EFFECT OF ANTIOXIDANTS ON LEVEL OF GENE MUTATIONS INDUCED BY BENZ(a)PYRENE IN MAMMALIAN CELLS *IN VITRO*

Yu. V. Pashin and L. M. Bakhitova

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Polycyclic aromatic hydrocarbons (PAH) and phenols are widely distributed environmental pollutants, and phenols often accompany PAH. Most PAH and, in particular, benz(a)pyrene (BP) have been found to possess mutagenic and carcinogenic properties. It has been shown that PAH which are primarily inactive in their physicochemical properties exhibit their mutagenic and carcinogenic potential only as a result of activation by microsomal enzymes and metabolic oxidation in the mammalian body. Phenols have a marked general toxic action and antioxidant activity. Investigations of the effect of phenols on mutagenic activity of PAH have virtually not been undertaken, and the results of the few studies of this problem which have been completed are contradictory. Some antioxidants carrying methyl groups, for instance, have been shown to inhibit metabolic transformation of BP and to reduce its mutagenic potential [4, 6]. Other workers observed opposite effects — antioxidants acted as powerful radiomimetic agents [5].

The aim of this investigation was to study the action of phenol and 5-methylresorcinol (5-MR), typical members of the antioxidant class, on the mutagenic potential of BP in mammalian cells.

EXPERIMENTAL METHOD

The V-79 line of pseudodiploid Chinese hamster cells used was obtained from Dr. J. Simons (Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, The Netherlands), BP was from Fluka (Switzerland), phenol (pure grade) and 5-MR were from Merck (West Germany). The mutagenic activity of BP, phenol, and 5-MR was estimated on the basis of their ability to induce direct gene mutations at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus on medium with 8-azaguanine by the method in [3] under conditions of metabolic activation by mouse liver microsomal enzymes [2]. BP was used in concentrations of 10 to 25 μg/ml and phenol and 5-MR in concentrations of 25, 50, 100, 250, and 500 μg/ml. Ethanol, in a volume of 0.1 ml, was used as the solvent. In the experiments of series I the mutagenic activity of BP, phenol, and 5-MR was investigated separately; in series II the mutagenic effects of mixtures of BP with phenol or 5-MR were studied.

EXPERIMENTAL RESULTS

Three different dose—effect curves were obtained in the experiments of series I during induction of gene mutations at the HGPRT locus, with different cell survival rates (Fig. 1). The greatest effect was observed with BP, with a survival rate of about 40%. Compared with BP, phenol and 5-MR inhibited cell viability less over a wide range of concentrations and a significant mutagenic effect for phenol was obtained only in the highest of the concentrations used (500 μg/ml), whereas 5-MR, in the same concentrations, produced no differences from the control (Fig. 1).

In the experiments of series II, on treatment with BP in a dose of 10 μg/ml, giving a mutation rate 4.4-6.4 times higher than the spontaneous level, substantial inhibition of cell viability was found as a result of mixing with different concentrations of phenol. For instance, when the phenol concentration was increased to 250 μg/ml the rate fell to 18%, but

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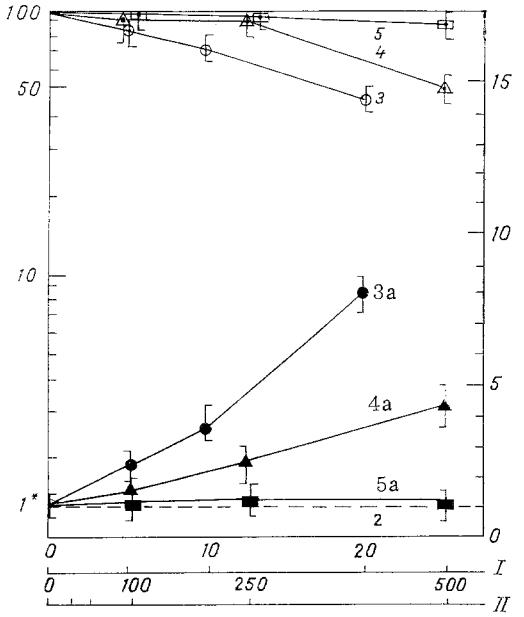

Fig. 1

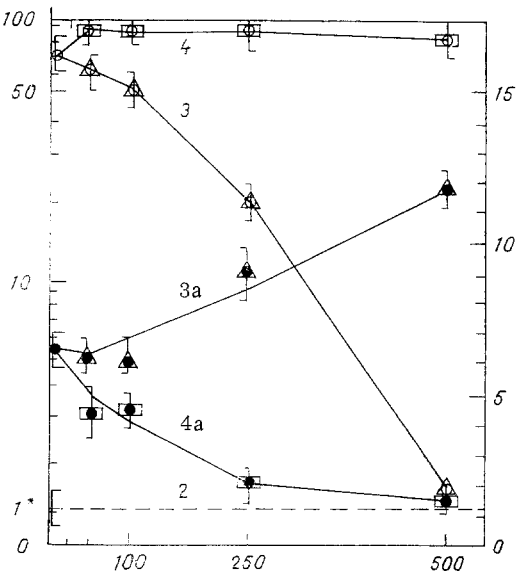

Fig. 2

Fig. 1. Effect of BP (I), phenol, and 5-MR (II) on cell survival rate and on induction of direct gene mutations at the HGPRT locus. 1) Level of spontaneous mutations in original cell population; 2) effect of microsomal activating mixture and solvent (ethanol) (negative control); 3) cell survival rate, 3a) number of induced mutations for BP; 4) cell survival rate; 4a) number of induced mutations for phenol; 5) cell survival rate, 5a) number of induced mutations for 5-MR. Two standard errors of arithmetic means are shown ($2S_{\bar{x}}$). Abscissa, concentration of BP, phenol, and 5-MR (in μg/ml); ordinate: left — cell survival rate (in percent of control), right — number of clones resistant to 8-azaguanine (per 10^5 cells).

