THE METABOLISM OF POLYCYCLIC HYDROCARBONS BY CULTURED HUMAN LYMPHOCYTES

Joan BOOTH, Gerald R. KEYSELL, Kalyani PAL and Peter SIMS
Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW3 6JB, England

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1. Introduction

One of the initial steps in the metabolism of the polycyclic hydrocarbons benz[a]anthracene [1], 7-methylbenz[a]anthracene [2] and benzo[a]pyrene [3] by rat liver preparations is by oxidation to 'K-region' epoxides that may rearrange to phenols, be converted into 'K-region' dihydrodiols by 'epoxide hydrase' [4] or into GSH conjugates by 'glutathione S-epoxide transferase' [5]. Phenols and dihydrodiols on bonds other than those of the 'K-regions' have also been detected [6,7] and presumably also arise through epoxide intermediates.

Recent work on the induction of the enzyme system that converts benzo[a]pyrene into 3-hydroxybenzo[a]pyrene [8,9] and on an 'epoxide hydrase' that catalyzes the hydration of styrene oxide to styrene glycol [10] have used cultured human lymphocytes as the enzyme sources. This report demonstrates that these cells metabolize aromatic hydrocarbons by pathways similar to those described for rat liver preparations [6].

2. Materials and methods

2.1. *Materials*

^{3}H-Labelled benz[a]anthracene, 7-methylbenz[a]anthracene and benzo[a]pyrene with specific activities 610, 457 and 475 mCi/mmole respectively were obtained from the Radiochemical Centre, Amersham, Bucks. [^{3}H]Benz[a]anthracene 5,6-oxide (specific activity 140 mCi/mmole) was prepared as described for the unlabelled compound [11]. Unlabelled reference compounds related to benz[a]anthracene [6,12], 7-methylbenz[a]anthracene [6,13] and benzo[a]pyrene [6,7] were prepared or obtained enzymically by published methods.

2.2. *Incubation of cultured lymphocytes with ^{3}H-labelled hydrocarbons*

Leucocytes from freshly drawn heparinized human blood from normal adults were cultured in the presence of foetal calf serum and phytohaemagglutinin for 72 hr [8,9]. Each culture tube contained approximately 4×10^6 cells in medium (5 ml). About 90% of the remaining cells are lymphoblasts that probably contain the hydrocarbon-metabolizing enzymes [10, 14]. After 72 hr, ^{3}H-labelled hydrocarbon (2 μg in 1 μl ethanol) and NADPH (1 mg in 0.1 ml sterile water) were added to each tube and the cells cultured for a further 24 hr. They were then removed by centrifugation and the medium extracted with ethyl acetate (7 ml). The extracts were co-chromatographed with unlabelled reference compounds on thin-layer chromatograms (0.25 mm layers of silica gel G) developed with benzene:ethanol, 9:1 v/v. The reference compounds were located and the amount of the radioactivity present was determined by liquid scintillation counting [6].

2.3. *Detection of 'epoxide hydrase' activity*

Lymphocytes, cultured for 96 hr, were treated with [^{3}H]benz[a]anthracene 5,6-oxide (2 μg in 1 μl ethanol) and maintained at 37°C for 1 hr. The medium was extracted and the products examined by t.l.c. as described above. The ethyl acetate extract was co-chromatographed with unlabelled *trans*-5,6-

dihydro-5,6-dihydroxybenz[a]anthracene and the radioactive dihydrodiol formed from the oxide determined by liquid scintillation counting [6].

