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Mechanisms involved in the induction of aneuploidy: the significance of chromosome loss*

A.I. Seoane, A.M. Güerci and F.N. Dulout

Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 60 y 118. CC 296. B1900AVW La Plata, Argentina. Send correspondence to F.N.D. E-mail: dulout@fcv.medvet.unlp.edu.ar

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Abstract

The induction of aneuploidy by physical and chemical agents using different test systems was evaluated. The effect of X-rays, caffeine, acetaldehyde, ethanol, diethylstilbestrol, propionaldehyde, and chloral hydrate was studied by chromosome counting in Chinese hamster embryonic diploid cells. Aneugenic ability of cadmium chloride, cadmium sulfate, potassium dichromate, chromium chloride, nickel chloride, and nickel sulfate was assessed by means of anaphase-telophase analysis in Chinese hamster ovary cells. Chromosome counting in human fibroblasts (MRC-5 cell line) was employed to evaluate the effect of cacodilic acid, cadmium chloride, cadmium sulfate, and potassium dichromate. Finally, the induction of kinetochore-positive and kinetochore negative micronuclei by cadmium chloride, cadmium sulfate, potassium dichromate, chromium chloride, and nickel chloride was studied using CREST antibodies. When the effect of different agents was determined by chromosome counting, an increase of hypoploid but not of hyperploid cells was observed. Anaphase-telophase analysis showed that metal salts increased the frequency of lagging chromosomes. This finding has been confirmed by the increment of kinetochore-positive micronuclei using CREST antibodies. Therefore, chromosome loss could be considered as the main cause of induced aneuploidy.

INTRODUCTION

Although aneuploidy is a serious health problem, the experimental methodology used to investigate the condition has not been completely validated. Development of a comprehensive test battery is necessary for the evaluation and detection of aneugenic chemicals. The reliability of any aneuploidy test is always challenged by the fact that the mechanisms involved in aneuploidy induction are poorly understood due, in part, to the multiple factors related with the occurrence of chromosome disjunction and nondisjunction.

Various test systems have been used to study chemical-induced aneuploidy. Chromosome counting in diploid cell lines (Danford, 1984, 1985; Dulout and Natarajan, 1987) was a validated test although it is time consuming. Fluorescence *in situ* hybridization with probes for entire chromosomes was recently used (van Diemen *et al.*, 1995; Dulout *et al.*, 1996; Natarajan *et al.*, 1996). Staining kinetochores in the cytokinesis-blocked micronucleus assay (Eastmond and Tucker, 1989; Lynch and Parry, 1993; Kirsch-Volders *et al.*, 1997; Thompson and Perry, 1988) or *in situ* hybridization with centromere specific DNA probes, followed by immunofluorescent staining (Eastmond and Pinkel, 1990; Farooqi *et al.*, 1993) are useful to discriminate between clastogens and aneuploidogens. Anaphase-telophase analysis, an ancilliary alternative test system (Nichols *et al.*, 1972; Dulout and Olivero, 1984) has been used to evaluate aneugenic damage by counting lagging chromosomes (Seoane and Dulout, 1994; Seoane, 1999).

In the present study, results obtained by chromosome counting, anaphase-telophase analysis and cytokinesis-blocked micronucleus assay were compared. Experiments were carried out to test the suspected aneugenic ability of different compounds or to validate the assay by studying known aneugenic chemicals. Chromosome counting reliability and usefulness was demostrated by Dulout and Natarajan (1987). The aneugenic ability of the synthetic estrogen diethylstilbestrol (DES), acetaldehyde, propionaldehyde, chloral hydrate, cadmium chloride, cadmium sulfate, potassium dichromate and cacodilic acid has been evaluated by employing this assay (Dulout and Natarajan, 1987; Dulout and Furnus, 1988; Furnus *et al.*, 1990, Güerci *et al.*, 2000). Anaphase-telophase analysis was employed to test the aneugenic ability of propionaldehyde, cadmium chloride, cadmium sulfate, potassium dichromate, chromium chloride (III), nickel chloride and nickel sulfate (Seoane and Dulout, 1994; Seoane, 1999). Cytokinesis-blocked micronucleus assay using anti-kinetochore antibodies was employed to evaluate the above mentioned heavy metal salts (Seoane, 1999).

