Genotypic, Sex, and Age Differences in the Clastogenic Action of Cyclophosphamide in Mice

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Interstrain genotypic, sex, and age differences in the clastogenic action of cyclophosphamide in various doses are established for C57Bl/6, MRL/1, and BALB/c mice.

Key Words: *cyclophosphamide; chromosome aberrations; sex; age; BALB/c, C57Bl/6, and MRL/1 mice*

Research on the biospecificity of action by mutagens is important for understanding the mechanisms of induced mutagenesis and for predicting the genetic risk arising from human exposure to mutagens [2-4]. There is experimental evidence pointing to a biospecificity of the cytogenetic effects produced by cyclophosphamide (CP) in certain fixed doses. Assays for sister chromatid exchanges and micronuclei revealed considerable interspecific differences in the damaging effects of CP between mice, rats, and hamsters [10], while assays for chromosome aberrations in mice disclosed interstrain differences in such effects of this compound in the dose of 100 mg/kg [7]; furthermore, the assays for sister chromatid exchanges, but not those for micronuclei, demonstrated that the harmful effects produced by CP (at 4.5 mg/kg) depend on the age and genotype of the mice.

The aim of the present study was to find out how the genotype, age, and sex of animals might influence the clastogenic activity of CP. To this end, cytogenetic effects of this agent were considered in relation to its dose in male and female C57Bl/6, BALB/c, and MRL/1 mice aged 1-2 and 5-6 months.

MATERIALS AND METHODS

Male and female mice of the strains BALB/c and C57Bl/6 from the *Stolbovaya* Nursery of the Russian Academy of Medical Sciences and of the MRL/1 strain from the Research Institute of Rheumatology were used. All mice were housed in the vivarium of the Institute of Pharmacology's Laboratory of Pharmacological Genetics using a 12-h light - 12-h darkness schedule; they were given standard food pellets and had free access to water.

Cyclophosphamide (Germed) was dissolved in physiological saline immediately before use and injected into the test mice once intraperitoneally in a volume of 0.01 ml/g at 10, 20, or 40 mg/kg 24 h before sacrifice; the control mice were injected with saline in the same volume.

The clastogenic activity of CP was assessed by counting bone marrow cells with chromosome aberrations. Cytogenetic preparations were made by the conventional technique [8]; 2.5 h before the end of the 24-h exposure to CP, animals were administered colchicine intraperitoneally (2.5 mg/kg) to allow for accumulation of metaphases. In the cytogenetic analyses, for which a Standart-20 microscope was used (at ×1000 magnification), metaphase plates with achromatic lesions (gaps), single and paired fragments, and chromosome exchanges were recorded, as were cells with multiple chromosome aberrations (>5 per cell). A total of 100 metaphases were examined for each mouse, and preparations from no less than 4-5 mice were examined in each group. For statistical analysis, the ϕ test was used, comparing the proportions of

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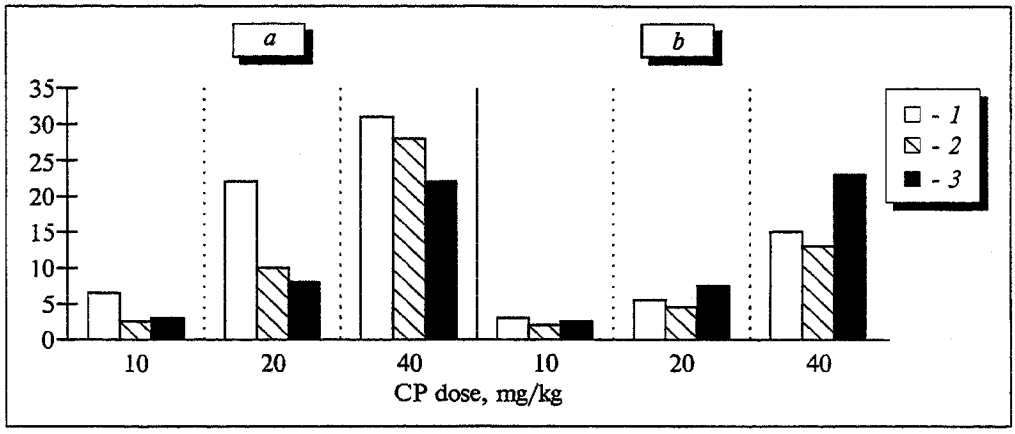

