Effect of Cisplatin treatment on death and survival of C6 glioma cells in culture: an ultrastructural and cytochemical study

G. Santin[1], D. Krajčí[2], M.G. Bottone[1], V. Lisa[3], V. Mareš [3,4] and C. Pellicciari[1]

1. Dip. Biologia Animale, Università di Pavia, Italy
2. Dept. Histology & Embryology, Palacky University Olomouc, Czech Republic, and Dept. Anatomy, Faculty of Medicine, Kuwait University, Kuwait
3. Inst. Physiology, Academy of Science (LBNB), Prague
4. Faculty of Science, University of J.E. Purkinje, Usti n. Labem, Czech Republic

pelli@unipv.it
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INTRODUCTION. Studies of the response of astrocytes to pathogenic stimuli *in situ* are hindered by the complex structure of the brain parenchyma. Therefore, we developed a simple in vitro model represented by C6 glioma cells treated with Cisplatin [1]. In this study we demonstrate the occurrence of early- and late-timed programmed cell death (PCD) in different forms, as well as changes in the morphology, cytokinetics, and chemistry of surviving cells; these resemble reactive glial cells *in situ* and their features may represent the cytological signs of a defense to cytostatic-induced stress.

MATERIALS AND METHODS. Astrocyte-like C6 glioma cells (ATCC CCL 107) were grown, either on flasks or on glass coverslips in Petri dishes, in Eagle´s MEM with 10% bovine serum and gentamycin (LEK, Ljubljana, Slovenia), at 37°C in a humidified air atmosphere with 5% of CO_2. On day 3 after seeding, the cells were exposed to 1.66 x 10^{-5} M cis-dichlorodiammine-platinum II (cisplatin, Sigma, St.Louis) for 90 min. After brief washing, the cells were re-fed on a fresh medium and cultured for one to several day post-treatment (p-t) intervals. Cisplatin-treated and control cells were examined by cytometry, immunofluorescence and electron microscopy. To detect early and late apoptotic cells, control and cisplatin-treated cells were harvested by mild trypsinization and incubated for 10 min in complete culture medium containing FITC-conjugated Annexin V and 2 μg/ml propidium iodide; flow cytometric measurements were taken with a Partec PAS II with argon laser and mercury lamp excitations. Flow cytometry was also performed on cell suspensions stained for DNA with Hoechst 33258. The cells were also immunolabeled to detect different proteins (α-tubulin, actin, and the Glial Fibrillary Acidic Protein, GFAP) in fluorescence microscopy. For electron microscopy, the cells were fixed in 2.5 % glutaraldehyde, 2% OsO4, embedded in Araldite CY212, stained with uranyl acetate and lead citrate, and examined by JEM-1200EXII (for details, see [1]). γ-glutamyltranspeptidase (γ-GT) was detected by enzyme cytochemistry [2].

RESULTS. Since 24 h, the cells were slowing down cycling (as already reported, [3]) and died by canonic apoptosis (Type I PCD) with nuclear condensation, formation of heterogeneous ectopic RNP-derived structures (HERDS), and cell shrinkage, resulting in the formation of apoptotic bodies [1]. At 48 h, in some cells intranuclear bundles of microtubules and microfilaments appeared (Figure 1a,b), without apoptotic chromatin condensation and nuclear fragmentation: these cells were undergoing an atypical form of PCD, we have previously described [4]. By 72 h p-t, the cells with nuclear microtubules disappeared, and most of the surviving cells became hypertrophic and their nuclei became large, multi-lobulated and often underwent massive fragmentation into micronuclei (Figure 2); in their cytoplasm, hetero and auto-phagosomes were found suggesting the possible occurrence of

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autophagic (Type II) PCD. Surviving hypertrophic cells (about 30%, present up to 192 h) up-regulated γ-GT and became more positive for GFAP, which suggests their defense to metabolic and oxidative stress. Indeed, more than 95% of these cells survived repeated cisplatin treatment. The cytometric evaluation of DNA content in Hoechst 33258-stained samples demonstrated that from 5 to 8 days p-t the cell fraction with DNA values exceeding 4C increased in cisplatin-treated cultures, indicating that first a G_2-block possibly took place, then that tetraploid cells occurred.

