HIGH MUTAGENICITY AND TOXICITY OF A DIOL EPOXIDE DERIVED FROM BENZO[a]PYRENE

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SUMMARY: (±)-7β,8α-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BP 7,8-diol-9,10-epoxide) is a suspected metabolite of benzo[a]pyrene that is highly mutagenic and toxic in several strains of Salmonella typhimurium and in cultured Chinese hamster V79 cells. BP 7,8-diol-9,10-epoxide was approximately 5, 20 and 40 times more mutagenic than benzo[a]pyrene 4,5-oxide (BP 4,5-oxide) in strains TA 98 and TA 100 of S. typhimurium and in V79 cells, respectively. Both compounds were equally mutagenic to strain TA 1538 and non-mutagenic to strain TA 1535 of S. typhimurium. The diol epoxide was toxic to the four bacterial strains at 0.5-2.0 nmole/plate, whereas BP 4,5-oxide was nontoxic at these concentrations. In V79 cells, the diol epoxide was about 60-fold more cytotoxic than BP 4,5-oxide.

Since benzo[a]pyrene (BP)[1] and other carcinogenic polycyclic aromatic hydrocarbons are widely distributed in the environment, there is much interest in determining how these compounds cause cancer. Polycyclic hydrocarbons are believed to exert their carcinogenic effects after activation to reactive metabolites (ultimate carcinogens) that bind to critical cellular constituents. Of the many metabolites of polycyclic hydrocarbons, arene oxides have been proposed as candidates for the ultimate carcinogens (1-4). Particular attention was given to K-region oxides because they are easily synthesized, relatively stable, and are more potent than the parent hydrocarbons in causing malignant transformations in cultured mammalian cells (4,5).

[1]Abbreviations used are BP, benzo[a]pyrene; BP 7,8-diol-9,10-epoxide, (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; BP 7,8-dihydrodiol, trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene; 8-AG, 8-azaguanine.

Since BP and other polycyclic hydrocarbons undergo metabolism at multiple sites, we have initiated studies on the synthesis and biological activity of many of the potential and known BP metabolites. Most carcinogens are mutagenic in suitable test systems either directly or after metabolic activation (6). Thus, one aspect of our evaluation of biological activity of BP derivatives has been to determine their potential mutagenicity in S. typhimurium and in cultured hamster V79 cells. Among almost 30 compounds tested, BP 4,5-oxide was the most potent mutagen (7,8).

Recently, Borgen et al. (9) observed that BP 7,8-dihydrodiol, in the presence of liver microsomes and NADPH, binds to DNA to a tenfold greater extent than BP. Sims et al. (10) reported evidence for the metabolic formation of a BP 7,8-diol-9,10-epoxide and its ability to bind to DNA. The purity and stereochemistry of the diol epoxide, which was also synthesized by the action of a peroxyacid on BP 7,8-dihydrodiol (10), were not provided. The expected stereochemistry of this diol epoxide is such that the epoxide and the 7-hydroxy group would lie on opposite sides of the molecule (11). We have recently synthesized the opposite stereoisomer (11), (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, in which the 7-hydroxy group and the epoxide ring are on the same side of the molecule (see reference 13 for structures), with the expectation that this arrangement would enhance the susceptibility of the molecule to attack by nucleophiles, as is the case with triptolide (12). A similar conclusion has been reached by Hulbert (13). As anticipated, (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene is greater than 100-fold more reactive toward opening of the epoxide ring by a thiol nucleophile than is the stereoisomer produced by a peroxyacid (11). The present report establishes that the BP 7,8-diol-9,10-epoxide with triptolide-like stereochemistry is a far more potent mutagen than any previously tested metabolite of BP. Indeed, this diol epoxide is one of the most potent mutagens that has ever been tested in S. typhimurium and in V79 Chinese hamster cells (14,15).

MATERIALS AND METHODS: BP 4,5-oxide (16) and BP 7,8-diol-9,10-epoxide (11) of analytical purity were prepared as reported and were stored at -90° in dimethyl sulfoxide (DMSO)/NH_4OH (1000:1) and anhydrous DMSO, respectively. Manipulation of the compounds was performed under subdued light.

The four S. typhimurium strains were obtained from Dr. Bruce Ames (University of California, Berkeley, California). Strains TA 1535 and TA 1538 detect mainly base pair and frameshift mutagens, respectively (17). TA 100 and TA 98 are the same respective strains with an added R-factor plasmid which increases the sensitivity of these strains to certain mutagens through error-prone recombinational repair (14).

The procedure used for testing the mutagenicity of chemicals in S. typhimurium has been described in detail elsewhere (17). Bacteria (2.0×10^8) were added to 2.0 ml of top agar consisting of 0.6% NaCl, 0.6% agar, 0.05 mM biotin and 0.05 mM histidine. After addition of the hydrocarbon in 100 µl of solvent, the entire contents were mixed and poured onto a petri dish containing Vogel-Bonner medium with 2% agar base.