MATERIAL AND METHODS

Chinese hamster embryo cells (CHED) and human diploid fibroblasts (MRC-5) were employed to carry out the chromosome counting assay. Chinese hamster ovary cells were employed in the anaphase-telophase test. MRC-5 cells were used in the cytokinesis-blocked micronucleus assay. Experimental procedures, compound dilutions and doses assayed have been described in previous papers (Dulout and Furnus, 1988; Furnus *et al.*, 1990; Seoane and Dulout, 1994; Seoane and Dulout, 1999; Seoane, 1999; Güerci *et al.*, 2000)

RESULTS

Irradiation increased the frequency of aneuploid cells with a corresponding decrease of diploid cells, except for the dose of 100 rad (Figure 1). Pulse treatments with different doses of DES also increased the frequency of aneuploid cells in relation with the dose employed but not with the duration of the treatment.

In cells treated with acetaldehyde, ethanol and DES the frequency of aneuploid cells increased significantly compared to controls (Figures 2,3). In addition, the frequency of aneuploid cells observed in the three treatments with acetaldehyde was higher than that detected in cells treated with DES. The increase of aneuploid metaphases was mainly due to the increment of hypoploid cells. Whereas the relation between hypoploid/hyperploid cells was 0.81 in the controls, in treated cells this relation varied from 2.72 to 3.82.

In the treatments with propionaldehyde and chloral hydrate a significant increase of aneuploid cells compared to untreated controls was observed (Figure 4). Although the frequencies of hypoploid cells were higher than the frequencies of hyperploid cells in all treatments, the differences were not significant.

An increase of lagging chromosomes was found in cells treated with cadmium salts, although significant differences from untreated controls were only found with the highest dose of cadmium sulfate. Whereas potassium dichromate increased the frequencies of lagging chromosomes at all the doses employed, only the two highest doses of chromium chloride gave positive results. Increments of lagging chromosome frequencies induced by nickel salts were lower than those induced by the other heavy metal salts. However, significant differences from controls were found only with the two highest doses (Figure 5).

Results of chromosome counting using MRC-5 cells (Figure 6) showed significant increments of aneuploidy in cacodilic acid- and cadmium chloride-treated cells. Cadmium sulfate induced significant increases at the two highest doses. Potassium dichromate induced significant increments of aneuploidy at all doses tested although at a lower degree than the other compounds.

Cadmium salts induced significant increases of kinetochore-positive and kinetochore-negative micronuclei when the cells were treated with the two highest concentrations of each compound. The lowest doses induced borderline increases in the frequency of kinetochore-positive micronuclei but no differences with respect to control values were found in the frequency of kinetochore-negative micronuclei. The highest increments of total micronuclei frequency were induced by chromium chloride. Most of these micronuclei were kinetochore-positive. Potassium dichromate also induced higher increases of kinetochore-positive micronuclei than kinetochore-negative ones. Nickel salts induced statistically significant increases of kinetochore-positive micronuclei with the three doses employed. On the other hand, increases in kinetochore negative micronucleus frequency were only slight (Figure 7).

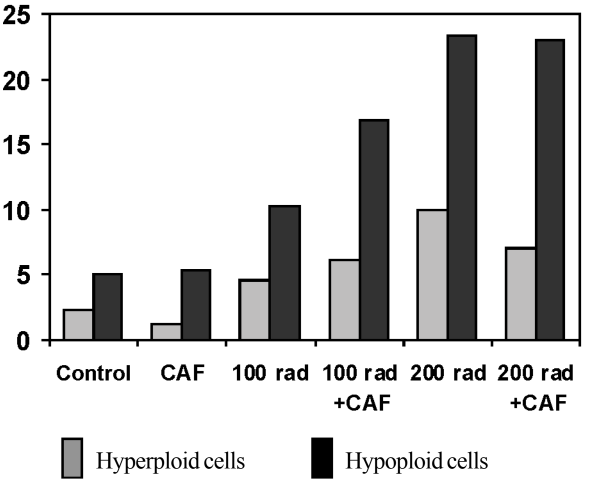

Figure 1 - Frequencies of hyperploid and hypoploid metaphases in CHED cells treated with X-rays (100 and 200 rad) and caffeine (CAF) (200 μg/ml).

