Cytotoxic effects of cisplatin, cis-dichlorodiammineplatinum(II), on Tetrahymena

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Summary

A study was made of the effects of cisplatin, cis-dichlorodiammineplatinum(II) (5–250 mg l⁻¹), on the physiology and fine structure of Tetrahymena. The physiological effects observed were dose-dependent. Endocytosis was inhibited reversibly in all, but late in the high, concentrations. After an initial dose-related increase, due to division of cells most advanced in the cell cycle, proliferation ceased for at least two normal cell generations (6 h) in 50 and 100 mg drug l⁻¹, but for 24 h in 250 mg l⁻¹, after which multiplication was resumed in a dose-dependent manner. Exposure to cisplatin resulted in the appearance of small, refractive granules and platinum (i.e. electron-dense material) accumulated in these granules. Fine structural observations of cells exposed to 250 mg drug l⁻¹ showed nucleolar fusion and appearance initially of lipid droplets, dense granules and autophagosomes. A time-dependent redistribution of cell organelles was revealed by morphometry; in particular, the mitochondria increased in number, but decreased in size. Moreover, after prolonged treatment (24 h) and without cell division, the inner mitochondrial membrane had diminished and the ratio of the inner to the outer mitochondrial membrane was only half of the value for control mitochondria. Concomitantly with this decrease, the cell content of ATP was reduced to a similar extent. The findings indicate a specific action of cisplatin on mitochondria, resembling that induced in Tetrahymena by chloramphenicol and methotrexate.

Key words: cisplatin, cis-dichlorodiammineplatinum(II), mitochondria, cell proliferation, detoxication, Tetrahymena pyriformis.

Introduction

Cisplatin (cis-dichlorodiammineplatinum(II)) is used widely in cancer chemotherapy either alone or in combination with other drugs (Rosenberg, 1973, 1980; Sessa, 1986; De Lena et al. 1987; Safirstein et al. 1987).

The discovery that cisplatin inhibits cell proliferation was made during exposure of Escherichia coli to an electric field using platinum electrodes (Rosenberg et al. 1965). During electrolysis, the platinum dissolved and formed with inorganic salts a non-electrolytic platinum(IV) complex, which became reduced to cis-dichlorodiammineplatinum(II), the biologically active compound. The antineoplastic properties of cisplatin have been confirmed on transplanted tumours (Rosenberg et al. 1965, 1969; Rosenberg, 1973, 1980); moreover, the chemical and clinical properties of the drug have been studied extensively (Rosenberg, 1973, 1980; Stetsenko et al. 1981; Litterst, 1984). The drug binds, after loss of two chloride ions, to two adjacent guanosines in DNA (Pinto & Lippard, 1985; Butour et al. 1985; Sherman et al. 1985). The intrastrand bindings of cisplatin cause local conformational changes of the DNA molecule, the phenomenon believed to be responsible for the inhibition of DNA synthesis.

A major clinical complication of the use of cisplatin in chemotherapy is nephrotoxicity (Dobyan, 1985; Pinnaro et al. 1986). Acute renal failure may occur a few days after administration of the drug (Pinnaro et al. 1986; Safirstein et al. 1987) and irreversible nephrotoxicity may result when the drug is used in combination with methotrexate (Goren et al. 1986).

The purpose of the present study has been to...
investigate the general cytotoxic effects and also possible more specific effects of cisplatin on DNA-containing organelles, using axenic cultures of the ciliate, *Tetrahymena pyriformis*. These cells have a short generation time, which is an advantage in growth experiments. This model cell system had been used to study the effects of, for example, methotrexate, which permits a limited number of cell doublings and affects the mitochondrial substructure (Nilsson, 1983).

**Materials and methods**

*Tetrahymena pyriformis* GL (Nanney & McCoy, 1976) was grown axenically at 28°C in 2% proteose peptone enriched with 0·1% liver extract and inorganic salts (Plesner et al. 1964). The cell density of the aerated and agitated cultures was determined as described (e.g. see Nilsson, 1986).

Cisplatin (*cis*-dichlorodiammineplatinum(II)) was obtained from two sources. The synthesized compound (gift from the Chemical Department, Risø Research Center, Denmark) was dissolved in 1% NaCl and the clinical, NaCl-containing Platinol (Bristol Laboratories, Bristol-Myers Co., Syracuse, USA) was dissolved in redistilled water; both compounds dissolved rapidly on slight warming. The stock solutions were 2·5 mg cisplatin ml⁻¹ and the appropriate amount of NaCl was added to the control cultures.

