bably accessible for mobilization for transfer to these bacteria with the aid of other conjugative plasmids. This hypothesis is supported by data on mobilization of factor pAP42 for transfer with the aid of plasmid RP4. The results also suggest that in the course of mobilization of plasmid pAP42 by plasmid RP4, cointegrative structures are formed in which the two component plasmid parts (pAP42 and RP4) are capable of expression. However, the problem of how replication of these cointegrates takes place requires special study.

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# EFFECT OF PHENOBARBITAL ON THE CYTOGENETIC ACTIVITY OF CYCLOPHOSPHAMIDE

V. S. Zhurkov, R. V. Merkur'eva, N. P. Burmantova, A. V. Votyakov, and I. F. Vyskubenko

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KEY WORDS: cyclophosphamide; phenobarbital; chromosomal aberrations; mixed function oxygenases.

We now know that the late effects of some chemicals are determined by their active metabolites formed with the participation of mixed function oxygenases (MFO), enzymes located in membranes of the endoplasmic reticulum. Hence the need to study the influence of substances modifying MSO activity on the mutagenic effect of chemicals in experiments on mammals.

The modifying effect of phenobarbital (PB), a known inducer of the MFO system, on the frequency of chromosomal aberrations was studied in rat bone marrow cells under the influence of cyclophosphamide (CP). The cytogenetic activity of CP has been well studied in experiments on mammals, the results of which have shown that the active principle of CP consists of its metabolites formed through the participation of MFO [1, 2, 8].

## EXPERIMENTAL METHOD

CP (from Jenapharm, East Germany) and PB sodium (from Farmakon, Czechoslovakia) were used and were dissolved in sterile distilled water. Experiments were carried out on noninbred male albino rats weighing 160-200 g. Six groups of animals were used, with 5-8 rats in each group. Animals of group 1 received a single intraperitoneal injection of CP in a dose of 25 mg/kg; animals of groups 2 and 3 received PB by three intraperitoneal injections in doses of 2 and 80 mg/kg, respectively, at intervals of 24 h; animals of groups 4 and 5 received PB by the same scheme, and also CP in a dose of 25 mg/kg 24 h after the last injection of PB. The animals of group 6 served as the control.

For cytogenetic analysis rats were killed 24 h after the last injection of the substances. Films of metaphase chromosomes of bone marrow cells were prepared by the standard method. In each animal 100 metaphases were analyzed. Cytogenetic analysis was carried out on numbered slides. Single and paired fragments and chromatid and chromosmal exchanges were counted. Cells with 10 chromosomal aberrations or more were classed as metaphases with multiple aberrations. Differences between frequencies of cells with aberrations in different groups were evaluated by the chi-square test.

Laboratory of Medical Genetics and Laboratory of Biochemistry, A. N. Sysin Research Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow.
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TABLE 1. Activity of MFO Enzymes and Frequency of Cells with Chromosomal Aberrations in Rat Bone Marrow after Isolated and Combined Action of PB and CP

| Parameter | Statistical index | Control | CP | PB, 2 mg/kg | PB, 2 mg/kg + CP | PB, 50 mg/kg | PB, 50 mg/kg + CP |
|---|---|---|---|---|---|---|---|
| Cytochrome P-450, nmoles/mg protein | $M$ | 1,19 | 1,42* | 1,31 | 1,43 | 3,21‡ | 3,89‡ |
| | $\pm m$ | 0,05 | 0,09 | 0,10 | 0,11 | 0,29 | 0,28 |
| | $n$ | 8 | 6 | 5 | 6 | 7 | 5 |
| Cytochrome $b_5$, nmoles/mg protein | $M$ | 0,84 | 0,86 | 0,74 | 0,78 | 1,19† | 1,25‡ |
| | $\pm m$ | 0,04 | 0,04 | 0,10 | 0,07 | 0,10 | 0,13 |
| | $n$ | 8 | 6 | 5 | 6 | 7 | 5 |
| Rate of p-hydroxylation of aniline, nmoles/min/mg protein | $M$ | 0,94 | 1,57* | 0,97 | 1,10 | 2,42‡ | 2,78‡ |
| | $\pm m$ | 0,09 | 0,09 | 0,13 | 0,09 | 0,31 | 0,25 |
| | $n$ | 8 | 6 | 5 | 6 | 7 | 5 |
| Frequency of cells with chromosomal aberrations, % | $M$ | 0,6 | 7,0‡ | 1,0 | 11,2‡ | 0,8 | 10,3‡ |
| | $\pm m$ | 0,34 | 1,04 | 0,44 | 1,29 | 0,44 | 1,24 |
| | $n$ | 5 | 6 | 5 | 6 | 5 | 6 |

*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

To investigate MFO activity the rats were killed 24 h after the last injection of PB was given alone, or 1 h after injection of CP (alone or in combination with PB). The microsomal fraction of the liver was obtained by ultracentrifugation of the postmitochondrial supernatant in a VAC 601 centrifuge (East Germany) at 105,000g for 1 h. Activity of the MFO system was judged from the level of cytochromes P-450 and $b_5$ [9] and the rate of p-hydroxylation of aniline [3]. Quantitative determination of cytochromes was carried out on a Hitachi 556 dual beam two-wave spectrophotometer. The microsomal protein content was determined by Lowry's method [7]. The experimental results were subjected to statistical analysis by Student's t test.