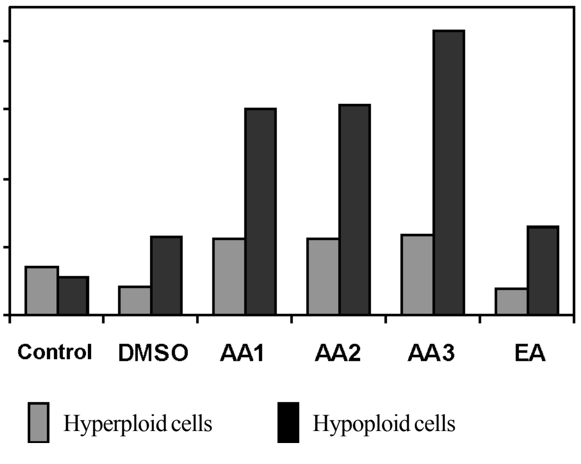

Figure 2 - Frequencies of hyperploid and hypoploid metaphases in CHED cells treated with acetaldehyde doses of 0.002% (AA1), 0.004% (AA2), and 0.006% (AA3) and 1% ethanol (EA).

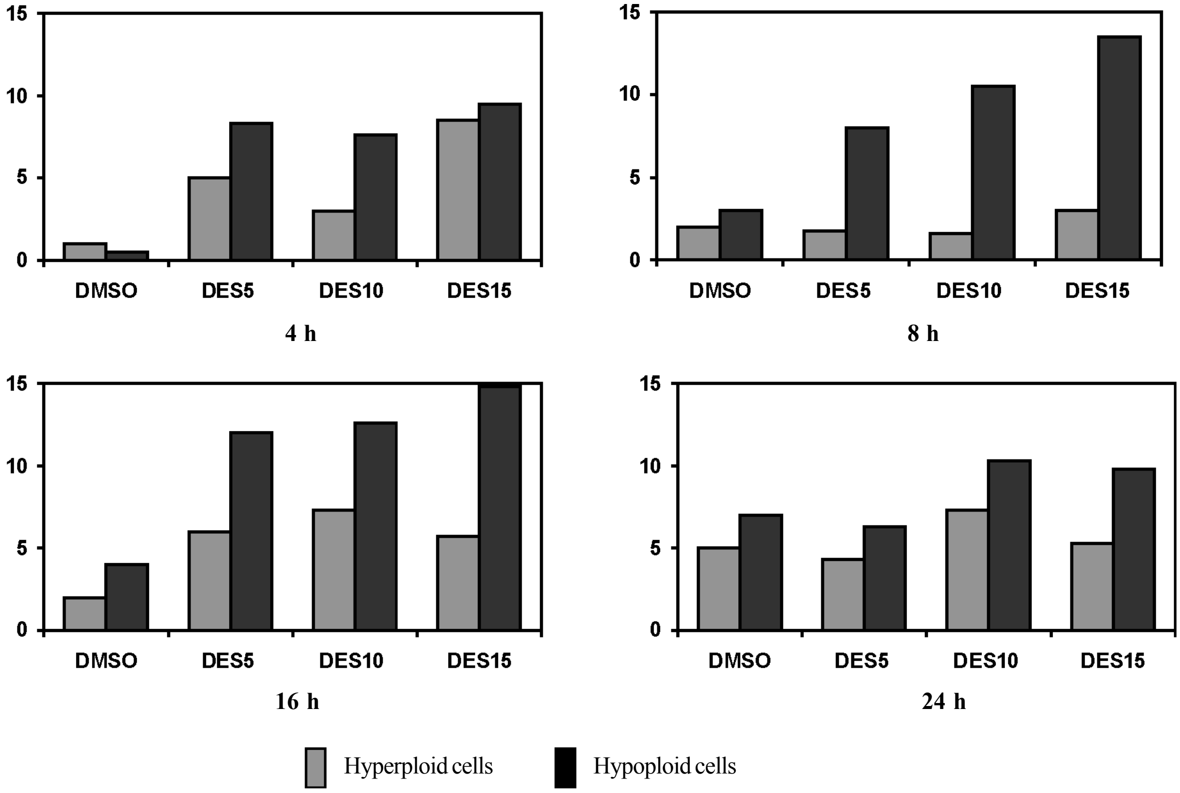

Figure 3 - Percentages of hypoploid and hyperploid CHED cells after treatment with diethylstilbestrol (DES) doses of 5, 10, and 15 µg/ml for different periods.

