DEPENDENCE OF ANTIMUTAGENIC ACTIVITY OF SIMPLE PHENOLS ON THE NUMBER OF HYDROXYL GROUPS

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Metabolic activation of mutagens in biological systems is accompanied by intensification of free-radical processes. The free radicals formed are groups of atoms with an unpaired electron, which remains in an unchanged form in certain reactions. During interaction with antioxidants, reactions of radical formation are interrupted, and mutagen metabolism is thus slowed. Phenols, which have an easily removed hydrogen atom in their structure, are effective antioxidants [1].

The aim of this investigation was to study the effect of simple phenols on the mutagenic activity of benz(a)pyrene *in vivo* and *in vitro*.

EXPERIMENTAL METHOD

Mono-, di-, and trihydric simple phenols were used: phenol itself (PH), resorcinol (RE), and pyrogallol (PY). In an *in vivo* system, mutagenic activity of the chemicals and their mixtures was tested relative to induction of micronuclei in polychromatophilic bone marrow erythrocytes of (CBA × C57BL/6J)F_1 mice, which reflects damage to chromosomes at the erythroblast stage. BP in a dose of 150 mg/kg was injected intraperitoneally into the animals in sunflower oil, and aqueous solutions of the phenols were injected simultaneously in a ratio of 1:0.5 with BP. To determine the optimal time of recording the frequency of induction of micronuclei, samples of bone marrow were taken from the animals 1, 2, 3, and 4 days after a single injection of the substances. Films were fixed in methanol and stained with 7% Giemsa solution, pH 6.8.

The action of PH, RE, and PY on mutagenic activity of BP was studied in an *in vitro* system by counting induced direct gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGP) locus in cultures of Chinese hamster V-70 somatic cells. The mutagenic activity of the chemicals and their mixtures was studied by the method described in [3] under conditions of metabolic activation by mouse liver microsomes [2].

EXPERIMENTAL RESULTS

PH, RE, and PY, in the doses studied, had no mutagenic action as revealed by the induction of micronuclei test. BP induced 7 times more micronuclei than in the control. When the combined action of the chemicals was studied, the phenols inhibited the mutagenic activity of P by different degrees (Table 1). PH reduced the mutagenic activity of BP on the 1st and 4th days, but RE had no evident statistically significant antimutagenic activity. The antimuta-

TABLE 1. Changes in Number of Micronuclei in Polychromatophilic Mouse Bone Marrow Erythrocytes after Injection of BP and Phenols ($\bar{x} \pm S_{\bar{x}}$)

Substances, mg/kg	Dose or ratio between doses	Time of investigation, days			
		1	2	3	4
Control		1,3±0,3	1,3±0,3	1,3±0,3	1,3±0,3
BP	150 mg/kg	6,8±0,7	9,6±0,1	5±0,5	5,3±0,8
BP + PH	1:0,5	3,2±0,8	9,3±0,8	5,0±1,0	2,0±0,5
BP + RE	1:0,5	6,2±1,3*	7,25±1,8*	3,3±0,9	3±1,5
BP + PY	1:0,5	3,6±0,4	4,6±0,3	3,7±0,3	2,3±0,6

Legend. *P ≥ 0.05 compared with control.

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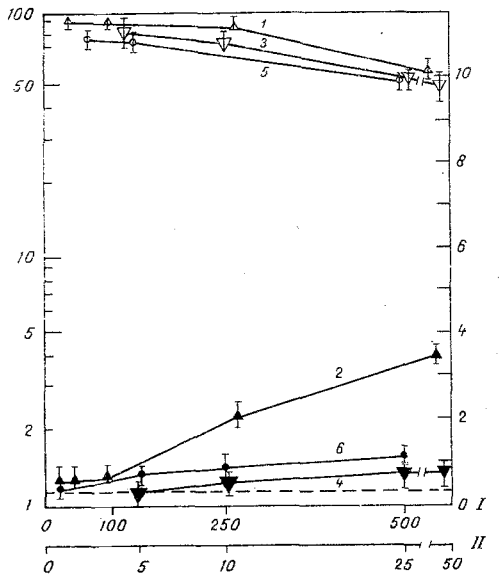