## EXPERIMENTAL RESULTS

The cytogenetic and biochemical data are given in Table 1. Administration of PB alone did not affect the level of cytogenetic disturbances. CP induced a significant increase in the frequency of metaphases with chromosomal aberrations compared with the control ($\chi^2 = 26.9$; $P < 0.001$). The frequency of cells with aberrations after the combined action of the two drugs was significantly higher than after the action of CP alone (for comparison of CP alone and a combination of CP + PB in a dose of 2 mg/kg: $\chi^2 = 6.31$, $P < 0.05$ with CP + PB in a dose of 50 mg/kg: $\chi^2 = 4.21$, $P < 0.05$).

The cytogenetic effect after the combined action of the drugs was independent of the dose of PB ($\chi^2 = 0.22$; $P > 0.05$). After the combined action of CP and PB metaphases with multiple chromosomal aberrations were observed. The ratio between the types of chromosomal aberrations following the action of CP and of PB together with CP was similar: 70% of single fragments, 5% of paired fragments, 25% of chromatin exchanges.

Investigation of the activity of the MFO system after the isolated action of PB showed that a dose of 2 mg/kg, just as in the preliminary experiments, caused no significant changes compared with the control. PB in a dose of 80 mg/kg increased the cytochrome P-450 content by 2.7 times ($P < 0.001$) and the cytochrome $b_5$ content by 42% ($P < 0.01$), and increased the rate of p-hydroxylation of aniline by 2.2 times ($P < 0.01$).

After the action of CP alone the cytochrome P-450 content increased by 19% ($P < 0.05$) and the rate of p-hydroxylation of aniline by 67% ($P < 0.01$) compared with the control. There was no change in the content of cytochrome $b_5$ in these experiments.

The combined action of PB in a dose of 2 mg/kg and CP caused no significant increase in parameters of the MFO system compared with those following the isolated action of PB. The rate of p-hydroxylation of aniline after the combined action of PB in a dose of 2 mg/kg and of CP was significantly lower than after the action of CP alone. The levels of all parameters of the MFO system tested after the combined action of PB in a dose of 80 mg/kg and of CP were

higher than after the action of this dose of PB alone. However, the increase was not statistically significant.

Injection of PB, an inducer of the MFO system, thus caused an increase in the level of chromosomal aberrations induced by CP in rat bone marrow cells. Definite correlation could not be found between the activity of the MFO system and the cytogenetic activity of CP. Against the background of marked induction of the MFO system (PB in a dose of 80 mg/kg) and of its very mild activating effect (PB in a dose of 2 mg/kg) the increase in the cytogenetic effect of CP was equal. To explain this fact it can be postulated that administration of low doses of PB, which do not bring about any appreciable induction of the MFO system, "converts" it from a state of relative stability into a state of readiness to react, against the background of which the rate of metabolism of the different substrates is modified.

These results are in agreement with observations showing an increase in the teratogenicity of CP in rats pretreated with PB, and a reduction in the antitumor activity of CP as a result of inhibition of the MFO system [6, 10]. However, these results were not confirmed by other investigations [4, 5]. The contradictions thus arising may be attributed to the pharmacokinetics of CP and to differences in the experimental conditions. All these considerations point to a need for further experimental studies of the facts described above, including a study of the kinetics of CP associated with the action of MFO modifiers in different doses.

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# GENETIC DIFFERENCES IN SENSITIVITY OF MICE TO THE IMMUNODEPRESSIVE ACTION OF ALKYLATING AGENTS


Kim Nam Ir, L. Yu. Telegin, and L. A. Pevnitskii



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Regulation of the immune response of the organism by means of chemotherapeutic agents is a problem of great practical and theoretical interest. An urgent aspect of this problem is the study of the genetic basis of the immunodepressive action of different agents, for the end result of intervention, namely inhibition of the immune response. However, insufficient research of this kind has been undertaken so far. Previously [1] the writers studied the sensitivity of mice of different genotypes to the immunodepressive action of cyclophosphamide (CP), an alkylating agent belonging to the bis-β-chloroethylamine group. It was shown by the use of a model of the primary humoral immune response during immunization with sheep's red blood cells (SRBC) that DBA/2 mice are highly sensitive to the immunodepressive action of CP whereas BALB/c mice are relatively resistant.


Laboratory of Immunogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.


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