Cisplatin-induced Cytogenetic Alterations in V79 Cells

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Summary  Cisplatin is an antineoplastic agent used to treat solid malignancies, such as ovarian, testicular and bladder tumors. Studies have been shown that cisplatin induces genotoxic effects and chromosomal alterations that can result in genetic and chromosomal instability. In this work, we investigated the effects of cisplatin on the cytogenetic traits of cultured V79 cells based on the modal chromosome number, mitotic index, frequency of polyploidy and number of aneuploid metaphases. Cisplatin-treated V79 cells showed an altered chromosome number distribution, as well as an enhanced mitotic index, frequency of polyploidy and number of aneuploid metaphases. These alterations were probably related to the genetic instability produced by cisplatin. In addition, these cells showed characteristics associated with neoplastic development that corresponded to neoplastic processes related to the use of cisplatin in chemotherapy.

Key words  Cisplatin, Cytogenetic analyses, V79 cells.

The antineoplastic activity of cisplatin was described in the late 1960s (Rosenberg et al. 1969) and resulted in its widespread use as a chemotherapeutic agent to treat a wide variety of neoplasias. Cisplatin is highly effective in the treatment of several types of tumors, including testicular, ovarian and bladder carcinomas, as well as head and neck, uterine cervix, esophageal and lung cancers (Giacone 2000). Since the introduction of cisplatin in oncological practice, there have been considerable advances in our understanding of the molecular mechanism of action of this drug’s antitumoral effects. The biochemical mechanism of cisplatin cytotoxicity involves the binding of cisplatin to DNA and other cell components, with the subsequent induction of cell death by apoptosis, necrosis or both of these mechanisms (González et al. 2001).

However, cisplatin can cause genotoxic effects, chromosomal alterations, and mutations (Srb and Procházková 1983, Tandon and Sodhi 1985, Turnbull et al. 1979, Wiencke et al. 1979). These findings, and the observation that patients who survive cancer frequently develop a second malignancy after chemotherapy, suggest that cisplatin can exert a tumorigenic effect after its use in chemotherapy (Chambers et al. 1989, Mead et al. 1983, Van Imhoff et al. 1986). In view of this evidence that cisplatin can induce carcinogenesis in animal cells, tissues and organs, it is important to understand the effects of this drug in different models.

V79 cells, a diploid fibroblast line obtained from Chinese hamster (Cricetulus griseus) lung cells, is an excellent model for cytogenetic assays of its very stable karyotype and cytogenetic characteristics (Bradley et al. 1981). Although this cell line has been immortalized, the cells show a stable chromosome number, and a uniform size, shape and growth pattern. This stability and the very well defined properties of V79 cells mean that subtle alterations induced by chemicals are easily de-
tected as cytogenetically.

In this work, we investigated the cytogenetic traits of cisplatin-treated V79 cells by determining the modal chromosome number, the mitotic index, the frequency of polyploidy and the number of aneuploid metaphases compared to control V79 cells.

Materials and methods

Control culture conditions

Chinese hamster V79 fibroblast cells obtained from the Adolfo Lutz Institute (São Paulo, SP, Brazil) at passage number 54 were maintained in control culture conditions in Ham F10 medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal calf serum (FCS-Nutri-cell Nutrientes Celulares, Campinas, SP, Brazil), at 37°C.

Treatment with cisplatin

Monolayers of V79 cells grown in culture flasks were treated with 50 μg of cisplatin (Sigma)/ml, prepared in complete medium at the desired concentration (Allavena et al. 1990). After 24 h, the culture medium containing cisplatin was removed and the treated cells were then maintained in control culture conditions (without cisplatin) during 10 successive subcultures. This assay was done in triplicate using non-treated V79 cells as a control.

Cytogenetic analysis

Control and cisplatin-treated V79 cells were arrested in metaphase by adding 16 μg of colchicine (Sigma)/ml followed by incubation for 4 h at 37°C. The cells were then harvested using trypsin-EDTA (Nutricell) and isolated by centrifugation at 1000 rpm for 10 min. Chromosomal preparations were obtained by swelling the cells in 0.075 M KCl (Merck KgaA, Darmstadt, Germany) followed by fixation in methanol : acetic acid (3 : 1, v/v) (Merck). Slides were prepared according to standard techniques and stained with 5% Giemsa (Sigma) solution.

The modal chromosome number was determined by counting the chromosomes in 100 metaphases of intact control and cisplatin-treated V79 cells (Genari and Wada 2003), in a range of 18–23 chromosomes. The mitotic index (MI) and polyploidy frequency (PF) were determined according to Deitch and Sawicki (1979) and Gilvarry et al. (1990), respectively. The MI was calculated by counting the metaphases in 1000 cells and then dividing the number of metaphases by the total number of cells analyzed (MI [%]=number of metaphases/1000×100). The PF was determined by counting the polyploid metaphases (number of chromosomes ≥42) in 1000 metaphases and then dividing the number of polyploid metaphases by the total number of metaphases analyzed (PF [%]=number of polyploid metaphases/1000×100). The number of aneuploid cells was determined by counting the aneuploid metaphases (range of 24–41 chromosomes) in 1000 metaphases. The chromosome number distribution in aneuploid cells was determined by counting the chromosomes in 33 aneuploid metaphases of cisplatin-treated V79 cells.

