the culture dishes from the culture medium With the increased number of passages, the cells of female origin became more prone to develop of a block in cyto- and karyokinesis resulting in a massive formation of tetraploid cells In contrast, the cells of male origin remained diploid and cycling at a higher rate A small number of 4C DNA cells is present also in the vascular wall *in situ* under physiological conditions and it increases with age and during hypertension (Rosen *et al* 1985, Owens *et al* 1988) Earlier studies performed *in vitro* showed that endomitosis followed by formation of 4C DNA cells occur in cultures of SMCs treated with angiotensin, arginin-vasopressin, catecholamines and TGF-beta in absence or very low concentrations of serum-provided mitogens (Yamori *et al* 1987, Owens *et al* 1988) In our study, repeated passaging could therefore induce different sensitivity of the male and female SMCs to the molecules responsible for the completion of karyokinesis resulting in a different number of 4C DNA cells in both sex-derived populations

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Ultrastructural Response of the Nuclear Envelope (NE) of C6-Glioma Cells to Cisplatin-Induced Apoptosis

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Abstract. The early response of the nuclear envelope of C6-glioma cells ($t \leq 24$ h), treated with a cytostatic dose of cisplatin in culture (5 μg/ml) included formation of slim

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and deep invaginations formed by either the inner or both membranes The invaginations made of the complete NE often extended up to the enlarged nucleoli In some of them, nucleous-like material occurred at their cytoplasmic site suggesting its enhanced nucleus-to-cytoplasm transport Some nuclear pores in the invagination-forming cells were covered by dome-shaped "caps" protruding into the karyoplasm The "capped" pores were absent in the cells that were initially more damaged At 48 to 72 h, we found a small number of large and hyperlobulated cells with some small lobules containing a rarefied chromatin and focally disintegrated NE The lamina-free remnants of the NE with a swollen perinuclear cistern were still present at 72–96 h when the population entered the execution phase of apoptotic death

Key words: Nuclear envelope — Nucleolar canals — Cisplatin — Apoptosis — Glioma cells

Introduction

Cisplatin (cis-dichlorodiammineplatinum) is a cytostatic which inhibits cell division mainly via its binding to the nuclear DNA As shown earlier on glioma cells in culture, 48 h after administration of this drug the cells are arrested at G_2/M and later (72 to 96 h), they die by apoptosis (Mareš *et al* 1987, Kondo *et al* 1995) Irrespective of the cell type and the lesioning agent, the early stages of apoptosis are dominated by changes in nuclear morphology, namely redistribution and condensation of chromatin The NE is preserved till the terminal breakdown of cells into the apoptotic bodies Before this stage, only sliding of the nuclear pores away from the dense marginal chromatin regions has been reported (Wyllie *at al* 1980, Falcieri *et al* 1994) Final dissolution of the NE is preceded by disassembly of the lamina (Wyllie *et al* 1980, Earnshow 1995) Data on the NE in cells undergoing apoptosis are, nevertheless, rare and refer mainly to advanced stages of this process Here, we followed changes in the NE of C6-glioma cells exposed to Cisplatin in cultures, starting from the initial cell cycling inhibition period to the terminal apoptotic cell death

Materials and Methods

The 3-day-old cultures of C6-glioma cells (Eagle's MEM with 10% bovine serum) were exposed to a 90 min pulse of cisplatin (Lachema, Brno, 5 mg/ml) and further cultured in a fresh medium for 4 to 96 h After fixation (2 5% phosphate buffered glutaraldehyde, pH 7 2, 30 min), washing and postfixation with OsO_4 (2%, 2 h), the cells were flat-embedded in Araldite (CY 212) by a routine procedure The sections were double stained with uranyl acetate and lead citrate and examined in the JEM-1200EXII (JEOL) electron microscope at 80 kV

Results

As revealed by the number of cells, the cisplatin treated population ceased growing at time $\leq$ 24 h At this time, the population density in the drug-treated cultures was only 48 3% of that in the "age-matched" controls This difference further increased with the post-treatment time At 72 h, the population density counts were only 5 6% of those in control cultures Since 72 to 96 h, the cells underwent apoptosis indicated by the typical changes in their ultrastructure and DNA fragmentation (Mareš *et al* 1996) The nuclei of cells treated for 24 h were larger and their chromatin accumulated near the NE At 24 to 48 h, the NE formed slim tubular invaginations made of the inner or both membranes The

