Cellular Pharmacology of Liposomal cis-Bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II) in A2780/S and A2780/PDD Cells

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ABSTRACT

We studied the cytotoxicity, cellular accumulation, and DNA interactions induced by liposome-entrapped NDDP (L-NDDP) and cisplatin in A2780 human ovarian carcinoma cells sensitive (A2780/S) and resistant (A2780/PDD) to cisplatin. L-NDDP was 2-fold more cytotoxic than cisplatin against A2780/S cells with 5-h or 24-h drug exposure. Both cell lines were equally sensitive to L-NDDP, while A2780/PDD cells were 4-fold resistant to cisplatin (resistance index, 1.30–1.85 and 4.02–4.50, respectively). Using a drug exposure time of 24 h, L-NDDP cytotoxicity was independent of the liposome composition used, whereas with shorter drug exposure (5 h), the cytotoxicity of L-NDDP was directly related to the relative content of DMPG in the liposome carrier. However, changes in liposome composition or drug exposure time did not alter the resistance index of L-NDDP in this cell system. The cellular accumulation of L-NDDP was similar in both cell lines and 2- to 5-fold higher than that of cisplatin in A2780/S cells, whereas the cellular accumulation of cisplatin was reduced by 2- to 3-fold in A2780/PDD cells. The presence of DMPE in the lipid bilayer enhanced by 2-fold the cellular accumulation of L-NDDP, in good correlation with the direct relation between cytotoxic potency of L-NDDP and the presence of DMPE in the liposome carrier.

Pt/DNA levels were determined at different time points after drug exposure for 1 h. Peak Pt/DNA levels were observed at 6 h for cisplatin and at 9 h for L-NDDP. Peak Pt/DNA levels and Pt/DNA over time of L-NDDP were about 1.5- and 3-fold higher than those of cisplatin in A2780/S and A2780/PDD cells, respectively, when equimolar concentrations of both drugs were used. Cisplatin induced significant DNA interstrand and DNA-protein cross-links in A2780/S cells, and a good correlation was observed between cytotoxicity against both cell lines and both types of lesions. In contrast, equimolar concentrations of L-NDDP induced only minimal DNA interstrand cross-links in either cell line. These results indicate that (a) L-NDDP is not cross-resistant to cisplatin against A2780/PDD ovarian carcinoma cells, (b) the cellular accumulation of L-NDDP is similar in both cells and several-fold higher than that of cisplatin, (c) the cytotoxicity of both drugs correlates with the extent of DNA platination over time, (d) the non-cross-resistance properties of L-NDDP are associated with its ability to induce similar Pt/DNA levels over time in sensitive and resistant cells, and (e) DNA interstrand cross-link formation does not seem to play a role in the cytotoxicity of L-NDDP.

INTRODUCTION

Cisplatin, like the bifunctional alkylating antitumor agents, is known to react with DNA producing bis-adducts such as DNA intrastrand or interstrand cross-links (1–7) as well as DNA-protein cross-links (8). The most frequent DNA adducts (60 to 70%) are guanine-guanine intrastrand cross-links that are probably formed through two adenine and cytosine and, so far, no adducts with thymine have been detected (9, 10). It is believed that one or more of the platinum-DNA interactions are responsible for its cytotoxicity and antitumor activity. However, the contribution of each type of lesion to the cytotoxicity of cisplatin has not been fully elucidated and, in some cases, conflicting results have been reported. DNA interstrand cross-links have been proposed by different investigators as an important lethal lesion (11–16).

Among the numerous analogues of cisplatin synthesized during the last decade, the family of the 1,2-diaminocyclohexane compounds has triggered significant attention because of their reduced nephrotoxicity and ability to kill cells that have acquired resistance to cisplatin, both in vitro and in vivo (17–19). At the cellular level, the mechanisms of non-cross-resistance of the 1,2-diaminocyclohexaneplatinum(II) compounds have not been elucidated, and the type of DNA adducts induced by these compounds does not appear to differ significantly, both qualitatively and quantitatively, from those induced by cisplatin (5).