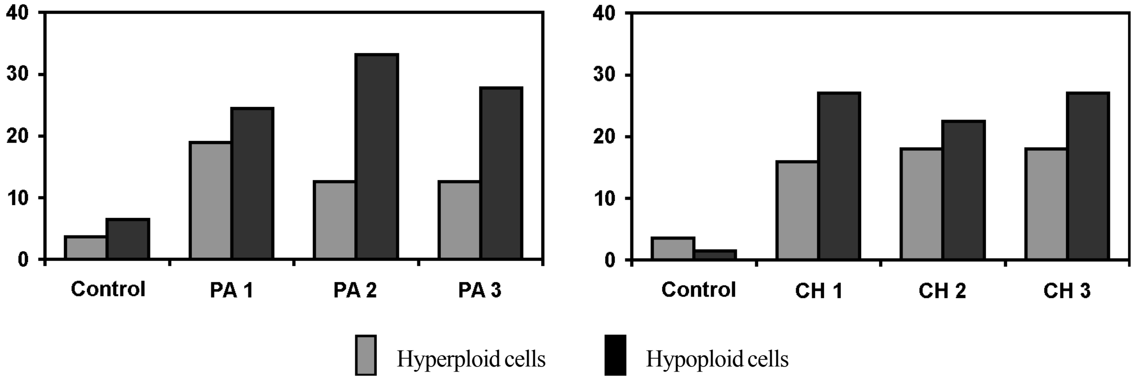

Figure 4 - Percentages of hypoploid and hyperploid CHED cells after treatment with propionaldehyde doses of 5 x 10^{-4}% (PA1), 1 x 10^{-3}% (PA2) and 2 x 10^{-3}% (PA3) for 3 h and chloral hydrate doses of 1 x 10^{-3}% (CH1), 2 x 10^{-3}% (CH2) and 3 x 10^{-3}% (CH3) for 1.5 h.

DISCUSSION

When a normal disjunction occurs in a mitotic division, the chromatids of a chromosome segregate to each cellular pole. Chromosomal missegregation can be produced by: 1) multipolar mitoses arisen from centriole alterations, 2) nondisjunction, when the chromatids of a chromosome do not separate correctly and the entire chromosome migrates to one pole, 3) chromosome loss, when a chromosome (or a chromatid) remains lagged at the equator and does not migrate to the corresponding pole.

If multipolar mitosis occurs, multinucleated cells with hypoploid nuclei are formed. Nondisjunction originates two aneuploid cells, one hypoploid and the other hyperploid. On the other hand, lagging chromosomes at mitosis produce two hypoploid daughter cells. Lagging chromatids originate a diploid and a hypoploid cell. In both cases