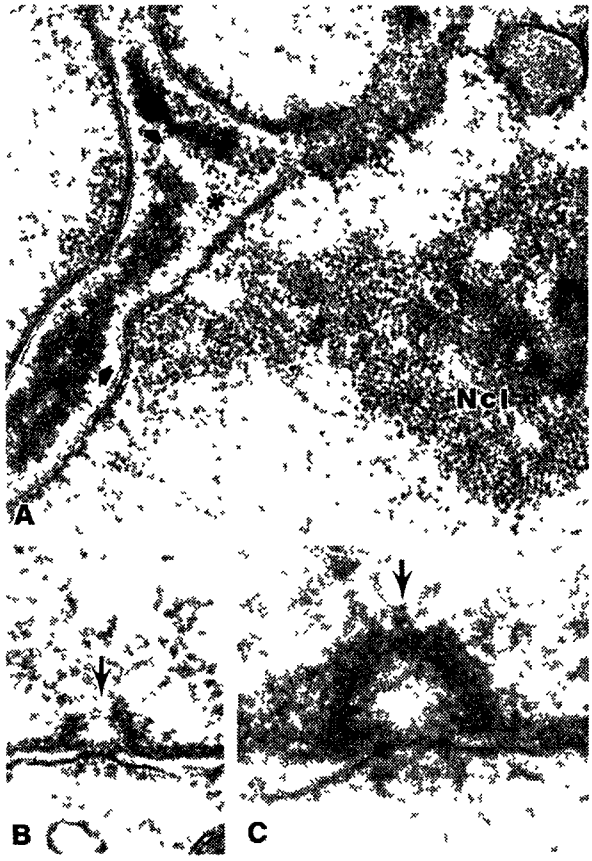

Figure 1. Nuclear envelope in C6-glioma cells in cultures 4 to 48 h after cisplatin (**A**) Deep part of a branched tubular "nucleolar canal" formed by both membranes of the NE contains cytoplasm (*) with an electron-dense granulo-filamentous material (arrows), identical with the dense fibrillar component of the nearby located nucleolus (Ncl) Magn ×58 000 (**B**) Early stage of the pore "cap" formation (arrow) represented by projections of the lamina over edges of the nuclear pore Magn ×90 000 (**C**) High magnification image of the complete dome-shaped "cap" over a nuclear pore (arrow) Magn ×280 000

latter type of invaginations often extended up to the nucleolus and enclosed a cytoplasmic matrix in which dense nucleolus-like material occurred at the different levels of their length, from the nucleolus to their cytoplasmic opening (Fig 1A) Numerous open pores were observed in the invaginated NE Some of the nuclear pores, located in the smooth parts of the NE, were covered by dome-shaped "caps" composed of a thin layer of fine fibrillar material (Figs 1B,C) These "caps" occurred in about 13% of the nuclear pores seen in the tangential sections of the NE in the invagination-forming cells They were not observed in smaller cells with pale nuclei and paramarginally clumped chromatin, separated from the NE by bundles of filaments or microtubules, indicating more profound damage of these cells Later (48–72 h), a subgroup of large cells with excessively lobulated nuclei appeared Their chromatin was, however, still only moderately condensed and the NE well preserved in the larger lobules In small lobules, the nuclear lamina, or even the NE membranes, were focally dissolved and the rarefied chromatin appeared "leaking" into the cytoplasm From 72 h, the cells with typical morphological signs of advanced apoptosis started to appear In brief, they displayed profound condensation of the nuclear content and its break-down into apoptotic bodies while retaining relatively well structured

organelles in the condensed cytoplasm In these cells, the lamina-free fragments of the NE were still visible between the regions with marginally condensed chromatin The nuclear pores were rare and the perinuclear cistern was slightly swollen

Discussion

Formation of deep and multiple NE invaginations was the first reaction of the NE to the damage by cisplatin The invaginations made of the complete NE were identical with the nucleolar (Bourgeois and Hubert 1998) or nuclear canals (Fricker *et al* 1997) believed to *facilitate transport of ribonucleoproteins in normal and neoplastic cells Since, the transfer* of ribosomal particles occurs only through the nuclear pores (Nigg 1997), such a deep and complex invaginations of the NE may provide larger total nuclear pore profile located close to the nucleolus. Transporting role of these invaginations in our study is indirectly indicated by the presence of the nucleolus-like material at their cytoplasmic site, both in the proximity of the nucleolus and near to their openings into the perinuclear cytoplasm, as well as by the increased quantity of ribosomes in the cytoplasm of these early reactive cells

Extranuclear and extracellular transport, and/or disposition, of the ribonucleoprotein-containing material was shown in thymocytes at advanced stages of spontaneous apoptosis (Biggiogera *et al* 1997) In our material, the cytoplasmic transfer of the nucleolus-like material occurred, however, in the cells with still well preserved morphology Moreover, we have not noticed extracellular extrusion of this material at this post-treatment interval Therefore, we assume that the tubular invaginations of the complete NE in our material reflect rather some activation of the protein synthesis apparatus than a regressive apoptotic change It is to be pointed out that these invaginations differed from the broad NE infoldings, which appeared in the large hyperlobulated cells at later intervals The inner nuclear membrane invaginations were previously described e g in tumor cells and classified as type 2 nuclear pockets (reviewed by Ghadially 1997) Similarly to the earlier studies, this type of invaginations was covered by the nuclear lamina and were free of nuclear pores

As it is shown in this study, the early nuclear response of C6-glioma cells to cisplatin included also formation of a novel structure, the nuclear pore "caps", attributed tentatively to the altered nuclear lamina and aberrant margination of chromatin Their functional significance remains to be established

Finally, we point out focal degradation of the NE in some small nuclear lobules of the hyperlobulated cells arising at later post-treament intervals This, together with the circumscribed rarefaction of chromatin, suggests a possibility of confined karyolysis in some cells condemned to apoptotic death induced by cisplatin

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Immunohistochemical Localization of Laminin-1 in the Acellular Nerve Grafts is Associated with Migrating Schwann Cells which Display Corresponding Integrin Receptors

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Abstract. The presence of laminin-1, collagen-IV, $\alpha 6$ and $\beta 1$ integrin chains was detected by indirect immunohistochemistry using biotin/streptavidin/HRP or gold-conjugated secondary antibody at the light and electron microscope level, respectively Cryo-treated segment of the peripheral stump without living Schwann cells (S-100$^-$) did not display immunoreactivity for laminin-1 and integrin's chains, while the migrating Schwann cells in the marginal regions were immunostained for the antigens Isolated acellular nerve segments protected from migration of Schwann cells (S-100$^-$) exhibited laminin-1$^-$, $\beta 1^-$, and $\alpha 6^-$ integrin chains immunoreactivities Position of the basal lamina was verified by collagen-IV$^+$ immunoreactivity Results indicate that presence of the laminin in the peripheral nerve is related with living Schwann cells

Key words: Acellular nerve graft — Laminin-1 — Integrins — Schwann cell migration

The basal lamina tubes of axon-Schwann cell units play an important role in the nerve regeneration preparing conduits for growing axons (Idé *et al* 1983) Laminin, the

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