For growth experiments, 100-ml cell cultures in the exponentially multiplying growth phase (about 4·×10⁴ cells ml⁻¹) were divided into two portions, the drug being added to one portion, while the other served as control.

The endocytic activity of the cells was tested as described (Nilsson, 1972), using carmine particles and counting of labelled food vacuoles in 100 cells/sample. The data were expressed as percentage of the control value (100%).

For determination of the cell content of ATP, the bioluminescent firefly luciferase assay (Myhrman et al. 1978) was used (ATP Monitoring Kit (1250-120) and Luminometer 1250, LKB-Wallace, Turku, Finland). Application of the technique to the *Tetrahymena* system has been described (Nilsson, 1980); cisplatin had no influence on the light output.

For electron microscopy, the cells were fixed as described (Nilsson, 1986). After embedding in Epon, the sectioned material was contrasted in zinc/uranyl acetate and lead citrate (Nilsson, 1986) or only in zinc/uranyl acetate. The sections were examined in Zeiss, EM9 and EM 109, electron microscopes.

Morphometry of control and drug-treated cells was performed using a Kontron Mini-Mop semi-automatic Image Analysis System equipped with a stylus (Kontron Bildanalyse GMBH, Munich, West Germany). For volume determinations, the cells were fixed for 10 min as for cell density, washed, and photographed in a Reichert anoptral phase-contrast system (×16 objective). An alternative method was to use Epon-embedded cells, mounted between slide and coverslip simultaneously with embedding for electron microscopy. After photographic enlargement, the volume of 100 cells/sample was measured; for both methods, the data were expressed as the percentage of the control cells (100%).

**Results**

**Growth characteristics of cisplatin-treated cells**

To determine dose-related effects of cisplatin, the cells were exposed to 5–250 mg drug l⁻¹ (i.e. up to 0·83 mM); higher concentrations were toxic.

The effect of cisplatin on the rate of endocytosis after a 1-h exposure is shown in Fig. 1. The linear, dose-dependent inhibition of endocytosis was reduced maximally to 30% of the control value (100%). The rate of endocytosis returned to the normal value after 3 h in up to 100 mg drug l⁻¹, whereas it was still inhibited after 6 h in the high concentrations (Fig. 1), but normal again after 24 h.

The rate of cell proliferation in 50, 100 and 250 mg drug l⁻¹ was followed for 72 h, corresponding to 25 normal cell generations. The initial response was a dose-dependent effect on the progress of cells most advanced in the cell cycle at the time of addition of the drug (Table 1); in the highest concentration only about 10% of the cells, i.e. those with initiated fission furrowing, completed division during the first 1·5 h,
Table 1. Number of cell doublings during continuous exposure to cisplatin

<table>
<thead>
<tr>
<th>Concentration of cisplatin</th>
<th>Time of exposure (h)</th>
<th>None</th>
<th>50 mg l⁻¹</th>
<th>100 mg l⁻¹</th>
<th>250 mg l⁻¹</th>
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<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>0.5</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>6.4</td>
<td>4.5</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>8.5</td>
<td>6.6</td>
<td>5.0</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>10.6</td>
<td>8.7</td>
<td>7.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>16.9</td>
<td>15.1</td>
<td>13.4</td>
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<tr>
<td></td>
<td>72</td>
<td>25.4</td>
<td>23.6</td>
<td>21.9</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Data based on four to six experiments for each concentration.

* Time after which cell proliferation occurred at normal rate.

Table 2. Effects of cisplatin on mitochondrial membrane ratio, cell content of ATP, and cell volume

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner/outer mitochondrial membrane*</td>
<td>100 ± 12.37 †</td>
<td>82.6 ± 15.00</td>
<td>83.1 ± 15.66</td>
<td>− †</td>
<td>49.2 ± 6.68</td>
</tr>
<tr>
<td>Cell content of ATP ‡</td>
<td>100</td>
<td>94.6 ± 2.02</td>
<td>89.3 ± 3.88</td>
<td>68.3 ± 6.67</td>
<td>52.9 ± 3.25</td>
</tr>
<tr>
<td>Cell volume §</td>
<td>100</td>
<td>74 ± 7</td>
<td>69 ± 10</td>
<td>65 ± 4</td>
<td>79 ± 4</td>
</tr>
</tbody>
</table>

