INDUCTION OF GENE MUTATIONS BY BENZ(a)PYRENE DURING ITS METABOLIC ACTIVATION

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The study of the mutagenic activity of polycyclic aromatic hydrocarbons (PAH) on eukaryote cell systems is a convenient model for examination of the role of mutations in carcinogenesis. Practically all carcinogens studied have been shown to be able to induce mutations, but not all mutagens induce malignant growth [8, 13]. Investigations have shown that PAH inactive as regards their physicochemical properties exhibit their mutagenic and carcinogenic potential only as a result of activation by microsomal enzymes and of metabolic oxidation in mammals. This primary metabolic reaction is brought about by the mono-oxygenases of various organs and tissues; the mono-oxygenase system of the liver, moreover, has the greatest activating capacity.

It has been shown on prokaryote cells that chemically reactive metabolites of PAH and of their most typical representative, benz(a)pyrene (BP) are able to form covalent bonds with DNA, to become incorporated into its molecule, and to lead to deletions, etc. At the same time, only a few studies have been carried out on eukaryote cells *in vitro*, the reasons being, first, difficulty of inducing gene mutations and, second, the absence or very low activity of their oxidative enzymes.

In recent years to activate BP *in vitro* a system using a fraction of mammalian microsomal enzymes, in which mono-oxygenase systems have been induced by preliminary treatment, has been suggested. By this approach, gene mutations responsible for changes in structure of the cell membranes [9, 12], and mutations at loci of hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) and thymidine kinase (TK), detected by measuring the resistance of induced clones to 8-azaguanine [11], 6-thioguanine [10], or trifluorothymidine [7], have been obtained in mammalian cell cultures.

The object of this investigation was to study the mutagenic activity of small doses of BP *in vitro* on mammalian cells.

EXPERIMENTAL METHODS AND RESULTS

The method of induction of direct gene mutations at the HGPRT locus on pseudodiploid Chinese hamster V-79 cells, obtained from Dr. Simons (University of Leyden, The Netherlands), was used, with detection of the induced clones by their resistance to 8-azaguanine [3, 5].

To induce liver microsomal enzymes, phenobarbital sodium was injected intraperitoneally into five mice daily for 3 days in a dose of 40 mg/kg body weight. The mice were killed by decapitation. Under aseptic conditions the liver was removed and cut into small pieces, and a homogenate was prepared in the cold in a glass homogenizer with 3 volumes of 0.15 M KCl and centrifuged in a sterile jar at 12,000 rpm for 15 min at 5°C. To obtain the microsomal activating mixture (MAM) the supernatant was used. In 1 ml of the MAM there were 0.3 ml of microsomal fractions and the cofactors: 4 mM NADP, 5 mM glucose-6-phosphate, 33 mM KCl, 8 mM $MgCl_2$, and 0.1 M potassium phosphate, pH 7.4 [6]. To prevent infection of the medium by bacterial flora antibiotics were added: penicillin 100 i.u./ml and streptomycin 50 i.u./ml.

BP was used in concentrations of 2.5, 5, 12, 20, and 25 μg/ml. To study the toxicity of BP and MAM, $1 \cdot 10^2$–$2 \cdot 10^2$ cells were seeded on Petri dishes. After they had adhered, me-

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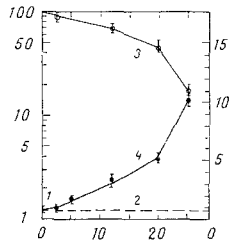

Fig. 1. Effect of BP on survival rate of cells and induction of direct gene mutations for the HGPRT locus. 1) Spontaneous level of mutations in original cell populations; 2) effect of MAM (negative control); 3) survival rate of cells; 4) number of induced mutants. Standard error of arithmetic mean is shown ($S_{\bar{x}}$). Abscissa, BP concentration (in μg/ml); ordinate: left — survival rate of cells (in % of control), right — number of colonies resistant to 8-azaguanine (per 10^5).

dium containing MAM × BP or MAM alone was added to the dishes. The cells were washed off 2 h later with Hanks' solution and incubated for 1 week in medium with 10% calf embryonic serum. The media was removed from the dishes after 7 days and the colonies were dried, stained with methylene blue, and counted. The survival rate of the cells was calculated as the ratio in percent of the number of cells surviving after treatment with BP + MAM or with MAM alone and the number of cells in the control (Fig. 1).