NDDP (Fig. 1) is a lipophilic cisplatin derivative of the 1,2-diaminocyclohexane family which has been formulated in a liposome composed of DMPC and DMPE for its in vivo administration (20–24). This lipid combination was used because it allows for the preparation of the liposomes in a lyophilized form. The chemical and biological characteristics of L-NDDP have been extensively studied. L-NDDP lacked cross-resistance with cisplatin in two experimental systems, was non-nephrotoxic, and was more active against experimental in vivo models of liver metastases (20). L-NDDP is currently undergoing clinical evaluation. In an initial Phase I study, the dose-limiting toxicity was myelosuppression, and no nephrotoxicity was observed (21).

We have previously reported that the presence of DMPE in the lipid bilayer plays an important role in modulating the stability of NDDP as well as its antitumor activity (22, 23). The higher the DMPE content in the lipid bilayer, the higher its in vivo antitumor potency. We hypothesized that DMPE might alter the pharmacokinetics and/or cellular pharmacology of NDDP or react with NDDP, resulting in more active platinum complex(es).

In this work, we attempted to understand the cellular pharmacology of L-NDDP by studying the cytotoxicity, cellular accumulation, DNA platination, and DNA cross-links induced by cisplatin and L-NDDP in various lipid formulations in A2780/S and A2780/PDD cells. Our results indicate that L-NDDP is not cross-resistant with cisplatin in this system and that the lack of cross-resistance is associated with its ability to induce significant Pt/DNA levels over time, compared with cisplatin in A2780/PDD cells.

MATERIALS AND METHODS

Drugs. Cisplatin was purchased from Bristol-Myers Company (Evansville, IN). Multilamellar L-NDDP were prepared as previously described using either only DMPC and only DMPE or both in a molar ratio of DMPC:DMPE = 7:3 and 3:7 (20–23). DMPC and DMPE were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL).

Cell Lines. A2780/S and its resistant counterpart A2780/PDD cells were obtained from Dr. Zahid Siddik at M. D. Anderson Cancer Center. Both cells

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3 The abbreviations used are: NDDP, cis-bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II); L-NDDP, liposomal NDDP; DACH, 1,2-diaminocyclohexane; DMPC, dimystyrl phosphatidylcholine; DMPE, dimystyrl phosphatidylglycerol; HPLC, high-performance liquid chromatography; lD50, 50% inhibitory concentration; RI, resistance index; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; AAS, atomic absorption spectrophotometry.
were grown for no more than 3 mo in RPMI Medium 1640 (Gibco, Grand Island, NY) containing 10% fetal calf serum plus penicillin and insulin. During this interval, resistance was retained.

Cytotoxicity Assay. Cells were trypsinized, counted, and seeded in 96-well plates (1 × 10⁴ cells/ml). Cells were allowed to attach overnight and then exposed to various concentrations of drugs (5.0 to 150 μM), in quadruplicate wells, for 1, 5, and 24 h at 37°C. At the completion of drug treatment, the medium was removed, the cells were washed 3 times with PBS (8.0 mM Na₂HPO₄-1.5 mM KH₂PO₄-0.14 mM NaCl-2.6 mM KCl), and fresh medium was added. After overnight (20–24 h) culture, the cell survival fractions were determined with MTT dye reduction as described by Hansen et al. (25).

The assays using 24-h drug incubation were performed directly after continuous drug exposure for 24 h. All experiments were repeated at least 5 times.

Drug Accumulation Studies. Cells (2 × 10⁴ cells/well) were seeded in 6-well plates and incubated for 48 h. Cells in exponential growth phase were then exposed to various concentrations of drugs (0 to 600 μM) or a single concentration (38 μM) for various times (0 to 6 h). Immediately after drug exposure, the medium was removed, and the cells were washed twice with cold PBS. Cells were detached with 100 μM EDTA solution in PBS for 5 min, harvested, centrifuged, and resuspended in 0.1 ml of saline. After 0.2 ml of 2 N NaOH solution was added, cells were digested at 50°C for 1 h. The cellular derived protein was determined with a protein assay kit (Sigma, MO; Catalog No. 56556), and the amount of elemental platinum was measured by AAS. All experiments were performed in triplicate. Cellular drug accumulation was expressed as ng of platinum per mg of protein.

