Mutagenesis in Chinese Hamster Cells by Cyclopenta(a)phenanthrenes Activated by a Human Hepatoma Cell Line

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ABSTRACT

The cyclopenta(a)phenanthrene, 15,16-dihydro-11-methyl-cyclopenta(a)phenanthren-17-one, had potent mutagenic activity in cell-mediated mutation assays with V79 Chinese hamster cells as targets, and cells of the human hepatoma line HepG2 as mediators of activation. The compound was inactive when low-passage hamster embryo cells were used as activators. When the mutagenic activity of a series of cyclopenta(a)phenanthrenes was compared in mutation assays with HepG2 cells as activators, there was a good correlation between mutagenic activity in this system and carcinogenic activity in mouse skin in vivo. One exception was a noncarcinogenic compound, which is mutagenic in the Ames’ test, and was also mutagenic in the mammalian cell assay.

INTRODUCTION

Cyclopenta(a)phenanthrenes are a class of chemicals structurally related both to steroids and to polycyclic hydrocarbons of the phenanthrene series. Their carcinogenic and mutagenic activity have been investigated in rodent skin by painting or injection (5-8) and in the Ames’ test with the Salmonella typhimurium tester strain TA 100 (9). Several cyclopenta(a)phenanthrenes are potent carcinogens. For example, 15,16-dihydro-11-methylcyclopenta(a)phenanthren-17-one (Chart 1, Compound Ib) and the analogue, 11-methyl-1,2,3,4-tetrahydrochrysen-1-one (Compound II), have activity similar to that of B(a)P\(^2\) in mouse skin (7). Of 40 derivatives related to Com pound Ib to metabolites that were mutagenic for the V79 Chinese hamster target cells. However, a human hepatoma cell line, HepG2 (1, 14), which we recently reported to be capable of activating B(a)P (11), did activate Compound Ib. We now report those results and the screening of several cyclopenta(a)phenanthrenes in the HepG2-mediated mutation assay.

MATERIALS AND METHODS

Chemicals. The cyclopenta(a)phenanthrenes and chrysene tested (Chart 1) were synthesized in the Chemical Laboratory of the Imperial Cancer Research Fund (4, 10). Stock solutions in acetone were freshly prepared as needed.

Cells and Media. All stock cultures were grown in Eagle’s minimum essential medium (AutoPow, Flow Laboratories, Rockville, Md.), containing an additional mixture of vitamins, as formulated for Eagle’s basal medium, and 10% fetal bovine serum (Reheis, Armour Pharmaceuticals, Phoenix, Ariz.). Eagle’s basal medium (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 10% fetal bovine serum, was used for all steps in the mutation assays.

Primary HE cell cultures were prepared from 13-day-old Syrian hamster embryos (Lakeview Hamster Colony, Newfield, N. J.). The V79-16 subline of the Chinese hamster lung cell line was cloned in this laboratory (12); it was subcultured twice weekly at 1:25 split ratios. The human hepatoma cell line, HepG2 (1, 14), was subcultured every 10 days at a split ratio of 1:2. Routine monitoring (3, 15) has shown the HepG2 and V79 cells to be Mycoplasma-free.

Cell-mediated Mutation Assay. The assay has been described in detail (11, 12). Briefly, HepG2 cells were irradiated with 2500 rads and 2.5 \( \times 10^6 \) cells were seeded together with 2.5 \( \times 10^5 \) V79-16 cells in 25-sq cm plastic flasks ( Falcon Plastics, Oxnard, Calif.). After 2 hr, when the cells had attached to the substrate, the test compound was added to the desired final concentration; acetone was added to control flasks to give the same concentration as in the highest concentration of compound being tested. After the desired treatment period, the cells were harvested by trypsinization and the V79 cells, which were easily distinguished morphologically from the HepG2 cells, were counted. The extent of cytotoxicity induced in the V79 cells was determined by seeding 100 V79 cells in each of ten 60-mm plates (Falcon Plastics), fixing the colonies with methanol 7 days later, and staining with Giemsa. For determining mutation frequencies, 1.2 \( \times 10^6 \) treated V79 cells were seeded first into 75-sq cm flasks and, after the desired expression time, usually 4 days, the V79 cells were again harvested. Cell viability at that time was determined by seeding 100 cells into ten 60-mm plates and staining 7 days later. The number of mutants was determined by seeding 4 \( \times 10^5 \) cells into twenty-five 60-mm plates in medium containing 0.1 mm 6-TG, refedding with 6-TG 7 days later, and staining the colonies 7 days after that. Mutation frequencies are based on cell viability under nonselective conditions at the end of the phenotypic expression time.

For mutation assays mediated by HE cells, secondary cultures were irradiated, 2.3 \( \times 10^5 \) cells were seeded in 25-sq cm flasks, 2.7 \( \times 10^5 \) V79 cells were added 24 hr later, and the test compound 2 hr after that. The rest of the procedure was as described above.

1 Supported, in part, by USPHS Grants CA 21778, CA 30446, and CA 10815 from The National Cancer Institute, Department of Health and Human Services.
3 The abbreviations used are: B(a)P, benz(a)pyrene; HE, hamster embryo; 6-TG, 6-thioguanine.