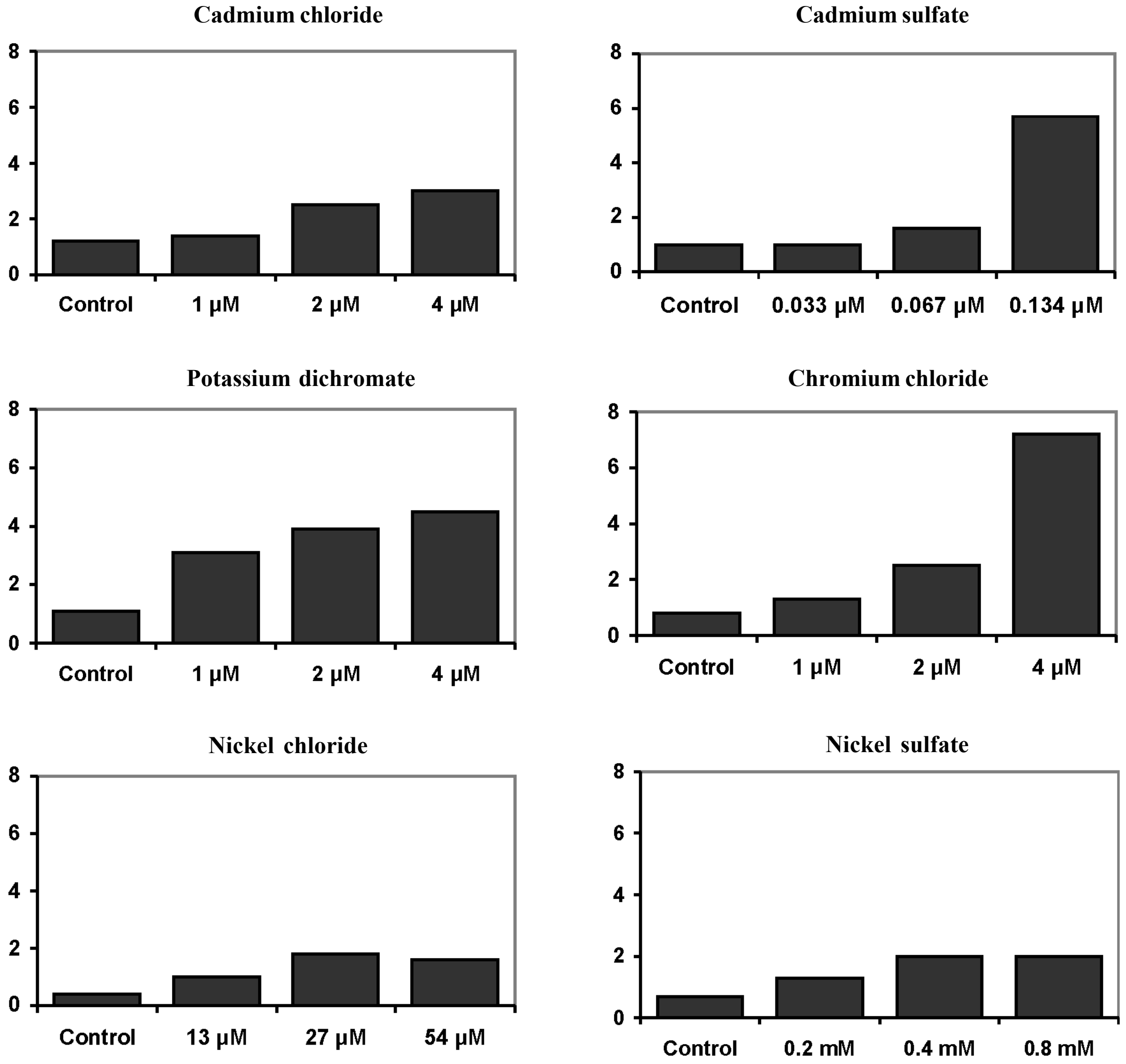

Figure 5 - Frequencies of lagging chromosomes induced by different metal salts in anaphase-telophase of CHO cells.

micronuclei containing either the lagged chromosome or the lagged chromatid are formed.

When cells were analyzed by chromosome counting, higher frequencies of hypoploid than hyperploid cells were found. These results seem to be in conflict with the belief that aneuploidy is a result of the induction of equal numbers of these two kinds of cells. Nevertheless, other evidence indicated that the increase of hypoploid cells is not a result of chromosome loss caused by technical factors: a) the chromosome counts were only made in metaphases surrounded by cytoplasm, as an indication of the integrity of the plasma membrane; b) the average chromosome number per cell in the different treatments was constant (data not shown); c) as the frequency of aneuploid cells increased, a correlative increment of multinucleated interphase cells was observed in the cultures treated with the chemicals. When the frequencies of hypoploid cells were compared with the frequency of hyperploidy plus the frequency of multinucleated cells a positive correlation and a good fit with the regression line were found.

The kinetochore-stained micronucleus test is based upon the assumption that a micronucleus containing a kinetochore presumably contains the centromere and the entire chromosome. This micronucleus will segregate with only one of the daughter cells but both have a high probability of being aneuploid (Thompson and Perry, 1988; Eastmond and Tucker, 1989). It must be considered that only malsegregation events (when a chromosome or a chromatid fails to migrate correctly and remains at the metaphase plate) could be detected in the cytokinesis-blocked micronucleus assay by using CREST antibodies. When nondisjunction occurs, two aneuploid daughter cells are formed (one hypoploid and one hyperploid), but neither lagging chromosomes nor micronuclei are induced if the entire chromosome migrates correctly to one pole. Positive-kinetochore micronuclei are formed only when a chromo-