Fig. 2. Effect of mixtures of BP (10 μg/ml) with different concentrations of phenol and 5-MR on cell survival rate and induction of direct gene mutations at the HGPRT locus. 3) Cell survival rate, 3a) number of induced mutations for BP + phenol mixtures; 4) cell survival rate, 4a) number of induced mutations for BP + 5-MR mixtures. Abscissa, concentration of phenol and 5-MR (in μg/ml). Remainder of legend as to Fig. 1.

later, when the phenol concentration was increased to 500 μg/ml, it fell sharply to 1%, and the rate of cell proliferation was slowed. The mutagenic effect of the mixture (BP + phenol) in this case can be regarded as the sum of two values characterizing each component separately (compare Figs. 1 and 2). During combined treatment with a constant concentration of BP (10 μg/ml) and increasing doses of 5-MR (50-250 μg/ml) the cell survival rate was maintained at the control level during a 3-4-fold increase, and it fell to 46% only when the highest of the concentrations tested (500 μg/ml) was used. In this case the mutagenic activity of BP was not exhibited (Fig. 2).

The ratios between concentrations of BP and antioxidants chosen correspond to those actually found in industry (from 1:5 to 1:25). With a further increase in the concentration of 5-MR relative to BP (more than 1:25) the cell survival rate fell, but no precise results could be obtained evidently because of the high toxicity of the mixture.

Inhibition of the mutagenic activity of BP by 5-MR discovered in these experiments agrees with results obtained with other antioxidants [16]. It can be postulated that this protective action is linked with ability to inhibit the formation of chemically active metabolites from BP. Direct correlation also has been found between the structure, intensity of antioxidative properties, and antimutagenic activity of chemicals [1]. 5-MR, with a methyl group in its chemical structure, like other powerful antioxidants [4], effectively inhibits the formation of mutagenic metabolites from BP, whereas phenol, a less active antioxidant, did not possess these properties. Conversely, in concentrations at which phenol exhibited its own mutagenic effect, its action was additive relative to BP.

The use of powerful bioantioxidants may thus be a promising approach to reducing the mutagenic potential of PAH. Less detrimental genetic consequences may also be expected from PAH if they are liberated into the environment mixed with powerful bioantioxidants.

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ROLE OF RECESSIVE LETHAL GENES IN SPONTANEOUS EMBRYONIC MORTALITY IN NONINBRED MOUSE AND RAT POPULATIONS IN CUBA

Kh. Kh. Semenov and D. Walker

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The frequency of recessive genes, lethal in the homozygous state, is a more objective criterion of latent variation, although recessive lethals are a very limited group of variants which cannot give an adequate idea of the general level of genetic variation. Meanwhile, recessive lethal genes are expressed if in the homozygous state and they make a definite contribution to spontaneous embryonic mortality. This latter parameter, a biological characteristic of the population, is very important for research workers who use this feature as an indicator of the genetic and teratologic effect of physical and chemical agents (mutagenesis and teratogenesis). The main method of determining genetic variation is by the use of inbreeding, which leads to an increase in homozygosity which, in turn, favors the manifestation of recessive alleles, hitherto concealed in heterozygotes.

The object of the present investigation was to study the frequency of recessive lethal genes in the genetic pool of noninbred mouse and rat populations in the laboratory animals nursery of the National Scientific Research Center (CENIC), Academy of Sciences of the Republic of Cuba.

EXPERIMENTAL METHOD

Small colonies of noninbred mice and rats from the CENIC Nursery served as test material. A colony of random-bred mice consisted of 240 parents (ratio 2:1) and a colony of rats consisting of 110 parents (ratio 1:1). Two crossing versions were used in the investigation: 1) autobred crossing, 2) inbred or closely related (crossing between sibs). Virgin females aged 2-2.5 months were crossed with males of the same age. In both versions of crosses the females were autopsied on the 15th-17th day of pregnancy and the number of living and dead embryos and the number of corpora lutea in the ovaries were counted. The spontaneous embryonic mortality level was determined as the total number of pre- and postimplantation embryos lost for the autobred crossing group. The frequency of recessive lethals was determined as the difference between embryonic mortality in progenies from sib crossing (F = 1/4) and crossing of unrelated males and females (F = 0), multiplied by 4. Altogether 150 female mice and 112 female rats were used in the experiment. Since parameters of normal ovulation, the level of spontaneous embryonic mortality before and after implantation, and the frequency of reces-

Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow.

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