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Use of the Anaphase-Telophase Test To Detect Aneugenic Compounds: Effects of Propionaldehyde and Cadmium Chloride

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Aneuploidy is a numerical chromosomal disorder originated from non-disjunction during cell division (Dellarco 1985). Aneuploid cells exhibit altered chromosome numbers varying from nulisomy (absence of a chromosome pair), monosomy (only one member of a chromosome pair), trisomy (three homologous chromosomes instead of two), tetrasomy (four homologous chromosomes instead of two), etc. In an aneuploid complement, one or more chromosome pairs can be involved.

In human beings aneuploidy is the main cause of pregnant loss, congenital malformation and mental retard (Hook 1983; Epstein 1988). Although the consequences of meiotic non-disjunction are clear, the relation between mitotic non-disjunction and neoplastic transformation susbsists uncertain despite there is increased evidence indicating that it is a significant factor during somatic malignancy development (Kondo 1984; Tsutsui 1983; Cavenee 1983). In addition, aneuploidy is frequently associated with infertility and abortion in domestic livestock (Halnan 1989).

Whereas DNA is the target molecule for the induction of point mutations and chromosome aberrations, several cellular components can be involved in the induction of aneuploidy. Thus, aneuploidy can arise from spindle disorders or from alterations at kinetochore level. Chromosomal missegregation can be produced: 1) when both sister chromatids migrate to the same pole after separation; 2) when one or more chromosomes, with or without separation of sister chromatids, do not migrate to the poles remaining in the equator. In the first case two aneuploid cells (one hypodiploid and the other hyperdiploid) will be originated (Rieder and Alexander 1989). In the second case, lagging chromosomes or chromatids will appear as micronuclei in the cytoplasm of interphase cells, similar to those derived from chromosomal fragments (Dulout and Furnus 1988; Van Hummelen et al. 1992). In this sense, it has been shown that acetaldehyde increases the frequency of hypodiploid cells in

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the second mitosis after treatment with a correlated increase of interphase micronucleated cells (Dulout and Furnus 1988).
The anaphase-telophase test has been used as an ancillary test system to analyse the induction of chromosomal damage in cultured cells. Chromosomal alterations that can not be detected in metaphase plates using routine procedures can be observed during anaphase-telophase (chromatin bridges originated from entanglement of chromatin fibers or exchange-type aberrations, multipolar mitoses arisen from alterations of the mitotic spindle, lagging fragments derived from chromosome breaks, and lagging chromosomes) (Dulout and Olivero 1984). As lagging chromosomes can be easily detected in the anaphase-telophase test, the method has been used to check their reliability for the study of aneuploidy. Propionaldehyde (PA), a well known aneugenic compound (Furnus et al. 1990), and cadmium chloride (CC) were used in this experiment. Cadmium is a metallic toxicant of great environmental and occupational concern. A potent carcinogen in animals, Cadmium is also suspected human carcinogen (Waalkes 1990). Selypes and coworkers (1992) reported the presence of hypo and hyperdiploid, triploid and tetraploid meiosis in CC ***in vivo*** treated mice. Lakkad and coworkers (1986) found various mitotic abnormalities like micronucleus formation, lagging chromosomes, chromatid bridges, etc. in CC ***in vitro*** treated CHO cells.

MATERIAL AND METHODS

Chinese hamster ovary (CHO) cells obtained from the American Type Collection were used in the experiment. Cells were cultured in Ham F10 medium (Gibco) supplemented with 10% fetal bovine serum and antibiotics (penicillin and streptomycin),

PA was purchased from Carlo Erba and CC was obtained from Sigma Chemical Company (Saint Louis, Mo.). PA was dissolved in Dimethyl Sulfoxide (DMSO) and CC in bidistilled water. Solutions were made in order to add 100 µL to each culture and final concentrations were 0.25×10^{-5}%, 0.5×10^{-5}%, and 0.75×10^{-5}% of PA and 0.5×10^{-7}, 1×10^{-6}%, 2×10^{-6}%, and 4×10^{-6}% of CC. Untreated cultures and DMSO-treated cultures were used as controls.

Cells were cultured as monolayer in 24 x 36 mm cover glasses attached with a small drop of siliconized grease to the bottom of 90 mm Petri dishes. Three cover glasses were placed in each Petri dish. Each cover glass was seeded with 1.5 mL of culture medium containing about 50,000 cells. After one hour 8.5 mL of culture medium was added to each Petri dish. Cultures were incubated al 37°C in a humidified atmosphere of 5% CO_2. Treatments were performed during the logarithmic growth phase. The set of cultures for each experiment was treated simultaneously 8 hours before cell harvesting. To avoid the detachment of cells from cover slides, cell harvesting was acomplished by adding an equal volume of fixative (methanol-acetic acid 3:1) to the culture medium. After 10 min, two changes of fixative were made. Coverglasses were stained with carbol fuchsin and attached with DPX mountant medium to coded slides. Each treatment was performed in cuadruplicate.

Coded slides were analysed by one investigator. A total of 100 cells per cover glass were scored. To avoid the erroneous scoring of chromatin bridges and lagging chromosomes or fragments cells were analysed at late anaphase-early telophase. In early or middle anaphase, it is not possible to distinguish chromosome arms from chromatin bridges and lagging chromosomes or fragments could be masked by chromosome arms. The frequency of cells with the following alterations were scored: 1) chromatin bridges; 2) lagging chromosomes and 3) lagging chromosomal fragments. The mitotic index (MI) was established in each experiment by determining the number of mitotic cells per 1000 cells on each cover slide. The MI was expressed as the percentage of mitotic plates.