Fig. 1

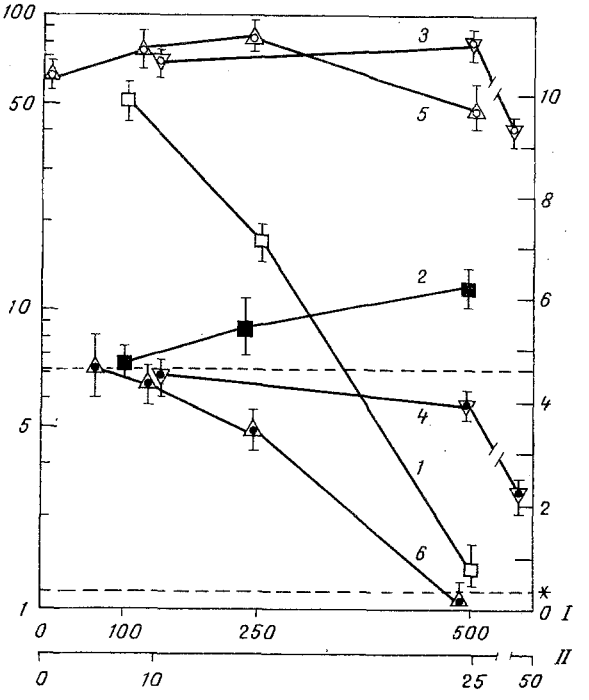

Fig. 2

Fig. 1. Effect of PH, RE, and PY on survival rate of cells and induction of direct gene mutations at the HGP locus. Abscissa, concentration (in μg/ml) of PH (I), RE, and PY (II); ordinate: on left — survival rate of cells (in % of control), on right — number of clones resistant to 8-azaguanine (per 10^5 cells). 1) Survival rate of cells; 2) number of induced mutations for PH; 3) survival rate of cells; 4) number of induced mutations for RE; 5) survival rate of cells; 6) number of induced mutations for PY. Broken line — level of sponteneous mutations in original cell population. Values of twice the standard error of the arithmetic mean ($2S_{\bar{x}}$) are shown.

Fig. 2. Effect of mixtures of BP with different concentrations of PH, RE, and PY on survival rate of cells and induction of direct gene mutations at the HGP locus. Abscissa, concentration (in μg/ml) of PH (I), Re, and PY (II); ordinate: left — survival rate of cells (in % of control), right — number of clones resistant to 8-azaguanine (per 10^5 cells). 1) Survival rate of cells; 2) number of induced mutations for mixture of BP + PH; 3) survival rate of cells; 4) number of induced mutations for mixture of BP + RE; 5) survival rate of cells; 6) number of induced mutations for mixture of BP + PY. Broken line shows level of gene mutations induced by BP. Remainder of legend as to Fig. 1.

genic activity of PY was observed after all time intervals (reduction by 50-60%).

In the *in vitro* system a constant dose of BP was used (10 μg/ml), with which the mutagenic effect was an increase in the number of mutations to 5 times the control level, with a survival rate of 62-67% of cells. A small but significant increase in the number of spontaneous mutations was observed after administration of PH in doses of between 250 and 500 μg/ml, with a 50% survival rate of the cells (the mean toxic concentration TC_{50} = 500 μg/ml; Fig. 1). The mixture of BP + PH reduced the survival rate of the cells synergistically but had no appreciable inhibitory effect on the mutagenic activity of BP in subtoxic concentrations. Conversely, when PH was used in a dose equal to TC_{50}, its own mutagenic effect increased the mutagenic activity of BP additively (Fig. 2).

TC_{50} for RE was 50 μg/ml, and for PY 25 μg/ml. In the concentrations studied, the mutagenic effects of these substances were not statistically significant. Dose-dependent inhibition of toxic effects and of mutagenic activity of BP by increasing concentrations of RE and PY was observed. A marked antimutagenic effect for BP + RE was observed only when in the ratio of 1:5.

A mixture of BP + PY in the ratio of 1:1 reduced the toxic effect of BP. Under these circumstances the mutagenic effect of BP was inhibited by 50%, but if the ratio of BP to PY was 1: 2.5 the level of induced mutations came close to the control value.

Inhibition of the mutagenic activity of BP by polyhydric phenols, observed in these experiments, is thus evidently connected with the presence of reactable hydrogen atoms in these compounds, which inhibit free-radical self-oxidation reactions of the chemical mutagen. The number of reactable hydrogen atoms in a series of polyhydric phenols increased with an increase in the number of hydroxyl groups, and was responsible for the antimutagenic properties of the simple polyhydric phenols (for example, the trihydric PY compared with the monohydric PH). Our results are in agreement with data in the literature on reduction of the mutagenic effect of BP by pyrogallol in a bacterial system [4]. Its antimutagenic properties may also be explained by its ability to form semiquinone derivatives, which bind free radicals most actively.

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IDENTIFICATION OF MUTAGENS BY FREQUENCY ANALYSIS OF MICRONUCLEAR NORMOCHROMIC ERYTHROCYTES

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Analysis of the frequency of polychromatophilic erythrocytes (PCE) with micronuclei in mammalian bone marrow is a rapid and simple test used at the stage of detection of potential mutagens and carcinogens [1, 2]. Reports have recently been published that normochromic erythrocytes (NCE) with micronuclei may accumulate in the peripheral blood of mice exposed repeatedly to chemical mutagens [3, 4]. The authors cited recommend that this modified micronuclear test be used for the intravital detection of mutagens in experiments on mammals.

To assess the potential of this method, the frequency of NCE (mature) and PCE (young) with micronuclei in the peripheral blood and the frequency of chromosomal aberrations in the bone marrow cells of mice, receiving cyclophosphamide with their drinking water, were compared.

EXPERIMENTAL METHOD

Experiments were carried out on random-bred male SHK mice. Each experimental and control group consisted of 5-6 animals. Cyclophosphamide (CP, from Jenapharm, East Germany), given to the animals with their drinking water in a concentration of 0.01% for 2 weeks, was used as the model mutagen.

In experiment I peripheral blood films were prepared by mixing a drop of blood from the subclavian vein on a slide with a drop of embryonic calf serum. The films were fixed in methanol and stained by the Giemsa method (pH 7.0). In experiment II, colchicine was injected intraperitoneally into the animals in a dose of 2.5 mg/kg 2 h before fixation, after which a parallel series of peripheral blood films and preparations of metaphase chromosomes of bone marrow cells was made.

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