* Morphometric measurements of 30 mitochondria per sample.
† S.D.
‡ Firefly luciferase assay; means of five experiments.
§ Morphometric measurements of 100 cells per sample; means of four experiments.

whereas in the low concentration about 30% of the cells, i.e. those past the S-phase (Nilsson, 1976) at the time of addition of the drug, had divided. No further proliferation occurred for at least 6 h, and for 24 h at the highest concentration (Table 1). Cell proliferation was resumed gradually and the normal rate was found after 18, 30 and 72 h in 50, 100 and 250 mg drug l⁻¹, respectively (indicated by asterisks in Table 1); all cultures reached maximal cell density (about 10⁶ cells ml⁻¹). The gradual resumption of cell proliferation might be ascribed to a gradual decrease in the drug concentration in the medium. This can be assumed, since transfer of cells to fresh drug-containing medium after 24 h in 50 mg drug l⁻¹ resulted in an immediate arrest of cell proliferation for another 6 h.

In vitro observations revealed small, refractile granules in all drug-treated cells. Some of the granules stained with Neutral Red, indicating their lysosomal nature. The number of granules increased with time, less at the low than in the high drug concentration; however, in proliferating cells the granules fuse with digestive vacuoles and their number remained constant. At the highest concentration, the cells moved slowly after about 3 h; it has been reported that cisplatin affects cell motility of Tetrahymena (Bergquist et al. 1986) in a 2-4 times higher concentration. With time, conspicuous defecation balls accumulated in the culture flasks, but were not seen in control cultures. Cell size decreased initially during exposure to the highest concentration, partially due to inhibition of endocytosis, but increased again after 24 h prior to initiation of cell proliferation (see Table 2).

Since cisplatin had a dose-dependent effect, during the first hours, on progress of the cells most advanced in the cell cycle (Table 1), the rate of cell recovery was studied after a 1-h exposure, corresponding to one third of a normal cell generation. After transfer to fresh growth medium, cells treated with 50 and 100 mg drug l⁻¹ resumed the normal rate of proliferation after a lag period of 2 and 3 h, respectively. Cells treated with 250 mg drug l⁻¹ exhibited, however, a 5-h lag period before proliferation recommenced at a decreased rate for about three generations; full recovery was found 24 h after the treatment. Recovery of 3-h treated cells followed much the same pattern.

Fine structure of cisplatin-treated cells

To study the maximal effect of cisplatin, cells exposed to 250 mg drug l⁻¹ were examined in the electron microscope.

The structure of mitochondria and peroxisomes (Nilsson, 1981b) changed during treatment. In proliferating control cells, both organelles were of the light type (Fig. 2). After 1 h in the drug, mitochondria showed little change (Fig. 3), whereas peroxisomes appeared as the very light type. After 2 h, peroxisomes remained light and mitochondria were of the dense type (Fig. 4). After 24 h, peroxisomes had changed to the dense type, whereas mitochondria appeared less

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Fig. 2. Electron micrograph showing mitochondria (m) and peroxisomes (p) of a control cell. Note the numerous tubules in the mitochondria. Bar, 1 μm. ×18000.

Fig. 3. Portion of cytoplasm of a cell after 1 h in cisplatin (250 mg l⁻¹). Mitochondria (m) are almost unchanged but the peroxisomes (p) are of an extremely light type. Electron-dense autophagosomes (arrows). ×18000.

Fig. 4. Portion of a cell treated for 2 h with cisplatin. The mitochondria (m) are of the dense type and peroxisomes (p) are still of the very light type. The ribosomes may form helices (arrow). ×18000.

Fig. 5. Cytoplasm from a cell treated for 24 h with cisplatin. The mitochondria (m) appear less dense (compare with Fig. 4) and with a reduced number of tubules (see Fig. 2). The peroxisomes (p) are of the dense type. Lipid droplets (l) and a small vacuole with dense material (arrow). ×18000.

dense and with a reduced number of tubules (compare Fig. 5 with Fig. 2). The reduced number of mitochondrial tubules (Figs 5, 6) was not uniform in mitochondria within a single cell. Measurements of the ratio of the inner to the outer mitochondrial membrane revealed that the mean value for 24-h-treated cells was only half of that of control cells (Table 2). Moreover, the ATP content of 24-h-treated cells was reduced to a similar extent (Table 2); although the volume of treated cells was reduced 20% (Table 2), the content of ATP per unit volume was still only 60% of the control value.

