MUTAGENICITY OF NON-K-REGION DIOLS AND DIOL-EPOXIDES OF BENZ(a)ANTHRACENE AND BENZO(a)PYRENE IN *S. TYPHIMURIUM* TA 100

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Summary: When incubated with a 9,000 x g rat-liver supernatant, benzo(a)pyrene 7,8-diol and benz(a)anthracene 8,9-diol were more active than the parent hydrocarbons in inducing *his*$^+$ revertant colonies of *S. typhimurium* TA 100. Benzo(a)pyrene 9,10-diol was less active than benzo(a)pyrene; the K-region diols, benz(a)anthracene 5,6-diol and benzo(a)pyrene 4,5-diol, were inactive. None of the diols was active when the cofactors for the microsomal monooxygenase were omitted. The diol-epoxides benzo(a)pyrene 7,8-diol 9,10-oxide, benz(a)anthracene 8,9-diol 10,11-oxide and 7-methylbenz(a)anthracene 8,9-diol 10,11-oxide and the K-region epoxides, benzo(a)pyrene 4,5-oxide and benz(a)-anthracene 5,6-oxide, were mutagenic without further metabolism.

The effectiveness of non-K-region diols derived from some aromatic hydrocarbons in inducing malignant transformation of cultured cells (1), as well as evidence that oxidation of the adjacent olefinic double bonds can yield diol-epoxides that react with DNA (2,3,4), has led to the suggestion that these non-K-region diol-epoxides may be more important biologically than the simple K-region epoxides examined earlier (5,6). Because of the close empirical correlation that exists between the mutagenicity and carcinogenicity of numerous chemicals, we have examined the mutagenicity of a series of benzo(a)pyrene and benz(a)anthracene derivatives towards *S. typhimurium* strain

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TA 100, which has been found effective in detecting carcinogens as mutagens (7) using, where appropriate, a tissue mediated assay (8).

MATERIALS AND METHODS

Benz(a)anthracene and benzo(a)pyrene purchased from Sigma Chemical Co., St. Louis, Mo., USA, were purified by chromatography on alumina and by recrystallization. 3-Methylcholanthrene was obtained from Fluka, Buchs, Switzerland. 8,9-Dihydro-8,9-dihydroxybenz(a)anthracene, 8,9-dihydro-8,9-dihydroxy-7-methylbenz(a)anthracene, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene were obtained from the relevant hydrocarbons by metabolism (9,10); the corresponding diol-epoxides, 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide, 8,9-dihydro-8,9-dihydroxy-7-methylbenz(a)anthracene 10,11-oxide and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide, were prepared from the diols by oxidation with *m*-chloroperoxybenzoic acid in benzene. *trans*-5,6-Dihydro-5,6-dihydroxybenz(a)anthracene and *trans*-4,5-dihydro-4,5-dihydroxybenzo(a)pyrene were prepared from the *cis* isomers (11,12); benz(a)anthracene 5,6-oxide and benzo(a)pyrene 4,5-oxide were also synthesized (13,14). All compounds were stored at -30° under nitrogen; acetone solutions were prepared immediately prior to mutagenicity assay.

Animals and pretreatment

Adult female BD-VI rats (120-140 g), bred in our laboratories, were kept on a Charles River CRF diet; groups of three animals received one intraperitoneal injection of 3-methylcholanthrene (40 mg/kg) two days before they were killed.

Mutagenicity assays

Post-mitochondrial supernatants were prepared from pooled rat-liver by centrifugation of an homogenate (3 ml of 0.15M KCl/g liver) as described (15, 16). *S. typhimurium* strain TA 100, derived from the histidine auxotroph TA 1535 strain by introducing an R factor plasmid (ampicilline resistance) p KM 101 (17) was generously provided by Professor B.N. Ames, Berkeley, California. The strain was grown overnight in nutrient broth (Difco). Unless otherwise stated, the liver supernatant, cofactors ($NADP^+$ and glucose 6-phosphate) bacteria and the substrate, which was added as a solution in acetone, were combined in a soft agar layer and plated in triplicate onto histidine deficient media as previously described (8). Control assays in which the cofactors for the microsomal mono-oxygenase were omitted were also carried out. The K-region epoxides and diol-epoxides were assayed for mutagenicity towards strain TA 100 directly in the absence of any metabolic activation system.