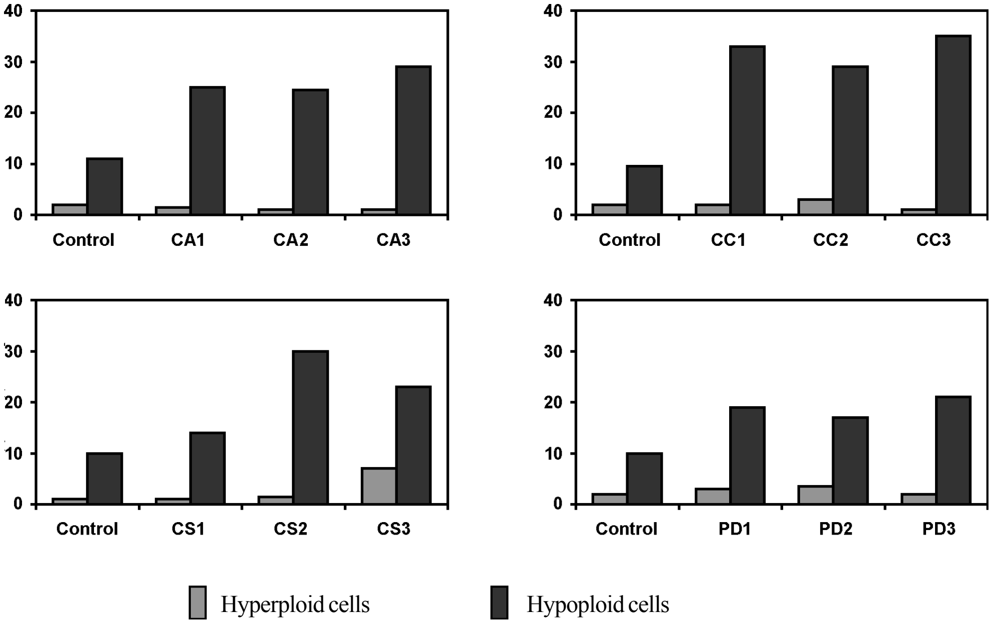

Figure 6 - Percentages of hypoploid and hyperploid MRC-5 cells after treatment with cacodilic acid doses of 1.25 x 10^{-2} mM (CA1), 2.5 x 10^{-2} mM (CA2) and 5.0 x 10^{-2} mM (CA3); cadmium chloride doses of 1.0 x 10^{-3} mM (CC1), 2.0 x 10^{-3} mM (CC2) and 4.0 x 10^{-3} mM (CC3); cadmium sulfate doses of 3.3 x 10^{-5} mM (CS1), 6.7 x 10^{-5} mM (CS2) and 1.3 x 10^{-4} mM (CS3), and potassium dichromate doses of 2.5 x 10^{-4} mM (PD1), 5.0 x 10^{-4} mM (PD2) and 1.0 x 10^{-3} mM (PD3).