Fig. 1. Clastogenic effects of cylophosphamide (CP) in doses of 10, 20, and 40 mg/kg in bone marrow cells from male (*a*) and female (*b*) mice aged 1 to 2 months. Here and in Fig. 2: *1)* MRL/1; *2)* BALB/c; *3)* C57Bl/6. Ordinate: % of damaged bone marrow cells.

cells with damaged chromosomes (including cells with gaps) in the different groups.

RESULTS

In the control group, the cytogenetic analysis revealed 1.4±0.5%, 2.5±0.8%, and 2.0±0.7% damaged cells in C57Bl/6, MRL/1, and BALB/c mice, respectively.

Figure 1, *a* shows dose-response relationships for 1- to 2-month-old male mice of the three strains. The CP dose of 10 mg/kg induced a statistically significant increase in the yield of cells with damaged chromosomes in MRL/1 males (6.7±1.3% abnormal cells), but virtually did not alter the proportion of cells with damaged chromosomes in mice of the other two strains.

At 20 mg/kg, CP led to a significant rise in the proportion of aberrant metaphases in the bone marrow of males of all three strains. Thus, C57Bl/6 mice had 8.0±1.2% such metaphases. BALB/c mice 10.2±1.4%, and MRL/1 mice 21.4±1.6%, i.e., the last strain was again significantly more sensitive to this clastogen than the other two.

After the highest CP dose (40 mg/kg), the proportion of cells with cytogenetic lesions in male C57Bl/6 mice was significantly lower (22.0±1.9%) than in BALB/c (28.8±2.0%) or MRL/1 (31.0±2.0%) males. Thus, the differences between MRL/1 and BALB/c males in the proportion of abnormal cells revealed after the CP doses of 10 and 20 mg/kg almost disappeared when the dose of the mutagen was increased to 40 mg/kg. Whereas at 20 mg/kg the cytogenetic effect of CP in BALB/c males was similar to that in C57Bl/6 males, at 40 mg/kg this effect was virtually no different from that recorded for MRL/1. On the whole, C57Bl/6 males proved to be the most sensitive and MRL/1 males the least sensitive to CP in the dose range used. These results are consistent with those of previous experiments in which C57Bl/6 mice were found to be less responsive to CP in a dose of 100 mg/kg than their BALB/c counterparts [7].

Figure 1, *b* shows dose-response relationships for 1- to 2-month-old female mice of the three strains. After the CP dose of 10 mg/kg, 3.0±0.9%, 1.5±0.6%, and 2.3±0.7% abnormal cells were recorded for MRL/1, BALB/c, and C57Bl/6 females, respectively. These percentages differ little from the control values (2.8±0.8, 1.3±0.7, and 1.6±0.7%). After the 20 mg/kg dose, the proportion of damaged cells significantly increased to reach 6.0±0.9% in MRL/1, 4.5±1.0% in BALB/c, and 7.6±0.9% in C57Bl/6, the difference between BALB/c and C57Bl/6 females being significant. After the 40 mg/kg dose, C57Bl/6 females had a significantly higher

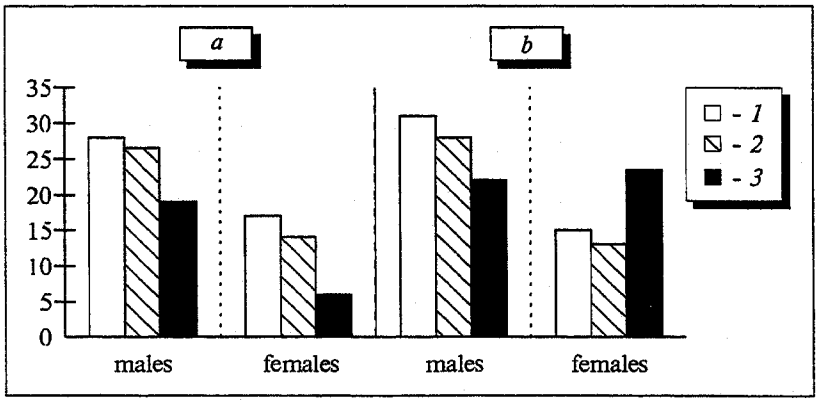

Fig. 2. Clastogenic effects of cyclophosphamide (CP) in a dose of 40 mg/kg in male and female mice aged 5–6 (*a*) and 1–2 (*b*) months.