Platinum Determinations. Atomic absorption spectra were recorded with the following Varian Technion units: Model SpectraAA-30 AAS with platinum hollow cathode lamp (No. 56-101041-00); GTA-96 graphite tube analyzer, and DS-15 data station. The platinum concentrations in the samples were determined from a calibration curve obtained with Na₃PtCl₆ solutions in 0.1 N HCl solution.

Determination of DNA Platinations. DNA was isolated according to the method of Kirby and Cooke (26). Exponentially growing cells (10 to 20 × 10⁶ cells) were exposed to various concentrations of drugs (30, 60, and 120 μM) for 1 h at 37°C. Subsequently, the medium was removed, and the cells were washed 3 times with PBS and postincubated in fresh medium at 37°C for 0 to 24 h. After cells were washed once, 100 μM EDTA solution was added. The cells were then harvested, centrifuged, and resuspended in 0.1 ml of PBS. The cellular derived protein was determined with a protein assay kit (Sigma, MO; Catalog No. 56556), and the amount of elemental platinum was measured by AAS. All experiments were performed in triplicate. Cellular drug accumulation was expressed as ng of platinum per mg of protein.

RESULTS

Cytotoxicity. Table 1 shows the results of cytotoxicity (ID₅₀ and RI) for cisplatin and L-NDDP in both cells using a 5- and 24-h drug exposure. L-NDDP was equally cytotoxic against both cells (RI, 1.30 to 1.85), whereas cisplatin was significantly less cytotoxic against A2780/PDD cells (RI, 4.02 to 4.50). L-NDDP is, therefore, not cross-resistant with cisplatin in these experimental systems. Moreover, the cytotoxicity of L-NDDP using 5-h drug exposure was directly related to the content of DMPG in the liposomal carrier, whereas using 24-h drug exposure, that was found to be independent of the liposome composition of L-NDDP. In other words, the presence of DMPG in the liposome did not enhance the cytotoxic effect of L-NDDP with 24-h drug exposure.

For example, when using 5-h drug exposure, the ID₅₀ of L-NDDP containing DMPG was 2- to 3- and 2- to 4-fold lower than that of L-NDDP containing DMPC alone in A2780/S and A2780/PDD cells, respectively. In contrast, when using 24-h drug exposure, the ID₅₀ of L-NDDP was similar in both cells, regardless of lipid composition. However, the RI values of L-NDDP and cisplatin were independent of the drug exposure time.

Table 1. MTT assay data using drug exposure for 5 h or 24 h

<table>
<thead>
<tr>
<th>Drug</th>
<th>5 h ID₅₀ (A2780/S)</th>
<th>5 h ID₅₀ (A2780/PDD)</th>
<th>24 h ID₅₀ (A2780/S)</th>
<th>24 h ID₅₀ (A2780/PDD)</th>
<th>RI ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>30.0 ± 2.0</td>
<td>16.7 ± 3.7</td>
<td>120.7 ± 10.7</td>
<td>75.1 ± 5.9</td>
<td>4.02</td>
</tr>
<tr>
<td>L-NDDP (7:3)</td>
<td>18.2 ± 2.9</td>
<td>8.2 ± 1.9</td>
<td>28.9 ± 2.9</td>
<td>10.7 ± 2.2</td>
<td>1.59</td>
</tr>
<tr>
<td>L-NDDP (3:7)</td>
<td>11.0 ± 1.9</td>
<td>8.5 ± 2.1</td>
<td>14.3 ± 3.0</td>
<td>12.3 ± 1.9</td>
<td>1.30</td>
</tr>
<tr>
<td>L-NDDP (DMPC)</td>
<td>9.3 ± 1.8</td>
<td>4.9 ± 1.0</td>
<td>11.4 ± 2.9</td>
<td>9.0 ± 0.9</td>
<td>1.23</td>
</tr>
<tr>
<td>L-NDDP (DMPC)</td>
<td>31.9 ± 3.0</td>
<td>4.9 ± 2.2</td>
<td>59.1 ± 5.2</td>
<td>8.9 ± 1.7</td>
<td>1.85</td>
</tr>
</tbody>
</table>