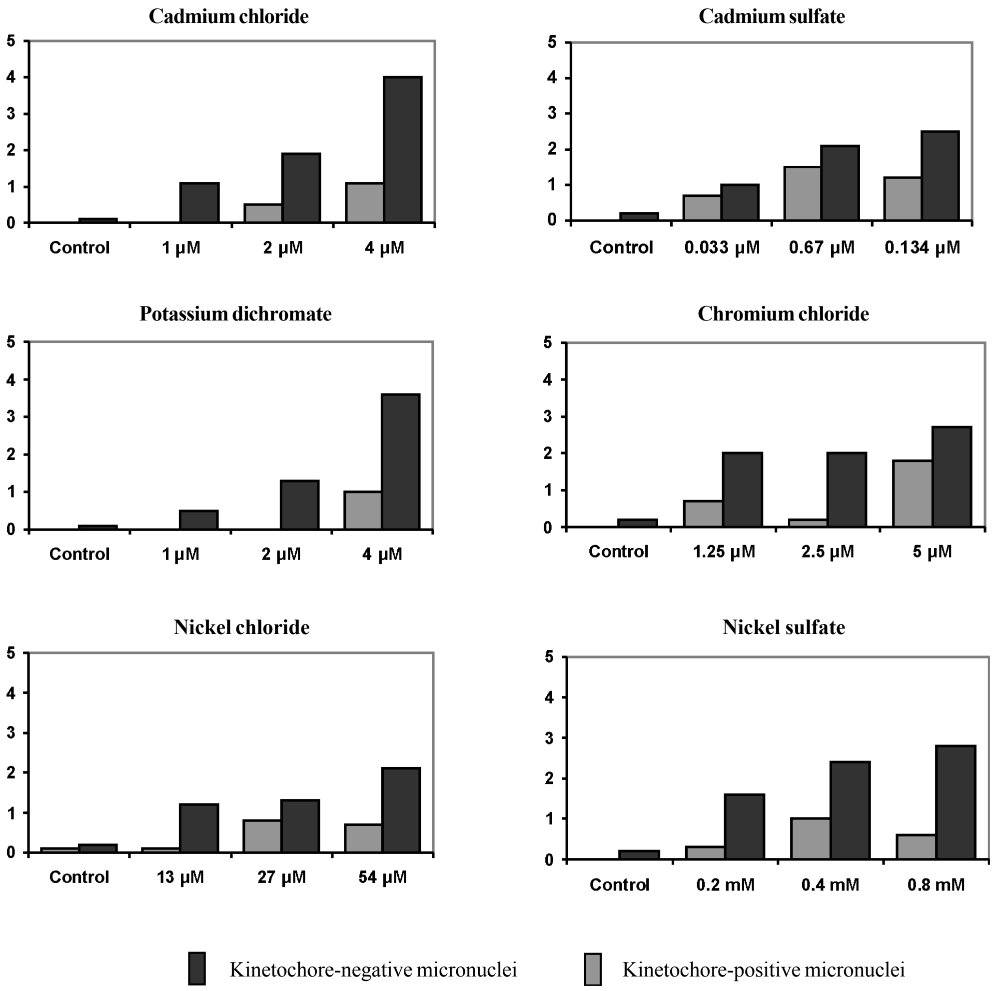

Figure 7 - Frequencies of kinetochore-negative and kinetochore-positive micronuclei in MRC-5 cells detected by using CREST antibodies.

some (or chromatid) remains lagged as a consequence of segregation errors (Ford and Correl, 1991; Van Hummelen *et al.*, 1992). Neither lagging chromosomes nor positive kinetochore micronuclei are indicative of nondisjunction (Lynch and Parry, 1993). These considerations are in agreement with findings obtained by chromosome counting and anaphase-telophase analysis. Recently, Natarajan and coworkers (1993) found that hypoploidy was induced more frequently than hyperploidy in cadmium chloride-treated CHED cells. Similar results were reported by Warr and coworkers (1993) in LUC2 cells treated with other aneuploidy-inducing agents. Taking into account these facts, it appears that nondisjunction is not the main mechanism in the generation of aneuploidy and it can be proposed that malsegregation is at least partially involved.

RESUMO

A indução de aneuploidia por agentes físicos e químicos usando diferentes sistemas de teste foi avaliada. O efeito de raios-X, cafeína, acetaldeído, etanol, dietilestilbestrol, propionaldeído e hidrato de cloral foi estudado por contagem cromossômica em células diplóides embriônicas de hamster chinês. A habilidade aneugênica de cloreto de cádmio, sulfato de cádmio, dicromato de potássio, cloreto de crômio, cloreto de níquel e sulfato de níquel foi avaliada por meio de análise de anáfase-telófase em células de ovário de hamster chinês. A contagem cromossômica em fibroblastos humanos (linhagem celular MRC-5) foi empregada para avaliar o efeito de ácido cacodílico, cloreto de cádmio, sulfato de cádmio e dicromato de potássio. Finalmente, a indução de micronúcleos positivos e negativos para cinetocoro por cloreto de cádmio, sulfato de cádmio, dicromato de potássio, cloreto de crômio e cloreto de níquel foi estudada usando anticorpos CREST. Quando o efeito de agentes diferentes foi determinado por contagem cromossômica, observou-se um aumento de células hipoplóides mas não de hiperplóides. A análise anáfase-telófase mostrou que sais metálicos aumentaram a freqüência de cromossomos “lagging”. Este achado foi confirmado pelo aumento de micronúcleos positivos para cinetocoro usando anticorpos CREST. Portanto, a perda cromossômica poderia ser considerada a principal causa de aneuploidia induzida.

REFERENCES

Danford, N. (1984). Measurement of levels of aneuploidy in mammalian cells using a modified hypotonic treatment. *Mutat. Res. 139*: 127-132.

Danford, N. (1985). Test for chromosome aberration and aneuploidy in Chinese hamster fibroblast cell line CH1-L. In: *Progress in Mutation Research* (Ashby, J., De Serres, F.J., Draoer, M., Ishidate Jr., M., Margolin, B.H., Matter, B.E. and Shelby, M.D., eds.). Vol. 5. Elsevier, Amsterdam, pp. 397-411.

Dulout, F.N. and **Furnus, C.** (1988). Acetaldehyde-induced aneuploidy in cultured Chinese hamster cells. *Mutagenesis 3*: 207- 211.

Dulout, F.N. and **Natarajan, A.T.** (1987). A simple and reliable *in vitro* test system for the analysis of induced aneuploidy as well as other cytogenetic end-points using Chinese hasmter cells. *Mutagenesis 2*: 121-126.

Dulout, F.N. and **Olivero, O.A.** (1984). Anaphase-telophase analysis of chromosomal damage induced by chemicals. *Environ. Mutagen. 6*: 299-310.