proportion of cells with damaged chromosomes than those of the other two strains (23.7±1.4% against 15.5±1.8% in MRL/1 and 13.6±1.5% in BALB/c).

The tests described above thus showed that the clastogenic effect of CP was dose- and strain-dependent in rats aged 1-2 months. It was not sex-dependent in the C57Bl/6 strain. In the MRL/1 and BALB/c females, however, the levels of damage caused by CP at 20 and 40 mg/kg were significantly lower than in the males of these strains. It can also be seen that MRL/1 and BALB/c males are more susceptible to this clastogen than C57Bl/6 males, and that females of those two strains are more susceptible than C57Bl/6 females.

In the next series of tests, 5- to 6-month-old mice were used, and the results are presented in Fig. 2, *a*.

Cytogenetic analysis of bone marrow cells from control males and females did not show significant differences from the results recorded for animals aged 1 to 2 months. Thus, the proportions of damaged cells amounted to 2.0±0.8% in C57Bl/6 males and to 1.5±0.6% in C57Bl/6 females, and the corresponding figures for MRL/1 and BALB/c males and females were 3.0±0.6 and 2.3±0.7% and 2.0±0.7 and 1.8±0.7%.

The CP dose of 40 mg/kg led to a significant rise in the yield of cells with chromosomal damage to 19.8±2.0%, 26.8±2.0%, and 28.7±2.6% in 5- to 6-month-old C57Bl/6, BALB/c, and MRL/1 males, respectively. As in the mice aged 1-2 months (Fig. 2, *b*), the proportion of damaged cells in C57Bl/6 animals was significantly lower than in the other two strains (Fig. 2, *a*). Intrastrain differences in the clastogenic effect of the 40 mg/kg dose between male mice aged 1-2 and 5-6 months were insignificant, suggesting that this effect of CP is not age-dependent in males of these three strains.

After the 40 mg/kg dose, significant increases in the proportion of damaged cells to 6.5±1.2, 14.4±1.6, and 17.2±2.0% were recorded for C57Bl/6, BALB/c, and MRL/1 females, respectively. Bone marrow cells from C57Bl/6 females were the least sensitive, exhibiting significantly lower levels of damage than those from females of the other two strains.

Comparison of the data obtained after the 40 mg/kg dose for females aged 1-2 and 5-6 months did not show a significant difference between these two age groups of BALB/c and MRL/1 mice (Fig. 2). For bone marrow cells from C57Bl/6 females, however, the age-related difference was found to be highly significant, the proportion of damaged cells being 23.7±1.4% in the younger group and only 6.5±1.2% in the older one. It is noteworthy that 1- to 2-month-old females of this strain are more sensitive to CP while those aged 5-6 months are less sensitive than BALB/c or MRL/1 females of the same age groups.

Males of all three strains and both age groups were in general more sensitive to the clastogenic action of CP at 40 mg/kg than were females, with the exception of C57Bl/6 mice aged 1-2 months (Fig. 2, *a*).

Interstrain differences in CP effects have been reported [2] and are usually attributed to metabolic features of CP, given that the latter is a promutagen and that the metabolism of promutagens may be genetically specific [1]. CP, however, has been shown capable of enhancing lipid peroxidation in tissues [5], which suggests the presence of a free-radical component in the damaging action of this compound [6]. On the other hand, there is solid evidence of interstrain, sex, and age differences in the organization and functioning of antioxidant systems [6,9]. Hence the importance of looking into the role of these systems in the clastogenic activity of CP.

The findings on interstrain differences in the dose-dependent clastogenic effects of CP and on the age and sex dependence of its cytogenetic activity call for pharmacogenetic studies.

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