Other structural changes were observed in drug-treated cells. Organelles such as lipid droplets, dense granules and autophagosomes appeared after 1 h (Fig. 3); their frequency increased with time but their distribution within the cell was not uniform (Fig. 7). Ribosomes decreased in number during the treatment (Figs 2–5) and they formed helices (Fig. 4). In the nucleus, nucleolar fusion was seen after 1 h and little additional change had occurred after 24 h (Fig. 7); however, at this time the nuclei contained fibrous bundles, previously reported in Tetrahymena exposed to dimethyl sulphoxide (Nilsson, 1980; Katsumaru &
Fukui, 1982) or heat, they consist of actin filaments (Hirono et al. 1987).

In cells recovering from a 1-h exposure to cisplatin, many of the same structural changes were seen (Fig. 8) as those observed during continuous exposure to the drug, even newly formed autophagosomes (Nilsson, 1984) were found after a 4-h recovery. However, mitochondria showed no indication of a reduction of tubules and the mean ratios of the inner to the outer mitochondrial membrane in 4-h recovering cells (4:2) and control cells (4:0) were very similar.

To obtain a semi-quantitative measurement of the drug-induced changes, the number of, and cytoplasmic area occupied by, the different cell organelles were
Table 3. Analysis of the distribution of cell organelles in cisplatin-treated cells

<table>
<thead>
<tr>
<th>Organelle (profile)</th>
<th>Control</th>
<th>1 h</th>
<th>1.5 h</th>
<th>2 h</th>
<th>24 h</th>
<th>Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number per 25 µm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondria:</td>
<td>7.6</td>
<td>9.6</td>
<td>9.2</td>
<td>9.6</td>
<td>9.0</td>
<td>6.4</td>
</tr>
<tr>
<td>% Cytoplasmic area</td>
<td>15.3</td>
<td>16.7</td>
<td>16.3</td>
<td>15.8</td>
<td>13.4</td>
<td>11.7</td>
</tr>
<tr>
<td>Mean size (%)</td>
<td>100</td>
<td>86.7</td>
<td>87.8</td>
<td>82.0</td>
<td>73.5</td>
<td>90.0</td>
</tr>
<tr>
<td>Peroxisomes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number per 25 µm²</td>
<td>2.1</td>
<td>2.7</td>
<td>2.3</td>
<td>1.7</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>% Cytoplasmic area</td>
<td>100</td>
<td>80.3</td>
<td>89.6</td>
<td>80.7</td>
<td>54.6</td>
<td>93.6</td>
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<tr>
<td>Mean size (%)</td>
<td></td>
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<td></td>
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<tr>
<td>Lipid droplets:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number per 25 µm²</td>
<td>0</td>
<td>0.5</td>
<td>0.7</td>
<td>0.9</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>% Cytoplasmic area</td>
<td>0</td>
<td>0.3</td>
<td>0.9</td>
<td>1.0</td>
<td>2.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Autophagosomes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number per 25 µm²</td>
<td>0</td>
<td>1.2</td>
<td>1.5</td>
<td>1.4</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>% Cytoplasmic area</td>
<td>0</td>
<td>1.4</td>
<td>2.7</td>
<td>2.8</td>
<td>2.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Dense granules:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number per 25 µm²</td>
<td>0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>% Cytoplasmic area</td>
<td>0</td>
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<td>0.2</td>
<td>0.5</td>
<td>1.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*After 1-h exposure to cisplatin.

Intracellular distribution of cisplatin

Since platinum is electron-dense, cisplatin and its possible platinum-containing metabolic products may be identified as electron-dense material not seen in control cells. Consistent with the bulk uptake by endocytosis, electron-dense material was present in food vacuoles either bound to less-dense matter or as finely granulated material. In the cytoplasm, attention was drawn to organelles of a size corresponding to that of the refractive granules seen in the light microscope (Fig. 9). Organelles of this size were typical dense granules (Fig. 11) and, for example, digestive stages of autophagosomes (Fig. 10, left), all limited by a thick membrane characteristic of the lysosomal system. Platinum was revealed as fine electron-dense dots that accumulated on the luminal side of the membrane and in less-dense, enclosed material (Figs 10–13); in the autophagosomes, the dots were distributed randomly (left in Fig. 10, bottom in Figs 12, 16). A coarse type of electron-dense material was found in the alveolar space below the plasma membrane (Fig. 14), in small vacuoles (Fig. 15), in vesicles (Fig. 16) and within endoplasmic cisternae (Fig. 17). In addition, similar material was embedded in translucent material in enlarged rough endoplasmic reticulum (not shown). Early during exposure to the drug, electron-dense dots were scattered free in the cytoplasm, but after 1.5 h they were concentrated at the sites just described. The amount of electron-dense material increased during treatment, but organelles containing scant amounts were seen always.

