

Using the Micronucleus Assay to Detect Genotoxic Effects of Metal Ions

by J. Bérces,¹ M. Ótos,¹ S. Szirmai,¹ C. Crane-Uruena,²
and G. J. Köteles²

The lymphocyte micronucleus assay was used to measure the average frequency of micronuclei in a population and thus assess genotoxic effects. Data from 174 persons give an average value of 16.4 ± 7.3 , and a slight age-dependence was observed. To detect combined environmental mutagen injuries the micronucleus assay was used to study the effects of metal compounds. Cadmium ions increased the micronucleus frequency linearly after incubation with whole blood *in vitro* with 10^{-6} – 10^{-3} M concentrations for 30 min. Similarly, a linear increase in micronucleus frequency was detected with 10^{-3} – 10^{-1} M mercury ions. Concerning the biological effect of selenium, it was found that neither sodium selenite nor selenium dioxide induced increases at concentrations of 10^{-7} – 10^{-6} M; 10^{-5} M caused a slight increase; 10^{-4} M, however, destroyed the cells. These results suggest that the human lymphocyte micronucleus test can be used to assess genotoxic injuries due to environmental effects in human lymphocytes.

Introduction

There are many genotoxic effects that result from occupational and residential exposures. The detection and the estimation of effects are important tasks in public health. For this reason, a suitable method is needed to select biological systems for testing.

Recently, the micronucleus test based on cultured human lymphocytes has been introduced. Reproducible dose-effect relationships were obtained after treatment of human blood with ionizing radiation (1,2). To detect clastogenic effects, it is necessary to know the average value of the micronucleus frequency over the population or, even better, to determine the personal reference values for the members of the group at risk. Therefore, a survey of frequency values in the general population was made. Our work was also aimed at studying the applicability of the micronucleus test in assessing the genotoxic effects of metal ions.

Materials and Methods

Blood samples were taken from 174 healthy donors, 20–80 years of age, under sterile conditions with sodium-heparine anticoagulant. Lymphocyte cultures were set up

in RPMI 1640 medium with 15% fetal calf serum. Culturing and evaluation were performed as described earlier (1). The chemicals applied were Na_2SeO_3 (Reanal, Budapest, Hungary), SeO_2 (Serva, Heidelberg, Germany), HgCl_2 (Reanal), and CdCl_2 (Reanal). All chemicals were of analytical grade. The applied statistical analyses are presented in "Results."

Results

Micronucleus Frequency in the General Population

The micronucleus frequency of 174 healthy donors of several ages from both sexes were determined. The distribution of the individual values are plotted in Figure 1. The average value \pm SD over the entire selected population is 16.4 ± 7.3 . Using chi-square statistical analysis, the distribution was found to be normal. As we had the opportunity to take samples of different ages, we could determine the micronucleus frequency and examine the age dependence. A slight age dependence was observed using statistical regression analysis (regression coefficient, 0.334). Fifteen values for young ages were taken from the literature (3). They are marked with diamonds in Figure 1, and it is interesting to note that these data fitted well in to our regression line.

We also examined the differences between the micronucleus frequencies of males and females. In Figure 2, the average values of both sexes are plotted in different age categories. It can be seen that there is no difference

¹Paks Nuclear Power Plant, Hungary.

²Frédéric-Joliot Curie National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

Address reprint requests to G. J. Köteles, H-1775, Budapest, POB 101, Hungary.

between the average micronucleus frequencies of the two sexes.

Effects of Cadmium Ions

Cadmium is frequently used in industry. Several pathological examinations suggest that cadmium can cause aneuploidy, and it poisons the mitotic spindle. The mitotic spindle has a great importance in the formation of micronuclei. Therefore, we examined whether there is any effect of cadmium on micronucleus formation.

Human blood samples were incubated with various concentrations of cadmium chloride for 30 min, and lymphocytes were cultured. It was found that cadmium ions at concentrations of 10^{-6} – 10^{-3} M induced a linear increase of micronucleus frequency (Fig. 3).

Effects of Mercury Ions

The clastogenic effect of Hg^{2+} ions was studied by applying mercury chloride. When a 30-min preincubation

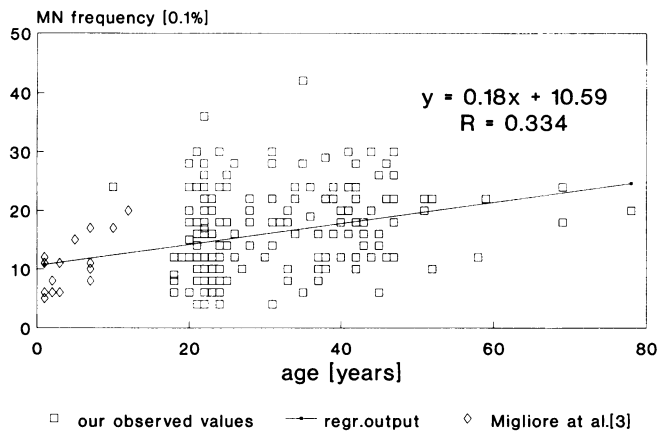


FIGURE 1. Age dependence of the micronucleus frequency in cytokinesis-blocked lymphocytes.

