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INDUCTION OF MALIGNANT TRANSFORMATION AND MUTAGENESIS BY DIHYDRODIOLS DERIVED FROM 7,12-DIMETHYLBENZ[a]ANTHRACENE

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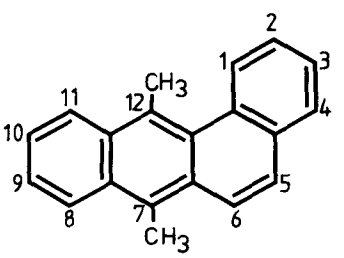
SUMMARY: Four trans-dihydrodiols derived from 7,12-dimethylbenz[a]anthracene have been tested, together with the parent hydrocarbon, for their abilities to induce mutations in V79 Chinese hamster cells and malignant transformations in M2 mouse fibroblasts. Both the 3,4- and the 8,9-diols derived from 7,12-dimethylbenz[a]anthracene induced mutations to 8-azaguanine resistance in V79 cells and malignant transformation in M2 mouse fibroblasts and were more active than the hydrocarbon itself. The K-region 5,6-diol and the non-K-region 10,11-diol were without activity. The biological activity of the 3,4-dihydrodiol is consistent with other data suggesting that the metabolic activation of 7,12-dimethylbenz[a]anthracene occurs through conversion of the 3,4-dihydrodiol into the related vicinal diol-epoxides, but the activity shown by the 8,9-dihydrodiol in these in vitro test systems is not.

A variety of different approaches have been used in order to identify the particular non-K-region dihydrodiols that are involved, through the formation of the related vicinal diol-epoxides, in the metabolic activation of the polycyclic hydrocarbons. Studies on mutagenicity (1,2) and on the ability of non-K-region dihydrodiols to induce malignant transformation in mammalian cells (3,4) and to initiate tumours in mouse skin (5,6) have been useful in helping to identify the vicinal diol-epoxides involved in the metabolic activation of benzo[a]pyrene and 7-methylbenz[a]anthracene. 7,12-Dimethylbenz[a]anthracene



(formula shown) is known to be converted into a variety of K-region and non-K-region dihydrodiols by rat-liver preparations (7,8) and by mouse skin in short-term organ culture (9). In this paper we describe the results of comparative tests that have been carried out on the activities of four trans-

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dihydrodiols derived from 7,12-dimethylbenz[a]anthracene in inducing mutations to 8-azaguanine resistance (10) in V79 Chinese hamster cells and malignant transformation in M2 mouse fibroblasts (11).

MATERIALS AND METHODS

Materials: N-Methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co., Milwaukee,Wis., USA), 8-azaguanine and 7,12-dimethylbenz[a]anthracene (Sigma Chemical Co., St. Louis, Mo., USA) and tissue culture media (Eagle's Basal medium and Dulbecco's MEM, both supplemented with 10% heat-inactivated foetal calf serum and penicillin-streptomycin) (Grand Island Biological Co., Grand Island, N.Y. USA) were purchased. trans-3,4-Dihydro-3,4-dihydroxy-7,12-dimethylbenz[a]anthracene trans-8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene and trans-10,11-dihydr 10,11-dihydroxy-7,12-dimethylbenz[a]anthracene were prepared by oxidation of the parent hydrocarbon with an ascorbic acid-ferrous sulphate-EDTA system (12,13) and characterized by their u.v., n.m.r. and mass spectral properties: trans-5,6-dihydrc 5,6-dihydroxy-7,12-dimethylbenz[a]anthracene was also prepared (14).

Mutagenesis in V79 cells. V79 Chinese hamster cells, supplied by Dr.E.H. Chu, Ann Ar Michigan, were used exactly as described previously (4) to determine cytotoxicity and the frequency with which mutations to 8-azaguanine resistance were induced. N-Methyl N'-nitro-N-nitrosoguanidine was used as a positive control in these experiments. In preliminary experiments a mutation expression time of 48 h. was found to be optimum.

Malignant transformation of M2 cells. The M2 clone of mouse fibroblasts, which was established as a line from C3H mouse prostate cells (15) was used in the malignant transformation assays precisely as described earlier (4). The properties of control cells, of morphologically-transformed cells and of cells of normal morphology from treated dishes were examined by reinjecting them (10^6 cells/mouse) into male inbred C3H/HeJ mice.

RESULTS AND DISCUSSION

The results obtained when four dihydrodiols derived from 7,12-dimethylbenz[a] anthracene were tested for biological activity in V79 Chinese hamster cells and in M2 mouse fibroblasts are given in Table I. Two non-K-region diols, the 3,4-diol and the 8,9-diol, were active in inducing mutations to 8-azaguanine resistance and, if the frequency of malignant transformation is considered in terms of the numbers of surviving cells, then both these diols appear to be more active than the parent hydrocarbon in inducing transformation. 7,12-Dimethylbenz[a]anthracene was inactive in inducing mutations in V79 cells and the K-region diol, the 5,6-diol, which cannot be converted into a vicinal diol-epoxide, was inactive in both cell lines. The other non-K-region dihydrodiol that was tested, the 10,11-diol, which can in theory be converted into a vicinal diol-epoxide by oxidation at the 8,9-bond, was also inactive as a mutagen and as a transforming agent at concentrations at which it did cause cytotoxicity.