Discussion

Consistent with the general action of cisplatin, proliferation of Tetrahymena ceased for two to ten normal cell generations, after an initial, dose-dependent progress only of cells most advanced in the cell cycle. The findings indicate an inhibition of cells in the S-phase, as reported for C6 glioma cells (Mares et al. 1987), consistent with binding of the drug to naked DNA rather than to chromatin (Kulamowicz & Walter, 1987). The concentrations used in the present study, i.e. 167–830 µM, are higher than the therapeutic dose of...
Fig. 9. Light micrograph of in vivo Tetrahymena exposed for 24 h to 250 mg l\(^{-1}\) cisplatin. Note the numerous refractive granules some of which are lysosomes (stain with Neutral Red). The nucleus (nu) along the periphery of which the fused nucleoli may be seen. Bar, 10 \(\mu\)m. ×900.

Fig. 10. Two organelles containing electron-dense ‘dots’ (arrows) representing platinum; one is an autophagosome (au). From a cell after 1.5 h in cisplatin. Bar, 0.5 \(\mu\)m. ×60 000.

Fig. 11. A typical dense granule with electron-dense material embedded in less-dense matter. From a cell after 2 h in the drug. ×60 000.

Fig. 12. Another dense granule with non-homogeneous contents, to which platinum is adsorbed (arrows). Part of an autophagosome (au) with scant amounts of platinum. ×60 000.

Fig. 13. An organelle believed to be an early dense granular stage. Note the accumulation of the dense (platinum) dots on the inner side of the limiting membrane and in the enclosed material. From a cell after 24-h in cisplatin. ×60 000.

about 25 \(\mu\)m (e.g. see Aggarwal et al. 1980) and the doses up to 33–133 \(\mu\)m used with tissue culture cells (Mareš et al. 1987; Eichholtz & Hietel, 1986), but near the 0.5–1 mm used in some in vivo experiments (Aggarwal et al. 1980; Fumarulo & Aresta, 1987). The apparently high tolerance of Tetrahymena could be ascribed to its capacity to synthesize metallothionein (Suzuki et al. 1987; Piccinni et al. 1987). This metal-binding protein may be induced, for example, by cadmium (Kagi & Nordberg, 1979) and it binds cisplatin (Litterst, 1984; Zelazowski et al. 1984; Krakre et al. 1985). Moreover, an enhanced resistance to cisplatin has been reported for cells with a high content of metallothionein (Bakka et al. 1981; Endresen et al. 1984; Andrews et al. 1987).

Cisplatin is taken up by endocytosis in tissue cells (Makita et al. 1986) and uptake is correlated with cytotoxicity (Eichholtz-Wirth & Hietel, 1986). Endocytosis in Tetrahymena was inhibited initially in a dose-dependent manner but only for a few hours, apart from

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Fig. 14. Accumulation of fine granular, electron-dense material (platinum) in the alveolar space (arrow) below the plasma membrane. From a cell after 24-h in cisplatin. No lead contrast. ×60000.

Fig. 15. Dense material, similar to that seen in Fig. 14, enclosed in a small vacuole (arrow). From a cell after 1-5 h in cisplatin. No lead contrast. ×60000.

Fig. 16. Scant amounts of electron-dense material (platinum) of the type seen in the two preceding figures in a small vesicle (arrow) and of the fine dot-type in the small vacuole (bottom). From a cell after 1-5 h in cisplatin. ×60000.