RESULTS AND DISCUSSION

Dose-response curves for mutagenicity were prepared with benz(a)anthracene and benzo(a)pyrene using varying amounts of post-mitochondrial liver supernatant so as to obtain optimal conditions for the tissue mediated mutagenicity assay since it had been reported that the mutagenic response from certain polycyclic aromatic hydrocarbons is influenced by the protein concentration of the tissue utilized (7). In the presence of 150 µl supernatant/plate, an increasing

mutagenic response was observed with both benz(a)anthracene and benzo(a)pyrene up to a concentration of 45 μM, although benzo(a)pyrene was always more active (Fig. 1). The addition of smaller amounts of supernatant decreased the number of mutant colonies of *S. typhimurium* that appeared. Neither hydrocarbon was mutagenic towards TA 100 when the cofactors for the mixed-function oxidase were omitted (Fig. 1). In quantitative comparisons of the mutagenicity of benz(a)anthracene, benzo(a)pyrene and related dihydrodiols we therefore utilized 150 μl of rat liver post mitochondrial supernatant per plate and substrate concentrations up to 45 μM.

The metabolically-mediated mutagenicity of the benz(a)anthracene series is shown in Fig. 2. The 8,9-diol was clearly more active than the hydrocarbon at 45 μM and the 5,6-diol, the K-region diol, was completely inactive. Benzo-(a)pyrene and three related dihydrodiols were assayed under comparable conditions; the results, which are shown in Fig. 3, reveal that whilst, on an equimolar basis, the 7,8-diol was more active than benzo(a)pyrene itself, the 9,10-diol

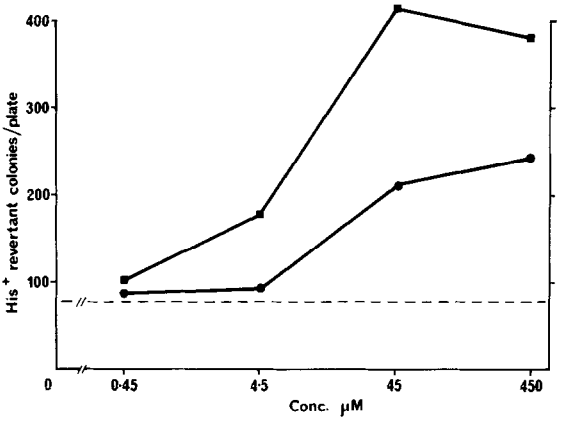

Fig. 1 Mutagenicity of benz(a)anthracene (●——●) and benzo(a)pyrene (■——■) towards *S. typhimurium* TA 100 mediated by a rat-liver microsomal preparation (150 μl/plate, ≡ 38 mg wet wt. liver). -----, Revertant colonies/plate appearing when $NADP^+$ and glucose 6-phosphate were omitted and when benz(a)anthracene or benzo(a)pyrene were or were not present.

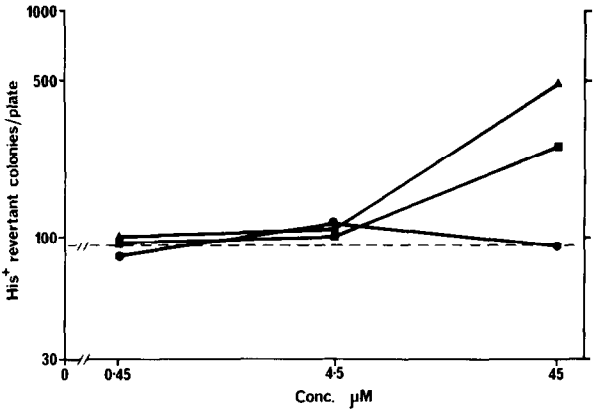

Fig. 2 Mutagenicity of benz(a)anthracene (■——■), 8,9-dihydro-8,9-dihydroxybenz(a)anthracene (▲——▲) and 5,6-dihydro-5,6-dihydroxybenz(a)anthracene (●——●) towards *S. typhimurium* TA 100 mediated by a rat-liver microsomal preparation. -----, Revertant colonies/plate appearing when NADP$^+$ and glucose 6-phosphate were omitted and when benz(a)anthracene or the diols were or were not present.

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was appreciably less active. The 7,8-diol was about 12 times more efficient than the isomeric 9,10-diol in reverting strain TA 100 to *his*$^+$. Again, the K-region diol, benzo(a)pyrene 4,5-diol, which does not possess an olefinic double bond adjacent to the hydroxyl groups, was not converted to metabolites mutagenic towards *S. typhimurium* TA 100. None of the benz(a)anthracene (Fig. 2) or benzo(a)pyrene (Fig. 3) diols were active when the microsomal cofactors were omitted from the plates.