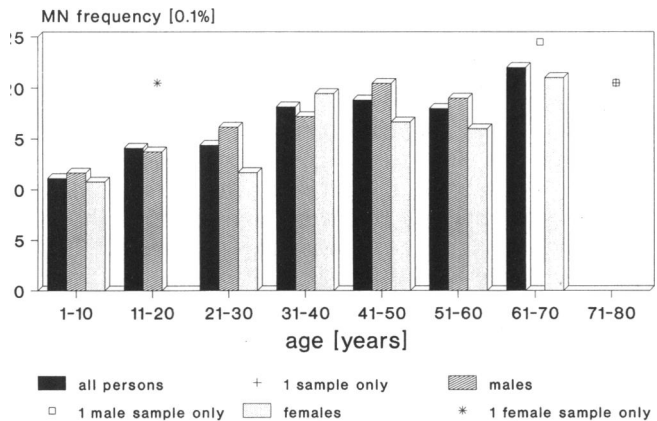


FIGURE 2. Micronucleus frequency of males and females in different age categories.

of human blood was performed and the lymphocytes were cultured, a linear increase of micronucleus frequency was found with Hg^{2+} concentrations of 10^{-3} – 10^{-1} M (Figure 4).

Effects of Selenium Compounds

Due to the controversy over the effect of selenium (4,5), we examined the effects of both sodium selenite and selenium dioxide. Human blood was preincubated for 30 min with concentrations of both compounds ranging from 10^{-7} to 10^{-6} M. Lymphocytes were cultured. It was found that neither sodium selenite nor selenium dioxide provoked considerable increases of micronucleus frequency at these concentrations; 10^{-5} M caused a slight increase; 10^{-4} destroyed the cells.

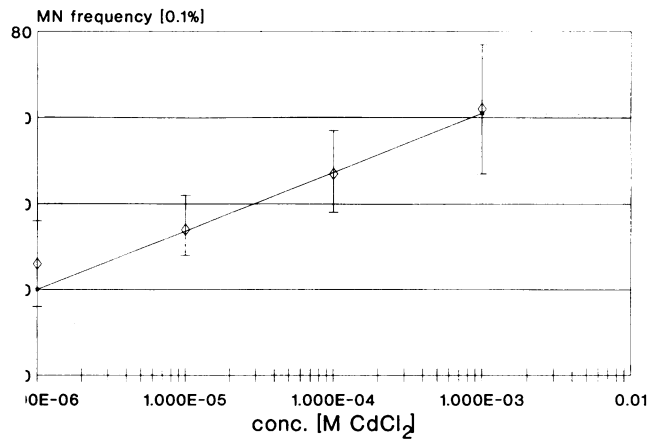


FIGURE 3. Micronucleus frequency in cultured lymphocytes after incubation of blood samples in the presence of various concentrations of cadmium chloride.

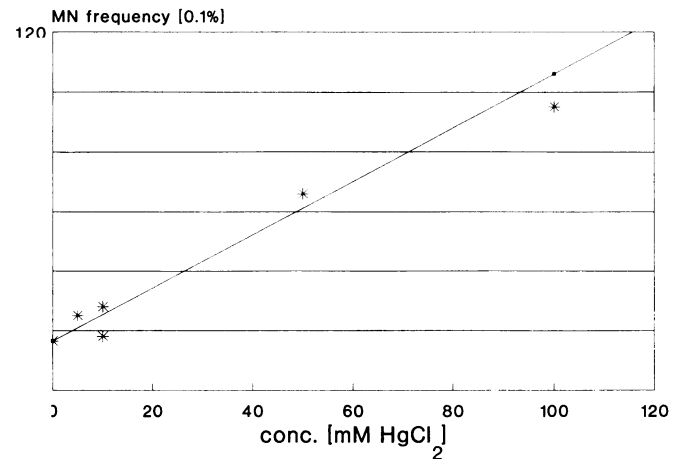


FIGURE 4. Increase of micronucleus frequency in CB cultured lymphocytes after incubation with various concentrations of mercury chloride.