Fig. 17. Scant amounts of electron-dense material (arrows) in membranous cistern. From a cell after 24-h in cisplatin. ×60000.

at the highest concentration. Since the drug contains platinum, the intracellular distribution of the metal-containing components could be observed as electron-dense dots often embedded in translucent material. The majority of platinum was found within the lysosomal membrane system, i.e. in food vacuoles, dense granules and autophagosomes. In proximal renal tubules, platinum was found in lysosomes, which increased in number with time after administration of cisplatin (Aggarwal et al. 1980) as do the dense granules in *Tetrahymena*. These granules, with a varying but high metallic content (Coleman et al. 1972; Dunlop & Chapman, 1981), accumulate in growth-inhibited cells, which indicates their role in the intracellular regulation of excess metal ions (e.g. see Nilsson, 1979, 1981a,b); the granules are lysosomes, since they are acidic compartments and fuse with digestive vacuoles in cells resuming proliferation. Scant amounts of platinum were found within the endoplasmic reticulum, in small vesicles, and initially free in the cytoplasm, which indicates that the drug binds to protein (metallothionein?) at any stage after synthesis in the endoplasmic reticulum and passage through the smooth endoplasmic reticulum over the Golgi complex to form the contents of small Golgi-derived vesicles of the lysosomal type. The vesicles may fuse with one another or with, for example, autophagosomes; the final elimination of their contents is through defecation. A continuous uptake of cisplatin by endocytosis and an apparent, continuous intracellular removal of the drug will reduce the extracellular concentration gradually; cell proliferation will then resume when the drug concentration is lowered sufficiently. In the kidney of drug-treated rats, the content of platinum/protein may be twice the administered dose/kg (Sharma & Edwards, 1983; Safirstein et al. 1987); although cisplatin also accumulates in liver, kidney function may be affected by repeated administration of the drug (Sharma & Edwards, 1983; Safirstein et al. 1987).

The inhibition of DNA synthesis by cisplatin may be irrelevant to the toxicity of the drug (Safirstein et al. 1987), since this is an early effect. Moreover, the early ultrastructural effects on *Tetrahymena*, as found in the present study and in drug-treated man and animals (Aggarwal et al. 1980; Gastaut & Pellissier, 1985), resemble general growth-inhibited changes; however, in *Tetrahymena* morphometric measurements revealed an early increase in number of mitochondrial profiles and a time-dependent decrease in their size, resembling the effects of methotrexate and chloramphenicol on *Tetrahymena* (Nilsson, 1983, 1986), but not that of starvation (Nilsson, 1984). After prolonged exposure to cisplatin, the mitochondria had changed to organelles with grossly diminished inner membranes in the absence of cell division; concomitantly, the cells had a considerably reduced ATP content.

This delayed effect of cisplatin on the mitochondria in *Tetrahymena* may be relevant to the nephrotoxicity induced by the drug in chemotherapy. Renal dysfunction occurs as a delayed effect (Pinnaro et al. 1986; Safirstein et al. 1987) and morphological damage is
This delayed effect on respiration during nephrotoxic cisplatin, remains normal for 4 days, it decreases restricted to the proximal tubules in which mitochondria become displaced and may show swollen cristae (Aggarwal et al. 1980; Aggarwal & Menon, 1981; Litterst, 1984; Gordon & Gattone, 1986). Although the basal respiration rate of proximal renal tubules, isolated daily from rats given a nephrotoxic dose of cisplatin, remains normal for 4 days, it decreases considerably on the 6th day (Safirstein et al. 1987). This delayed effect on respiration during nephrotoxicity correlates well with a changed mitochondrial substructure, like that observed in Tetrahymena where loss of the inner mitochondrial membrane, i.e. a reduced number of tubules and reduced cell content of ATP, were time-dependent phenomena. The present findings indicate that mitochondria are the target site of the drug action; moreover, in kidneys of drug-treated rats, Sharma & Edwards (1983) found an almost fivefold higher content of platinum/protein in mitochondria than in nuclei, although the nuclei contain 2-3-fold more DNA/protein than the mitochondria (Sharma & Edwards, 1983). The exact action of cisplatin on mitochondria remains to be shown, but a binding to DNA would affect mitochondrial transcription and translation equally. In Tetrahymena, loss of the inner mitochondrial membrane is induced by methotrexate (Nilsson, 1983), an inhibitor of a DNA precursor synthesis, and by chloramphenicol (Nilsson, 1986), an inhibitor of mitochondrial protein synthesis (Tzagoloff, 1982); in both instances the effect is seen only after prolonged treatment. The enhanced nephrotoxicity of cisplatin administered together with methotrexate (Goren et al. 1986; Jaffe et al. 1987) may well be ascribed to a combined action on the structure and function of mitochondria.

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References


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