Dulout, F.N., Grillo, C.A., Seoane, A.I., Maderna, C.R., Nilsson, R., Vather, M., Darroudi, F. and **Natarajan, A.T.** (1996). Chromosomal aberrations in peripheral blood lymphocytes from Andean women and children from northwestern Argentina exposed to arsenic in drinking water. *Mutat. Res. 370*: 151-158.

Eastmond, D. and **Pinkel, D.** (1990). Detection of aneuploidy inducing agents in human lymphocytes using *in situ* hybridization with chromosome specific DNA-probes. *Mutat. Res. 234*: 303-318.

Eastmond, D. and **Tucker, J.** (1989). Identification of aneuploidy inducing agents using cytokinesis-blocked human lymphocytes and an antikinetochore antibody. *Environ. Mol. Mutagen. 13*: 34-43.

Farooqi, Z., Darroudi, F. and **Natarajan, A.T.** (1993). The use of fluorescence *in situ* hybridization for the detection of aneugens in cytokinesis-blocked mouse splenocytes. *Mutagenesis 8*: 329-334.

Ford, J. and **Correl, A.** (1991). Chromosome errors at mitotic anaphase. *Genome 35*: 702-705.

Furnus, C., Ulrich, M.A., Terreros, M.C. and **Dulout, F.N.** (1990). The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate. *Mutagenesis 5*: 323-326.

Güerci, A.M., Seoane, A.I. and **Dulout, F.N.** (2000). Aneugenic effects of some metal compounds assessed by chromosome counting in MRC-5 human cells. *Mutat. Res.* (in press).

Kirsch-Volders, M., Elhajouji, A., Cundari, E. and **Van Hummelen, P.** (1997). The *in vitro* micronucleus test: a multi-endpoint assay to detect simultaneously mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction. *Mutat. Res. 392*: 19-30.

Lynch, A. and **Parry, J.** (1993). The cytochalasin-B micronucleus/kinetochore assay *in vitro*: Studies with 10 suspected aneugens. *Mutat. Res. 287*: 71-86.

Natarajan, A., Duivenvoorden, W., Meijers, M. and **Zwanenburg, T.** (1993). Induction of mitotic aneuploidy using Chinese hamster primary embryonic cells. Test results of 10 chemicals. *Mutat. Res. 287*: 47-56.

Natarajan, A.T., Boei, J.J.W.A., Darroudi, F., Van Diemen, P.C.M., Dulout, F.N., Hande, M.P. and **Ramalho, A.T.** (1996). Current cytogenetic methods for detecting exposures and effects of mutagens and carcinogens. *Environ. Health Perspect. 104* (Suppl. 3): 445-448.

Nichols, W.W., Moorehead, P. and **Brewen, G.** (1972). Chromosome methodologies in mutation testing. Report of the Ad-Hoc Committee on the Environmental Mutagen Society and the Institute for Medical Research. *Toxicol. Appl. Pharmacol. 22*: 269-275.

Seoane, A.I. (1999). Contaminación ambiental por metales pesados: evaluación del efecto genotóxico. Doctoral thesis, Faculty of Natural Sciences, National University of La Plata, La Plata.

Seoane, A.I. and **Dulout, F.N.** (1994). Use of anaphase-telophase test to detect aneugenic compounds: effects of propionaldehyde and cadmium chloride. *Bull. Environ. Contam. Toxicol. 53*: 924-929.

Thompson, E. and **Perry, P.** (1988). The identification of micronucleated chromosomes; a possible assay for aneuploidy. *Mutagenesis 3*: 415-418.

van Diemen, P.C.M., Maasdam, D., Vermeulen, S., Darroudi, F. and **Natarajan, A.T.** (1995). Influence of smoking habits on the frequencies of structural and numerical chromosome aberrations in human peripheral blood lymphocytes using the fluorescence *in situ* hybridization (FISH) technique. *Mutagenesis 10*: 487-495.

Van Hummelen, P., Deleener, A., Vanparys, Ph. and **Kirshc-Volders, M.** (1992). Discrimination of aneuploidogens from clastogens by C-banding, DNA and area measurements of micronuclei from mouse bone marrow. *Mutat. Res. 271*: 13- 28.

Warr, T., Parry, E. and **Parry, J.M.** (1993). A comparison of two *in vitro* mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens. *Mutat. Res. 287*: 29-46.

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