Discussion

To use the micronucleus assay as a cytogenetic monitor of environmental effects, it is essential to know the base level in a normal population. Our average value is in good agreement with published values (6–9). It is, however, more disputable whether there is an age-dependent increase in the micronucleus frequency of population. Some authors suggest a definite age-dependent increase (7,8), while others could not verify these observations. Our thorough study provides an explanation of these conflicting findings; there is a relation, but very slight; therefore, the significance can be demonstrated only after a large number of measurements.

No sex-dependent differences could be found in the basic level of micronucleus frequency in the control population. This fact, however, does not exclude the possibility that certain exogenous harmful effects might create differences due to different sensitivities of the sexes.

Our investigations also indicate that cadmium and mercury ions provoke the formation of aberration as detected by the increase in frequencies of micronuclei. These results are in good agreement with data from studies using metaphase aberrations as testing end points (12).

Conclusion

The detectability of genotoxic effects from environmental pollutants including chemicals like cadmium ions and physical genotoxins like ionizing radiation has come into the limelight. The micronucleus test in human lymphocytes offers a cytogenetic technique that can be performed easier than metaphase chromosome analysis (1). Our study illustrates several applications of this test: *a*) to determine the average micronucleus frequency over a population under risk; *b*) to detect effects in the concentration range of Cd^{2+} ions that induce alterations on two cellular targets [i.e., chromatin substances as indicated in the present work and cellular membranes (11)] and which are used in experiments and occur in polluted environments; and *c*) to detect the combined environmental effects of some heavy metal ions such as Cd^{2+} , Hg^{2+} , and Se^{2+} .

The micronucleus test proves to be reliable, easy to perform, and relatively quick for *in vitro* testing of geno-

toxic compounds. The method can be automated easily (12). Its advantages to make it a valuable assay that can be performed routinely.

The authors gratefully acknowledge the interest and support of L. B. Sztanyik, Director General of the Institute and the skillful technical assistances of Éva Tóth and Magda Nagy.

REFERENCES

1. Kormos, C., and Köteles, C. J. Micronuclei in X-irradiated human lymphocytes. *Mutat. Res.* 199: 31–35 (1988).
2. Almásy, Z., Krepinsky, B. A., Bianco, A., and Köteles, G. J. The present state and perspectives of micronucleus assay in radiation protection. A review. *Appl. Radiat. Isotope* 38: 242–249 (1987).
3. Migliore, L., Guidotti, P., Favre, C., Nardi, M., Sessa, M. R., and Brunori, E. Micronuclei in lymphocytes of young patients under antileukemic therapy. *Mutat. Res.* 263: 243–248 (1991).
4. Khalil, A. M. The induction of chromosome aberrations in human purified peripheral blood lymphocytes following *in vitro* exposure to selenium. *Mutat. Res.* 224: 503–506 (1989).
5. Norppa, H., Westermarck, T., Laasonen, M., Knuutila, L., and Knuttila, S. Chromosomal effects of sodium selenite *in vivo*. *Hereditas* 93: 93–96 (1980).
6. Prosser, J. S., Moquet, J. E., Lloyd, D. C., and Edwards, A. A. Radiation induction of micronuclei in human lymphocytes. *Mutat. Res.* 199: 37–45 (1988).
7. Migliore, L., Parrini, M., Sbrana, I., Biagini, C., Battaglia, A., and Loprieno, N. Micronucleated lymphocytes in people occupationally exposed to potential environmental contaminants: the age effect. *Mutat. Res.* 256: 13–20 (1991).
8. Fenech, M., and Morley, A. A. Cytokinesis-block micronucleus method in human lymphocytes: effect of *in vivo* ageing and low dose X-irradiation. *Mutat. Res.* 161: 193–198 (1986).
9. Littlefield, L. G., Sayer, A. M., and Frome, E. L. Comparisons of dose-response parameters for radiation-induced acentric fragments and micronuclei observed in cytokinesis arrested lymphocytes. *Mutagenesis* 4: 265–270 (1989).
10. Muramatsu, H., Hanada, H., and Himeno, K. Effects of cadmium chloride and X-rays on chromosome aberration induction in bone marrow cells and spermatogonia of mice. In: *Radiobiological Equivalents of Chemical Pollutants*. International Atomic Energy Agency, Vienna, pp. 61–69.
11. Zherbin, E. A., Chukhlov, A. B., Köteles, G. J., Kubasova, T. A., Vaschenko, V. I., and Hanson, K. P. Effects *in vitro* of cadmium ions on some membrane and nuclear parameters of normal and irradiated thymic lymphoid cells. *Arch. Toxicol.* 59: 21–25 (1986).
12. Szirmai, S., Bérces, J., and Köteles, G. J. Computerized image analysis for determining micronucleus frequency. *Environ. Health Perspect.* 101(Suppl. 3): 57–61 (1993).