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RESULTS

In cell-mediated mutation assays with irradiated HepG2 cells as mediators of carcinogen activation, Compound Ib was a very effective mutagen for V79 target cells (Chart 2). With a treatment time of 48 hr, there was a linear relationship between dose and mutation frequency within the concentration range 0.1 to 0.5 μg/ml, although at the lower concentrations the compound induced very little toxicity in the V79 cells. At a concentration of 1 μg/ml, the mutation frequency was slightly higher, but cell survival was reduced by 50%. Exposure to 0.5 μg/ml for 24 hr induced an average mutation frequency of approximately 34 mutants/10⁵ V79 cells and a 26% reduction in survival (Table 1).

In experiments described above, the selective agent, 6-TG, was applied 4 days after the end of the treatment period. This expression time was selected on the basis of experiments in which we determined, using the subcultivation procedure described in Diamond et al. (12), that with Compound Ib the mutation frequency reaches a peak at this time and then plateaus, remaining constant for at least 2 weeks thereafter. This is different from what we observed with polycyclic hydrocarbons such as compound 7,12-dimethylbenz(a)anthracene under the same assay conditions. In that case, after the optimum expression time (4 to 5 days), the mutation frequency decreased with increasing expression time (12).

In contrast to what was observed with HepG2 cells, when Compound Ib was tested in cell-mediated mutation assays with irradiated secondary HE cells as mediators, it was inactive. For example, the mutation frequency induced by exposure to 1 μg Compound Ib per ml for 72 hr was only 0.1 6-TG-resistant mutant/10⁵ V79 cells. Therefore, the mutagenic activity of a series of cyclopenta(a)phenanthenes was compared in mutation assays mediated by HepG2 cells. The expression time of 4 days was chosen for these assays, based on the determinations made with Compound Ib (see above), and the assumption that all compounds of this chemical class would have similar optimum expression times. As shown in Table 1, the structurally related chrysene derivative (Compound II) was as mutagenic as Compound Ib and, like Compound Ib, produced very little cytotoxicity in V79 cells when the treatment schedule shown in Table 1 was used. Compound la, the unsubstituted parent, also had some mutagenic activity, but much less than Compounds Ib or II. Two of the compounds tested (Compounds lc and ld) were inactive (Table 1), even when tested at 2 μg/ml (data not shown), a concentration close to the limits of solubility for these compounds in cell culture medium.

DISCUSSION

We have found that under the same conditions as those in which B(a)P (0.5 μg/ml for 48 hr) induces a mutation frequency of ~75 6-TG-resistant mutants/10⁵ cells (12), the cyclopenta(a)phenanthrene Compound Ib is not activated to mutagenic metabolites by HE cells. The hamster cell line, BHK21, and low-passage mouse embryo cells also do not activate this compound in cell-mediated assays. Thus, the human cell line HepG2 can activate both cyclopenta(a)phenanthenes and polycyclic aromatic hydrocarbons (11), whereas rodent embryo cells that are able to activate polycyclic hydrocarbons (12, 13, 16) do not appear to activate cyclopenta(a)phenanthenes. We have found quantitative differences in the metabolites produced when the polycyclic hydrocarbon 7,12-dimethylbenz(a)anthracene is metabolized by HepG2 and HE cells, and differences in the extent of its mutagenicity when activated by the 2 cell types. We are currently comparing the metabolism...
of Compound Ib in HepG2 and HE cells to try to find an explanation for the difference in the activation capabilities of these cells for this class of compounds.

The relative mutagenic activity of cyclopenta(a)phenanthrenes in V79 target cells and in Salmonella in the Ames' test (9) is shown in Table 2 and compared with carcinogenic activity in mouse skin (6, 7). The 2 mutation systems give very similar results, except for the somewhat higher activity of Compound II in the mammalian cells than in the bacteria, in agreement with its carcinogenic potency. For the most part, there is a good correlation between the carcinogenic and mutagenic activity of the 5 compounds that have been tested in all assays. However, with both in vitro assays, there is an important exception: a noncarcinogen (Compound Ia) has substantial mutagenic activity.

It is clearly of interest to extend this screening in V79 cells to other cyclopenta(a)phenanthrenes that have also been tested for mutagenicity in Salmonella. This would help determine if the mammalian cell assay is a valid in vitro system for assessing the carcinogenic potential of these compounds. Two questions need to be answered: (a) does mutagenic potency in V79 cells correlate with carcinogenic potency better than does mutagenic potency in Salmonella; and (b) are other non-carcinogens that are mutagenic in the Ames' test also mutagenic in mammalian cells?

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REFERENCES


Table 2

Comparative mutagenic and carcinogenic activity of cyclopenta(a)phenanthrenes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mutagenicity</th>
<th>Carcinogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella</td>
<td>V79 cells</td>
</tr>
<tr>
<td>Unsubstituted 17-ketone (Ia)</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>2-Methyl-17-ketone (Ic)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Methyl-17-ketone (Id)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11-Methyl-17-ketone (Ib)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>11-Methylchrysenone (IId)</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

a See Chart 1.
b Carcinogenicity in mouse skin as determined by topical application, twice weekly for 50 weeks, of 6 µl of toluene solutions (0.5% w/v) of Compounds I, a to d, and Compound II (6, 7).
c Mutagenicity in Ames' test with Salmonella typhimurium TA 100 in the presence of rat liver S-9 and NADPH; the experiments are described by Coombs et al. (9). Scores of +++ and -- are equivalent, respectively, to 22 and <0.2 revertant colonies/nmol.
d The data in Table 1 and Chart 2 of this report are summarized. Values of +++ and -- are equivalent, respectively, to approximately 30 and <1.5 6-TG-resistant mutants/10^6 V79 cells.
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