Statistical comparisons were made by means of the Sokal and Rohlf method (Sokal 1979). Regression analyses were performed to evaluate the mitotic index variations.

RESULTS AND DISCUSSION

Table 1 shows the results obtained. A significant increase of lagging chromosomes was found in cells treated with PA. However, only the treatments with the highest dose showed statistical significant differences in relation with the untreated controls ($G = 4.21$, $p < 0.05$). Comparing the frequencies of chromatin bridges and lagging fragments in PA- and untreated cultures the differences were also not significant. The effect of CC was evidenced by an increase of chromatin bridges, lagging chromosomes and lagging fragments with the dose of 4×10^{-6}% ($G = 12.15$, $p < 0.025$; $G = 8.45$, $p < 0.01$ and $G = 8.95$, $p < 0.01$ respectively). In addition, the dose of 1×10^{-6}% induced a significant increase of lagging fragments ($G = 5.55$, $p < 0.025$). The dose-response relationships were established by means of the correlation analysis. In PA-treated cells, only the frequencies of lagging chromosomes were correlated with the doses employed ($r = 0.90$, $p < 0.05$). In CC-treated cultures the frequencies of chromatin bridges, lagging chromosomes and lagging fragments were correlated with the doses ($r = 0.99$, $p < 0.01$; $r = 0.94$, $p < 0.05$ and $r = 0.92$, $p < 0.05$ respectively). The cytotoxicity of both compounds tested was established by scoring the mitotic index. In PA-treated cultures no correlation was found between the mitotic index and the doses employed ($r = 0.53$, $p > 0.1$). On the other hand, a highly significant negative correlation ($r = -0.96$, $p < 0.01$) was found between the different CC doses and the frequency of mitotic cells.

Chromosomal alterations observed in anaphase-telophase cells are produced by different events: chromatin bridges are originated from entanglement of chromatin fibers or exchange-type aberrations, multipolar mitoses are the consequence of mitotic spindle alterations, lagging fragments derived from chromosome breaks (Dulout and Olivero 1984), and lagging chromosomes are presumably produced by alterations at kinetochore level. Assuming that the increased frequency of lagging chromosomes in anaphase-telophase cells could indicate the aneugenic ability of PA, data obtained are in agreement with previous results. In a recent paper we demonstrated the induction of aneuploidy by PA in Chinese hamster primary cultures by chromosome counts in the second mitosis after treatment. The cytogenetic analysis of these cells

Table 1 Anaphase-telophase alterations in CHO cells treated with propionaldehyde (PA) or cadmium chloride (CC).

Treatment	Cells scored	Anaphase-telophase alterations per 100 cells CB(1)	LC(2)	LF(3)	MI(4)
Untreated control	1,000	0.40 (0.06)(5)	0.40 (0.06)	0.10 (0.03)	2.170
PA $0.25x10^{-5}$%	1,000	0.80 (0.08)	1.00 (0.09)	0.40 (0.06)	2.400
PA $0.50x10^{-5}$%	1,000	0.30 (0.05)	1.10 (0.10)	0.20 (0.04)	2.170
PA $0.75x10^{-5}$%	1,000	0.70 (0.08)	1.20* (0.10)	0.20 (0.04)	2.050
Untreated control	900	0.66 (0.08)	1.33 (0.11)	0.66 (0.08)	2.975
CC $0.5x10^{-7}$%	900	1.00 (0.09)	1.44 (0.11)	1.44 (0.11)	2.635
CC $1.0x10^{-6}$%	900	1.33 (0.11)	1.55 (0.12)	1.88* (0.13)	2.161
CC $2.0x10^{-6}$%	900	1.77 (0.14)	1.11 (0.10)	1.44 (0.11)	2.110
CC $4.0x10^{-6}$%	900	2.44* (0.15)	3.33* (0.17)	2.33* (0.15)	1.400

(1) Chromatin bridges
(2) Lagging chromosomes
(3) Lagging fragments
(4) Mitotic index
(5) Standard error of the mean is indicated between parentheses below the corresponding figures
(*) Significant difference from control

also showed the ability of PA to induce chromosomal aberrations, including chromosome breaks (Furnus et al. 1990). Accordingly, the chromosome-breaking ability of PA must have been evidenced in anaphase-telophase analysis by an increase of lagging fragments. However, no differences between the frequencies of lagging fragments in PA-treated cells and untreated controls were found. This apparent discrepancy could be due to the fact that the doses of PA employed for

anaphase-telophase analysis varied from 3.75 to 5% of those employed with Chinese hamster primary cultures. These lower doses were selected because the higher sensitivity of CHO cells and the results obtained could be considered as an indication that the doses employed were around a threshold effect level.

The effect of CC was evidenced by an increase of chromatin bridges, lagging chromosomes and lagging fragments. However, statistical significant differences with controls were observed only in cells treated with the higher doses. On the other hand, treatment with CC but not with PA induced a decrease of the mitotic index correlated with the doses. These results indicated that CC is able to induce not only chromosomal damage that can be detected in metaphase, but has also aneugenic properties.

Finally, results obtained can be considered as an indication that the analysis of alterations in anaphase-telophase of cultured cells could be considered as a useful test to evaluate the aneuploidogenic ability of chemicals. Recently, a new assay has been developed using fluorescence stain to identify kinetochore protein or centromere DNA for aneuploidy (Thomson and Perry 1988; Eastmond and Tucker 1989). However, the anaphase-telophase test has several advantages such as: 1) very low cost and short time consumption; 2) it can be developed using established cell lines like CHO instead of diploid primary cell cultures required to analyse aneuploidy by chromosome counts; 3) it allows a simultaneous qualitative and quantitative study of other chromosomal alterations.

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