CONCLUSIONS. Following treatment with a cytostatic dose of cisplatin, C6 astrocytoma cells in culture underwent a time-dependent and cell-cycle-phase-related damage resulting in either canonical or atypical forms of regulated cell death, for a large part of the cell populations. The surviving cells became hypertrophic and assumed a phenotype resembling reactive astrocytes *in situ*. The increased positivity for γ-GT enzyme activity and for GFAP indicates the occurrence of an oxidative stress and an antioxidative defense reaction. This study suggests that these changes led to an increased resistance of C6 cells and it is tempting to speculate that resistant reactive astrocytoma cells could also occur *in situ*, as a consequence of the chemotherapeutic treatment with cisplatin.

1. D. Krajčí et al., Eur. J. Cell Biol. **79** (2000) p365.
2. Z. Lojda, J. Histochem. Cytochem. **29** (1981) p481.
3. V. Mareš et al., Histochemical J. **19** (1987) p187.
4 D. Krajčí et al., Histochem. Cell Biol. **12** (2006) p183.
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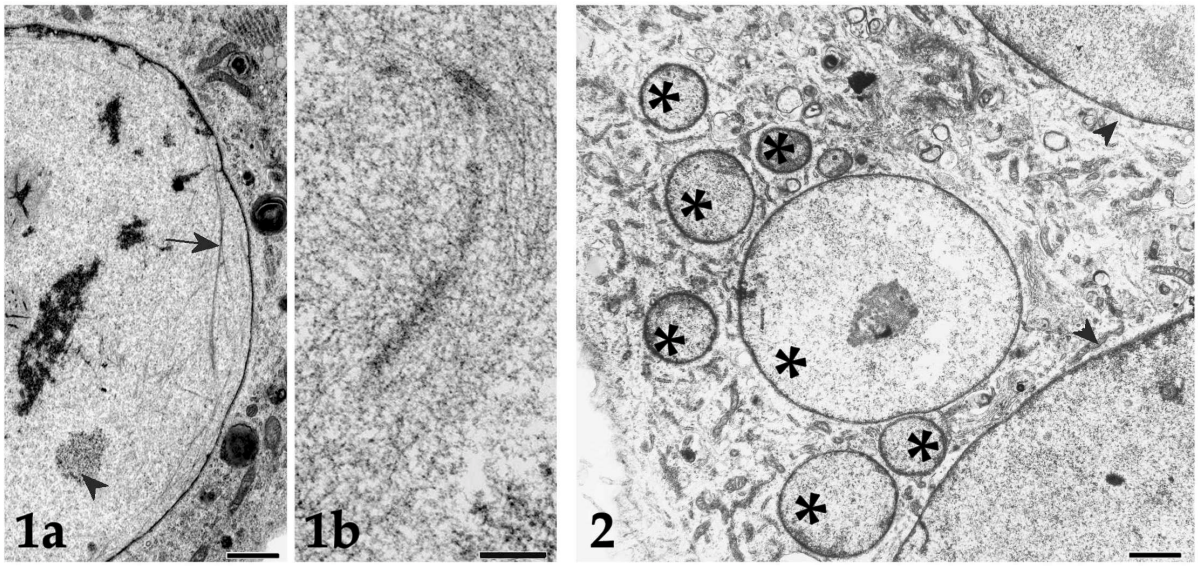

Figure 1. Cisplatin-treated C6 glioma cell at 48h p-t: **1a.** Small bundles of microtubules at the periphery of the nucleus (arrow) and intranuclear HERDS (arrowhead) are observed (scale bar 2 μm); **1b.** large intranuclear tuft of microfilaments are also found (scale bar 0.2 μm). **Figure 2.** In a giant hypertrophic C6 cell at 9 days after cisplatin-treatment, two large nuclear lobules (incompletely shown; arrowheads) and several micronuclei (asterisks) are observed (scale bar 2 μm).

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