When non-K-region diol-epoxides and K-region epoxides were assayed for their direct mutagenic action in *S. typhimurium* strain TA 100, the number of *his*$^+$ revertant colonies was found to increase as a function of the concentration (Table 1). At the highest concentration tested (45 μM), the relative mutagenicity of the compounds was in the following order where the number of histidine revertant colonies divided by the spontaneous mutation rate was benzo(a)pyrene 4,5-oxide = 26.9; benz(a)anthracene 8,9-diol 10,11-oxide = 20.9; benz(a)anthracene 5,6-oxide = 17.1; benzo(a)pyrene 7,8-diol 9,10-oxide = 13.5; 7-methylbenz(a)anthracene 8,9-diol 10,11-oxide = 7.0 and benz(a)-

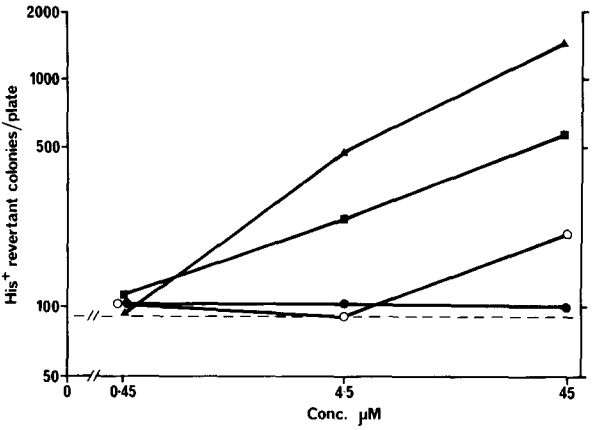

Fig. 3 Mutagenicity of benzo(a)pyrene (■——■), 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (▲——▲), 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene (O——O) and 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene (●——●) towards *S. typhimurium* TA 100 mediated by a rat-liver microsomal preparation. -------, Revertant colonies/plate appearing when $NADP^+$ and glucose 6-phosphate were omitted and when benzo(a)pyrene or the diols were or were not present.

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anthracene or benzo(a)pyrene = 1.0. These data demonstrate that non-K-region diol-epoxides are mutagenic for *S. typhimurium* TA 100, the activity being of the same order as that of the corresponding K-region epoxides. However, possible differences in the stabilities of these compounds in the aqueous incubation mixture may have influenced the mutagenic effects.

The mutagenicity of products formed by the further metabolism of non-K-region diols derived from benz(a)anthracene and benzo(a)pyrene towards *S. typhimurium* TA 100 is in accord with current ideas concerning the metabolic activation of polycyclic hydrocarbons. The results presented here support the findings of Marquardt *et al.* (1), who showed that some non-K-region diols were more active than the parent hydrocarbons in inducing malignant transformation of cultured cells and that the corresponding K-region diols were inactive. The present results with the benzo(a)pyrene diols also parallel those from other experiments in which benzo(a)pyrene diols were metabolized to derivatives that became covalently bound to DNA and where the 7,8-diol was

Table 1

MUTAGENICITY OF K-REGION EPOXIDES AND NON-K-REGION DIOL-EPOXIDES OF BENZ(a)ANTHRACENE AND BENZO(a)PYRENE IN *SALMONELLA TYPHIMURIUM* TA 100

Compound	Conc. (μM)	No. of *his*$^+$ revertant colonies/plate[a] (*S. typhimurium* TA 100)
None	0	60 ± 4
Benzo(a)pyrene 4,5-oxide	0.045	40 ± 5
	0.45	70 ± 3
	4.5	485 ± 40
	45	1071 ± 53
7,8-Dihydro-7,8-dihydroxy-benzo(a)pyrene 9,10-oxide	0.045	50 ± 5
	0.45	100 ± 3
	4.5	532 ± 33
	45	675 ± 90
Benz(a)anthracene 5,6-oxide	0.045	65 ± 5
	0.45	93 ± 4
	4.5	264 ± 18
	45	1110 ± 35
8,9-Dihydro-8,9-dihydroxy-benz(a)anthracene 10,11-oxide	0.045	57 ± 4
	0.45	56 ± 3
	4.5	133 ± 14
	45	1190 ± 57
8,9-Dihydro-8,9-dihydroxy-7-methylbenz(a)anthracene 10,11-oxide	0.045	52 ± 4
	0.45	48 ± 3
	4.5	84 ± 7
	45	362 ± 22

[a]Mean values from 4 to 8 plates ± SE. Direct plating test performed in soft agar (2.2 ml) containing 4.5% acetone (v/v) and the compound. Benz(a)anthracene and benzo(a)pyrene were not mutagenic at concentrations up to 45 μM.

by far the most effective substrate examined (18).

Non-K-region diols are most probably coverted by metabolism to diol-epoxides (2-4). Certain diol-epoxide isomers have been predicted on theoretical grounds to be particularly reactive towards nucleophilic centres (19); the direct mutagenic activity shown in the present experiments (Table 1) by three such diol-epoxides adds to the evidence that this type of product also possesses considerable biological activity. This is in contrast to the recent finding that two simple non-K-region epoxides, benzo(a)pyrene 7,8- and 9,10-oxides, were only one hundredth as mutagenic in *S. typhimurium* as the corresponding K-region epoxide (20).

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