To study the mutagenic effect of BP the cells were treated in 100-ml flasks in the presence of MAM. After treatment for 2 h the cells were washed off with Hanks' solution and reseeded in 250-ml flasks, allowing for the survival rate of the cells. The cells were incubated for 3-4 days at 37°C to allow phenotypic expression of the mutations. The cells were then reseeded in Petri dishes at the rate of $1 \cdot 10^5$ per dish. Four hours after adhesion of the cells to the surface of the glass 8-azaguanine was added at the rate of 30 μg/ml. The medium was removed 14 days later and the colonies were stained and counted.

It will be clear from Fig. 1 that MAM had no effect on survival of the cells and did not increase the number of mutant clones compared with that in the control. BP in concentrations of 2.5-5 μg/ml reduced the survival rate of the cells by 25%. With a further decrease in survival of the cells (BP concentration 12 μg/ml) the level of induction of mutations was 2-3 times higher than the spontaneous background level. Growth of mutant clones was sharply inhibited by BP in a concentration of 20-25 μg/ml. With these doses of BP the mutagenic effect was much higher still than the spontaneous level of mutations.

According to data in the literature, to induce mutations in cells of eukaryotes BP was used within a wide range of concentrations, depending on the test system, the effectiveness of the activating mixture, and the quality of the inducer of mono-oxygenase systems in the animals before preparation of the liver homogenate. For example, to induce mutations of resistance to 8-azaguanine, BP was used in concentrations of 10 to 20 mM. In the present experiments similar doses of BP were used, but these doses gave an appreciable mutagenic effect, which can be explained by the greater effectiveness of the activating system, as a result of the different composition of the activating mixture, the conditions of centrifugation, different conditions of addition of the inducer of the liver hydroxylase systems, the use of liver from an animal of a different species, etc.

In the search for an adequate activation system to test the effect of small doses of BP we chose an activation system in which the microsomal fraction accounted for 3% of the total

quantity of nutrient medium. A similar activation system enabled BP to exhibit a mutagenic effect in a concentration of 5 μg per Petri dish in experiments on salmonellas [2]. It must also be borne in mind that, despite its fairly wide distribution in the environment, BP acts on man in relatively low concentrations. For example, the total dose of BP which can enter the human body during the lifetime of a person living in a zone polluted by waste products of organic synthesis [1] is measured in micrograms. According to the available data [4], the maximal allowable concentrations for BP in the air of industrial premises approved in the USSR is 15 μg/100 m^3. Accordingly the present investigations may be of definite importance for the detection of the mutagenic effect of BP in concentrations actually encountered in the environment.

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CHROMOSOMAL ABERRATIONS AND MOLECULAR WEIGHT OF SINGLE-STRANDED DNA FRAGMENTS IN EMBRYONIC FIBROBLASTS OF 101/H AND CBA MICE

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Attempts have recently been made to use mice of certain lines in order to create models of human diseases associated with chromosomal instability [6, 7]. Mouse line 101/H, characterized by a high spontaneous and induced level of chromosomal aberrations in the bone-marrow cells [4], and high susceptibility to induction of DNA injuries in embryonic fibroblasts under the influence of 4-nitroquinoline-1-oxide [1], is interesting from this point of view. The nature of the molecular events determining the increased mutability of the chromosomes in mice of line 101/H is not known.

The object of this investigation was to study the frequency of chromosomal aberrations, the molecular weight (mol. wt.) of DNA, and DNA-protein interaction in intact embryonic fibroblasts of 101/H mice. For comparison, mice of CBA line, relatively more resistant to mutagenic factors, were used [1, 4].

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