Results and discussion

Cisplatin-treated V79 cells showed an altered chromosome number distribution: 59% of control V79 cells had 21 chromosomes (range of 18–23, n=100), whereas 42% of cisplatin-treated V79 had 21 chromosomes and 38% had 20 chromosomes (range of 18–23, n=100) (Fig. 1). Representative metaphases of control and cisplatin-treated V79 cells are shown in Fig. 2A and 2B, respectively.

The normal diploid chromosome number in V79 cells is 22, with a range of 20–23 chromosomes, according to the American Type Culture Collection. In this study, control and cisplatin-treat-
ed V79 cells had a modal chromosome number of 21, although 38% of the cisplatin-treated V79 cells had 20 chromosomes, indicative of genetic instability when compared to control V79 cells. Compounds that induce genetic or chromosomal instability in cells can produce multiple genetic alterations. Various studies have indicated that genetic or chromosomal instability induced by radiation or chemical compounds is an important mechanism in carcinogenesis (Chang and Little 1992, Harper et al. 1997, Little 1999).

Although it is not clear how and when the cisplatin-treated V79 cells acquired this instability, it is likely that the alteration occurred immediately after treatment and that it persisted for many generations. The induction of genetic or chromosomal instability may be a common pathway to carcinogenesis, but little is understood of the underlying mechanisms. One possible mechanism is that a mutation in a gene or genes that contribute to genomic or chromosomal stability is responsible for the change in phenotype (Ohshima 2003).

The mitotic index (MI) and polyploidy frequency (PF) were enhanced in cisplatin-treated V79 cells. The MI was 18.1% in control V79 cells and 22.8% in cisplatin-treated V79 cells. The higher
MI in cisplatin-treated V79 cells was related to altered cell proliferation and growth in culture, both of which are characteristic of cells transformed in vitro (Genari et al. 1996, Genari and Wada 1995, Hadnagy and Seemayer 1988). The PF (metaphases with ≥42 chromosomes) in control and cisplatin-treated V79 cells was 1.6% and 2.2%, respectively. Higher polyploidy frequencies have also been associated with cellular transformation in vitro (Genari and Wada 2000), and the presence of a large number of polyploid cells in tissue culture is characteristic of transformation and/or malignancy (Borenfreund et al. 1989, Gilvarry et al. 1990, Lothschutz et al. 2002).

Polyploid cells arise during a variety of pathological conditions. Genetic instability in polyploid cells may provide a route to aneuploidy and contribute to the development of cancer (Storchova and Pellman 2004). The number of aneuploid metaphases was increased in cisplatin-treated V79 cells; whereas control V79 cells contained 4 aneuploid metaphases (range of 24–41 chromosomes) per 1000 metaphases (0.4% of cells counted), cisplatin-treated V79 cells contained 33 aneuploid metaphases/1000 metaphases (3.3% of cells counted). Fig. 3 shows the chromosome number distribution in aneuploid metaphases of cisplatin-treated V79 cells.

Although the basic causes of the cellular alterations involved in neoplastic transformations are not fully known, chromosomal changes probably play a major role in the emergence of transformed lineages. Several alterations are observed early after exposure to a chemical carcinogen and may be accompanied by numerical and/or structural chromosomal changes (Barrett 1985). Malignancy is a multistep process in which cells acquire multiple genetic alterations followed by selective clonal expansion that results in the neoplastic phenotype. Hence, neoplastic transformation in vitro is a progressive, multiple-stage process in which genetic alterations are obligatory (Zhu and Gooderham 2002).

The mechanisms of the cisplatin-induced changes in the cytogenetic characteristics of V79 cells were not investigated here. However, the toxicity of cisplatin in chemotherapeutic procedures is a consequence of DNA damage caused by the formation of platinum-DNA adducts, and this injury is apparently responsible for the induction of cell death (Reedjik 1987). However, DNA-adduct-forming agents may induce chromosomal breaks that can result in chromosomal gains via fusion or unrepaired breaks, with subsequent nondisjunction or loss of the defective chromosomes during mitosis, leading to a change in DNA content. Hence, cells with an abnormal DNA content.
will not be effectively excluded from the cell cycle and may continue to divide. These cells will be subject to asymmetric chromosomal segregation every time they divide (Holliday 1989).

In conclusion, the results of this study show that the incubation of V79 cells with a single concentration of cisplatin altered the chromosome number distribution, mitotic index, polyploidy frequency and number of aneuploid metaphases of these cells. These alterations were probably related to the genetic instability induced by cisplatin treatment. The altered characteristics of these cells were similar to those associated with neoplastic processes in vivo following the chemotherapeutic use of cisplatin.

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References