)anthracene is nearly so. The 7-iodomethyl-12-methyl compound induced a severe local reaction (necrosis and bleeding) after each injection which could have caused loss of the injected material. This was the only compound that caused permanent scars in the skin. Tumors induced by this compound were several cm from the scars; none were observed in the scars. At this dose level, 25% of the rats given injections of 7-hydroxy-DMBA had sarcoma at the site of injection, and 48% had mammary fibroadenoma. The incidence of spontaneous mammary fibroadenoma is less than 10% in Sprague-Dawley strain females 1 year old (unpublished observation); the higher incidence is attributed to 7-hydroxy-DMBA.

The carcinogenicity of 7-iodomethyl-12-methyl-, 7-chloromethyl-12-methyl-, and 7-benzyloxymethyl-12-methylbenz(a)anthracene and
5-methoxy-DMBA has not been reported previously. Compounds that failed to induce cancer under these conditions might do so under other conditions. Similarly, active compounds will require additional testing to establish their relative potency.

**DISCUSSION**

These experiments were undertaken in an effort to distinguish between 2 hypotheses for the mechanism of action of DMBA. The 1st is the direct action hypothesis, according to which DMBA is the ultimate carcinogen (1, 15). The 2nd is that metabolic activation of DMBA is necessary. Current opinion favors the 1st hypothesis, and all biotransformations are thought to represent mechanism of detoxification. 7-Hydroxy-DMBA, a metabolite of DMBA, is an exception since it is more toxic than the parent compound (18). The 2nd hypothesis is supported by several lines of indirect evidence: (a) binding studies implicate the formation of cellular protein or nucleic acids in covalent linkage to the hydrocarbon, which implies that the compound undergoes some type of chemical transformation in order that binding may occur (5, 9, 13); (b) recent evidence suggests that metabolism is a prerequisite for binding to cellular constituents (12); and (c) 7-hydroxy-DMBA is carcinogenic for mice (2, 3) and rats (10, 11). In this study, all of the compounds [7-formyl-12-methyl-, 7-methoxymethyl-12-methyl-, 7-acetoxyethyl-12-methyl-, 7-benzyloxyethyl-12-methyl-, 7-iodomethyl-12-methyl-, 7-bromomethyl-12-methyl-, and 7-chloromethyl-12-methylbenz(a)anthracene and DMBA] which would be expected to be converted to 7-hydroxy-DMBA in vivo were carcinogenic. Compounds bearing substituents at positions 4 and 5 were not carcinogenic. Our preliminary (unpublished) observations indicate that each of the carcinogenic compounds was, in fact, transformed in part to 7-hydroxy-DMBA by rat liver homogenates, whereas compounds that failed to induce cancer were not transformed to 7-hydroxy-DMBA. The esters, which may be regarded as derivatives which mask the 7-hydroxymethyl group, are more lipid soluble than 7-hydroxy-DMBA. The lower incidence of sarcoma induced by 7-hydroxy-DMBA is attributable to its lower lipid solubility. We have previously demonstrated that after p.o. administration the concentration of hydrocarbon in fatty tissue such as mammary gland is much higher for DMBA than for 7-hydroxy-DMBA (11). It is therefore reasonable to suppose that the more lipid-soluble compounds might penetrate lipid-containing cellular membranes with greater ease.

Our observations are somewhat at variance with those of Dipple and Slade (7), who reported that a single s.c. injection of 2.5 mg of 7-bromomethyl-12-methylbenz(a)anthracene or DMBA induced sarcoma in 100% of Chester Beatty hooded rats. The lower tumor incidence observed with the 7-bromomethyl derivative in this study is not due to the shorter observation period of 200 days. Their failure to observe tumors after the administration of 2.5 mg of 7-hydroxy-DMBA is surprising but could be due to the strain of rats used for these tests, since the Chester Beatty strain is more resistant to the carcinogenic effects of DMBA (4).

**REFERENCES**

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