The Chinese hamster cell line V79-6 was kindly provided by Dr. E. H. Y. Chu (University of Michigan, Ann Arbor, Michigan). Our culture conditions for the maintenance of these cells were as previously described (7), except that a subclone of the cells with a lower spontaneous mutation frequency was used in this study. Procedures for assessing cytotoxicity and inducing 8-azaguanine-resistant cells were adapted from Chu et al. (15) and Huberman et al. (18), and the exact conditions used were described previously (7).

RESULTS: BP 7,8-diol-9,10-epoxide was highly mutagenic to several strains of S. typhimurium (Figure 1). Within the linear range of response, BP 7,8-diol-9,10-epoxide was approximately 5 and 20 times more mutagenic than BP 4,5-oxide in S. typhimurium strains TA 98 and TA 100, respectively. Both compounds were equally mutagenic to strain TA 1538 and nonmutagenic to TA 1535. The number of mutations per plate was proportional to the concentration of BP 7,8-diol-9,10-epoxide from 0.05-0.3 nmoles/plate. The toxicity of the diol epoxide lowered the number of mutations at higher concentrations (0.3-1.0 nmole) and produced an irregular lawn of bacteria above 1.0 nmole/plate. BP 4,5-oxide was not toxic to the bacteria, and the number of mutations induced per plate was proportional to the concentration of BP 4,5-oxide from 0.05-2.0 nmole/plate (Figure 1). Strains TA 100 and TA 98 were more sensitive to the mutagenicity of BP 7,8-diol-9,10-epoxide than strain TA 1538, whereas TA 98 and TA 1538 were more sensitive to BP 4,5-oxide than strain TA 100.

BP 7,8-diol-9,10-epoxide was highly mutagenic and cytotoxic in Chinese hamster V79 cells (Figure 2). Exposure to the diol epoxide at a concentration

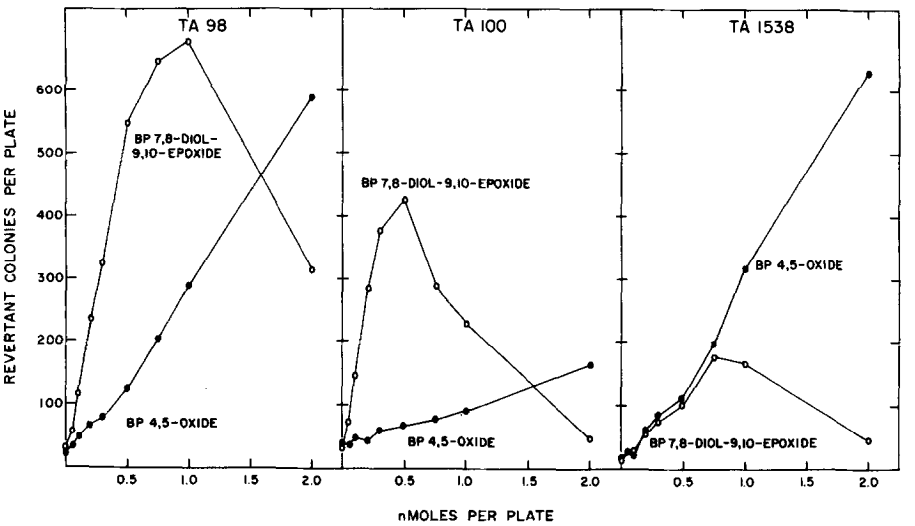

Figure 1. Mutagenic activity of BP 4,5-oxide and BP 7,8-diol-9,10-epoxide in S. typhimurium strains TA 98, TA 100 and TA 1538. Experimental conditions were as described in Materials and Methods. Each point is the average of three plates.

of 0.8 nmole per ml of medium killed 65% of the cells and induced 87 mutant colonies per 10^5 surviving cells. Three nmoles of the diol epoxide per ml of medium killed all the cells. We have previously reported (7) that BP 4,5-oxide was the most cytotoxic of nine BP derivatives studied with V79 cells and the only derivative with significant mutagenic activity. As shown in Figure 2, the diol epoxide is about 60 times more cytotoxic than BP 4,5-oxide and about 44 times as mutagenic in the V79 cells.

DISCUSSION: Earlier studies with the BP 4,5-, 7,8- and 9,10-oxides and their six corresponding phenols revealed that BP 4,5-oxide was greater than murium and in hamster V79 cells (8). Among these compounds, BP 4,5-oxide was the most mutagenic. The present study indicates that the diol epoxide is many-fold more mutagenic than BP 4,5-oxide. Indeed, the diol epoxide is one of the most mutagenic compounds ever tested in S. typhimurium or in V79 Chinese