2.4. *Detection of 'glutathione S-epoxide transferase' activity*

Lymphocytes cultured for 96 hr, were removed from the medium by centrifugation. Washed cells from two culture tubes were suspended in buffer (5 ml) (0.1 M phosphate buffer pH 7.4 : 0.154 M NaCl 1:1 v/v) and the suspension frozen and thawed five times. After centrifugation at 120 000 *g* for 1 hr, [^{3}H]benz[a]anthracene 5,6-oxide (20 μg in 4 μl ethanol) and GSH (1 mg) were added to the supernatant phase and the mixture incubated at 37°C for 10 min. Acetone (10 ml) was added, the precipitated protein removed by centrifugation and water (5 ml) added. The supernatant was extracted with diethyl ether (20 ml) and the aqueous layer evaporated to dryness. The residue, in water (1 ml), was passed through a column (60 X 0.5 cm) of Sephadex G-25 and the conjugate eluted with water. The fraction associated with radioactivity was chromatographed on silica gel G developed with butan-l-ol:propan-l-ol: 2 M-NH_3 (2:1:1, by vol.) in the presence of unlabelled S-(5,6-dihydro-6-hydroxybenz[a]anthracen-5-yl)glutathione [11]. The conjugate was located with ninhydrin and the radioactivity present in this and other areas of the chromatograms measured [6].

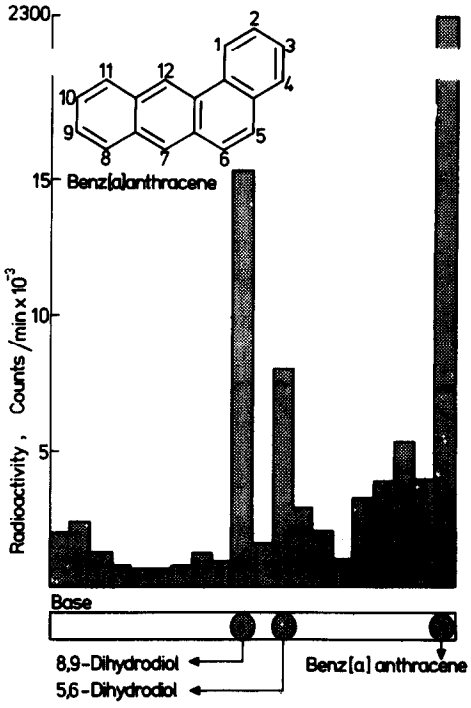

Fig. 1. Metabolism of [^{3}H]benz[a]anthracene. Unlabelled reference compounds were co-chromatographed with ethyl acetate extracts of incubation mixtures containing cultured human lymphocytes and [^{3}H]benz[a]anthracene as described in the text. The reference compounds were located in ultraviolet light and the profile of radioactivity obtained as described [6].

3. Results and discussion

Chromatographic examination of ethyl acetate extracts of incubation mixtures containing cultured human lymphocytes, NADPH and [^{3}H]benz[a]anthracene showed two major bands of radioactivity (fig. 1) that had the chromatographic properties of the 5,6- and 8,9-dihydrodiols of benz[a]anthracene respectively.

In similar experiments with 7-methylbenz[a]anthracene, bands of radioactivity were found, coincident on thin-layer chromatograms with the unlabelled 5,6- and 8,9-dihydrodiols of 7-methylbenz[a]anthracene. Fig. 2 shows the results of one such experiment in which cultured lymphocytes also formed a radioactive metabolite of [^{3}H]methylbenz[a]anthracene that had the chromatographic properties of 7-hydroxymethylbenz[a]anthracene. However, this metabolite was not detected in all samples of lymphocytes examined.

The radioactivity profile of similar thin-layer chromatograms from experiments using benzo[a]pyrene as substrate (fig. 3) showed one peak with the chromatographic properties of 3-hydroxybenzo[a]pyrene and others with those of the 4,5-, 8,9- and 10,11-dihydrodiols of benzo[a]pyrene.

None of the metabolites of any of the hydrocarbons was detected when lymphocytes were omitted from reaction mixtures. Thus cultured human lymphocytes convert the three hydrocarbons into the dihydrodiols that are also formed by rat liver preparations.