Morphologically-transformed cells were also tested for their ability to induce tumours in isologous, non-irradiated mice. Five weeks after the injection in C3H/HeJ mice (3 mice/clone) of cells from two clones transformed by the 3,4-dihydro-

Table I INDUCTION OF MALIGNANT TRANSFORMATION AND MUTAGENESIS IN MAMMALIAN CELLS BY DIHYDRODIOLS DERIVED FROM 7,12-DIMETHYLBENZ[a]ANTHRACENE[1]

Compound	Concentration (μg/ml)	Transformation in M2 Cells[2]			Mutagenesis in V79 Cells[3]	
		Plating Efficiency (%)	Transformed foci per number of dishes treated	Transformed foci per 10^3 survivors	Plating Efficiency (%)	8-Azaguanine-resistant colonies/10^5 survivors
Dimethylsulphoxide	0.5%	31	0/9	0	84	2.7
N-Methyl-N'-nitro-N-nitrosoguanidine	0.4	21	10/9	5.3	31	294.1
7,12-Dimethylbenz[a]anthracene	0.25	26	9/8	4.3	63	3.0
	1.0	24	19/11	7.2	39	4.4
trans-3,4-Dihydro-3,4-dihydroxy-7,12-dimethylbenz[a]anthracene	0.12	31	3/5	1.9	67	5.7
	0.25	26	9/10	3.5	58	19.8
	0.5	25	26/8	13.0	50	29.8
	1.0	15	20/9	14.3	43	44.1
trans-5,6-Dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene	1.0	28	0/10	0	80	2.0
	5.0	28	0/10	0	73	0.9
	10.0	20	0/10	0	57	0
trans-8,9-Dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene	0.25	26	4/4	3.8	62	1.9
	0.5	22	15/5	13.6	45	20.5
	1.0	7	11/6	26.2	32	42.5
trans-10,11-Dihydro-10,11-dihydroxy-7,12-dimethylbenz[a]anthracene	0.25	30	0/8	0	76	6.3
	0.5	26	0/12	0	68	5.6
	1.0	11	0/11	0	44	4.9

[1] Composite results from 2 separate experiments. [2] Cells were grown in medium containing the test compounds for 24 h.

[3] Cells were grown in medium containing the test compounds for 3 h.

diol derived from 7,12-dimethylbenz[a]anthracene, all six animals developed sarcoma Six mice that received injections of cells from normal cultures or of cells with normal morphology from treated dishes did not develop tumours.

7,12-Dimethylbenz[a]anthracene is a potent carcinogen and, when this hydrocarbon is incubated with rat-liver preparations, dihydrodiol formation at the 3,4-, 4,5-, 8,9- and 10,11-positions has been detected (7,8,13): these dihydrodiols are also formed by mouse skin that has been treated in short-term organ culture with the hydrocarbon (9), a tissue in which 7,12-dimethylbenz[a]anthracene is carcinogenic.

In V79 Chinese hamster cells, 7,12-dimethylbenz[a]anthracene was, like 7-methylbenz[a]anthracene (4), inactive in inducing mutations to 8-azaguanine resistance, presumably because these cells do not metabolize the hydrocarbons to reactive metabolites. They do appear, however, to possess some metabolic capability since some non-K-region diols, which require conversion to the related vicinal diol-epoxides to be active, do induce mutations in these cells. There is no information available as yet, however, as to whether the enzymes in V79 cells that are involved in the oxidation of the olefinic double bonds adjacent to the dihydrodiol grouping to epoxides are microsomal mono-oxygenases or some other enzymes. In the present experiments, the 3,4-diol and the 8,9-diol derived from 7,12-dimethylbenz[a]anthracene were equi-potent in inducing mutations to 8-azaguanine resistance in V79 cells and in inducing malignant transformations in M2 mouse fibroblasts (Table I). The incidence of transformation induced by the 8,9-diol in the present experiments is similar to that reported previously (3). Both the 3,4-diol and the 8,9-diol are non-K-region dihydrodiols that can be converted into vicinal diol-epoxides but, although there is evidence that activation of 7,12-dimethylbenz[a]anthracene occurs in the 1,2, 3,4-ring (16-18) and that the 3,4-diol is very potent as a mutagen in microsome-mediated tests with S.typhimurium (19), there is no evidence so far that implicates the 8,9-diol in metabolic activation in target tissues. The 1,2-dihydrodiol derived from 7,12-dimethylbenz[a]anthracene has not so far been prepared by synthesis nor has it been detected as a metabolite of the hydrocarbon either in liver preparations or in mouse skin and it is possible that it is not formed because of steric hindrance from the 12-methyl group. Preliminary results from experiments in which dihydrodiols derived from 7,12-dimethylbenz[a]anthracene are being tested for tumour-initiating activity on mouse skin indicate that both the 3,4-diol and the 8,9-diol are active (20). Much of the available evidence is consistent both with the idea that vicinal diol-epoxides are the important species involved in the activation of the polycyclic hydrocarbons as a class of chemical carcinogens (21) and with the hypothesis that the active diol-epoxides will be of the bay region type (22) but with 7,12-dimethylbenz[a]anthracen

more information, particularly on the nature of the nucleic acid products that are formed in target tissues treated with the hydrocarbon, is still required.

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