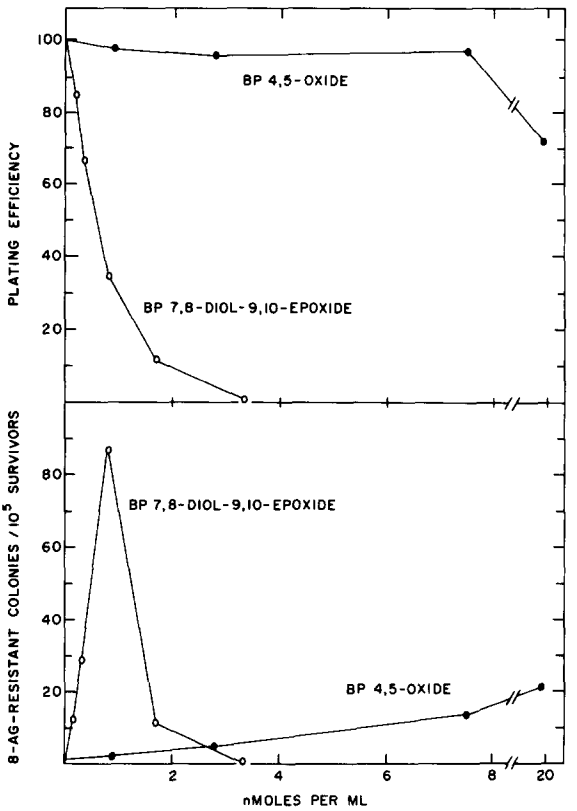

Figure 2. Cytotoxic and mutagenic activity of BP 4,5-oxide and BP 7,8-diol-9,10-epoxide on Chinese hamster V79 cells. Cells (10^2 for cytotoxicity and 10^4 for mutagenicity) were treated for one hour at 37°, 18 hours after the cultures were initiated in 60-mm culture dishes. Compounds were added in 20 µl of solvent. Cytotoxicity was determined seven days after treatment by counting the number of viable colonies of 50 or more cells in four replicate cultures. Values shown are expressed as a percentage of the number of colonies surviving treatment with solvent alone. The absolute plating efficiency of the solvent control culture was 93%. Mutagenicity was determined 14 days after treatment by counting the number of viable colonies of 50 or more cells in 16 replicate cultures that had been treated with 8-azaguanine (10 µg/ml) two days after treatment with the BP derivatives. The actual number of 8-azaguanine-resistant colonies induced by BP 4,5-oxide at concentrations of 0.9, 2.8, 7.5 and 19 nmole/ml was 2, 7, 20 and 22, respectively. BP 7,8-diol-9,10-epoxide, at concentrations of 0.17, 0.33, 0.82, 1.7 and 3.3 nmoles/ml, induced 16, 29, 46, 2 and 0 resistant colonies, respectively. Solvent-treated cultures had a total of two resistant colonies in 16 dishes.

100-fold more mutagenic than any of the other compounds tested (7). Our laboratory has now studied the mutagenic activity of all twelve BP phenols and several BP oxides, dihydrodiols and quinones in several strains of S. typhi-

hamster cells. In V79 cells, the diol epoxide is more mutagenic than MNNG, ethyl methanesulfonate or the several polycyclic hydrocarbon arene oxides that are commonly used as reference mutagens (15,18). Since BP 7,8-diol-9,10-epoxide is very unstable (half-life <30 seconds in the test systems), its intrinsic mutagenic and cytotoxic activity is probably even greater than what is described here. During the preparation of this manuscript, Malaveille et al. (19) reported on the mutagenic activity of a different BP 7,8-diol-9,10-epoxide (synthesized by a peroxyacid oxidation) in S. typhimurium TA 100. It was found that their diol epoxide was about equipotent to BP 4,5-oxide. The stereochemistry and purity of this compound were not provided.

McCann et al. (14) found that the metabolic activation of BP by the post-mitochondrial supernatant of rat liver caused more mutations in S. typhimurium strains TA 100 and TA 98 than in strain TA 1538. No mutations were obtained in strain TA 1535. This profile of mutagenic activity in the various strains is similar to that observed for BP 7,8-diol-9,10-epoxide and suggests the possible in vitro formation of this compound in the microsome-mediated assay.

Arene oxides have long been considered as possible ultimate carcinogenic metabolites of polycyclic hydrocarbons. However, recent data (9,10,20-23) on the binding of polycyclic hydrocarbon metabolites to DNA have indicated that K-region arene oxides were not the only reactive metabolites. In carcinogenicity studies from our laboratory, the non K-region BP 7,8-oxide was strongly carcinogenic (but less carcinogenic than BP) on mouse skin, whereas the BP 4,5- and 9,10-oxides showed little and no carcinogenic activity, respectively (24). BP 7,8-oxide may be carcinogenic per se, or it may undergo further metabolism to BP 7,8-diol-9,10-epoxide or other compounds which possess high biological activity. The strong mutagenicity and cytotoxicity of BP 7,8-diol-9,10-epoxide in both bacterial and mammalian cells indicate high reactivity of this compound with genetic material and suggest that the diol epoxide may be an ultimate carcinogen of BP. Studies are now in progress to determine the carcinogenicity of BP 7,8-diol-9,10-epoxide.

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