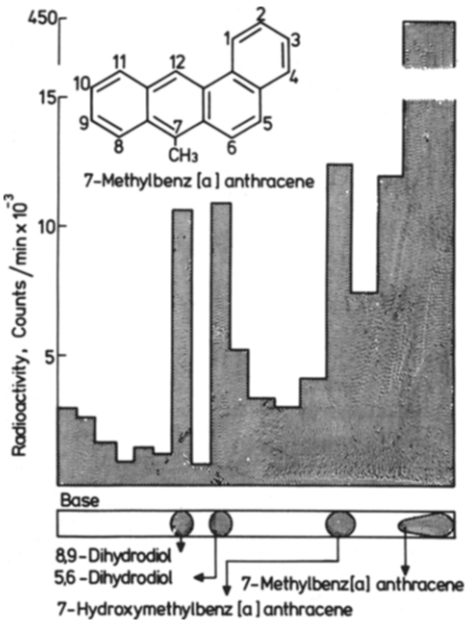

Fig. 2. Metabolism of [^{3}H]-7-methylbenz[a]anthracene. Ethyl acetate extracts of incubation mixtures containing cultured human lymphocytes and [^{3}H]-7-methylbenz[a]-anthracene were treated as described in the legend for fig. 1.

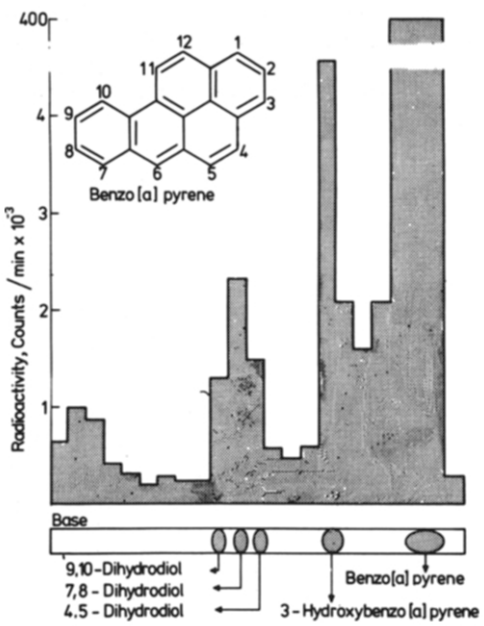

Fig. 3. Metabolism of [^{3}H]benzo[a]pyrene. Ethyl acetate extracts of incubation mixtures containing cultured human lymphocytes and benzo[a]pyrene were treated as described in the legend for fig. 1.

The presence of 'epoxide hydrase' was demonstrated by incubating cultured human lymphocytes with [^{3}H]benz[a]anthracene 5,6-oxide, when a radioactive product that was chromatographically indistinguishable from trans-5,6-dihydro-5,6-dihydroxybenz[a]anthracene was formed. Similar experiments in which the cell suspension was heated (10 min at 100°C) before incubation with the oxide showed that 88% of the activity measured was due to an enzymatic reaction.

Cultured human lymphocytes also contain 'glutathione S-epoxide transferase' activity since [^{3}H] benz[a]anthracene 5,6-oxide reacted with GSH in the presence of the soluble cell fraction to yield a radioactive product that was coincident on thin-layer chromatograms with S-(5,6-dihydro-6-hydroxybenz[a]anthracene-5-yl)glutathione. This reaction was essentially enzymic, since heating (10 min at 100°C) before incubation with the oxide destroyed 89% of the activity measured.

At least three enzyme systems are involved in the overall metabolism of aromatic hydrocarbons by rat liver preparations. Oxidation of the hydrocarbon double bonds to epoxides is catalyzed by the microsomal oxygenases [1–3] and the further metabolism of the oxides by microsomal 'epoxide hydrase' [4] and 'glutathione S-epoxide transferase' [5] present in the cytosol. The formation of [^{3}H]benz[a]anthracene 5,6-oxide from [^{3}H]benz[a]anthracene and its conversion into the 5,6-dihydrodiol and the GSH conjugate by human lung preparations [15] demonstrates that these reactions also take place in man. It is now shown that these enzyme systems are also present in cultured human lymphocytes by the identification of dihydrodiols as metabolites of the three hydrocarbons studied and the conversion of an epoxide(benz[a]anthracene 5,6-oxide) into the corresponding dihydrodiol and GSH conjugate by these cells.

The inducibility of 'benzo[a]pyrene hydroxylase' activity in cultured human lymphocytes by 3-methylcholanthrene is considered to be under genetic control [14,16] and its correlation with some forms of cancer has been demonstrated [17]. It is thus possible that other hydrocarbon biotransformations that are catalyzed by cultured human lymphocytes, a readily available human tissue, may also be of use in this type of investigation.

Acknowledgements

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