² All ID₅₀ values expressed as μM.  
² RI, resistance index, ratio of ID₅₀ in resistant cells to ID₅₀ in sensitive cells.  
² Mean ± SD from at least five separate experiments.  
² L-NDDP contained either DMPC, DMPG, or both in molar ratios of either 7:3 or 3:7.
Cellular Accumulation. Fig. 2 shows the concentration (A) and time course (B) of the cellular accumulation with L-NDDP of different lipid composition and cisplatin in A2780/S cells. The results indicate that the accumulation of L-NDDP in A2780/S cells is 2- to 5-fold higher than that of cisplatin. The difference is small at low drug concentration (6 to 40 μM) or short time of drug exposure (0 to 30 min), but markedly increased at high drug concentrations (50 to 600 μM) or long time of drug exposure (1 to 6 h). Under the former conditions, the cellular accumulation of L-NDDP was independent of liposome composition. In contrast, under the latter conditions, the mere presence of DMPG in the liposomes regardless of the DMPC:DMPG ratio enhanced the cellular accumulation of L-NDDP by 2-fold compared with L-NDDP formulation using only DMPC. The lower cellular accumulation of L-NDDP containing only DMPC is interesting in view of its decreased cytotoxic potency reported above and its decreased in vivo antitumor activity previously reported (23).

Cellular and DNA Platination Studies. In the cellular accumulation studies, we observed that L-NDDP resulted in a much higher accumulation than did cisplatin in both A2780/S and A2780/PDD cells. To ascertain whether the lack of cross-resistance of L-NDDP is due to a higher level of DNA platination secondary to the higher cellular accumulation of L-NDDP, we studied the levels of DNA platination achieved at different times after incubating the cells at various drug concentrations of both drugs. In all these experiments, the DMPC:DMPG ratio was 7:3.
Table 2  Cellular accumulation and DNA platination in A2780/S cells treated with cisplatin and L-NDDP

A2780/S cells were treated for 1 h with cisplatin or L-NDDP composed of DMPC:DMPG (7:3 molar ratio) and postincubated for 0, 6, and 24 h. The cellular accumulation (Pt/protein) and the DNA platination (Pt/DNA) were determined as described in "Materials and Methods."

| Drug (μM) | 0 h | 6 h | 24 h | 0 h | 6 h | 24 h | Pt/DNA* | Pt/protein | % cell kill
|----------|-----|-----|------|-----|-----|------|---------|------------|------------
| Cisplatin (30) | 28.8 ± 3.5 | 21.3 ± 6.1 | 20.9 ± 5.3 | 9.8 ± 4.1 | 23.3 ± 3.2 | 18.6 ± 3.9 | 1.09 | 16.5 ± 0.7 | 16.5 ± 0.7
| Cisplatin (60) | 59.7 ± 8.8 | 41.6 ± 8.6 | 43.1 ± 9.9 | 10.6 ± 3.9 | 80.0 ± 4.7 | 18.1 ± 3.6 | 1.92 | 34.5 ± 1.4 | 34.5 ± 1.4
| Cisplatin (120) | 103.5 ± 9.2 | 80.7 ± 12.9 | 51.1 ± 5.7 | 14.2 ± 5.5 | 152.5 ± 13.6 | 23.9 ± 4.5 | 1.88 | 49.5 ± 1.9 | 49.5 ± 1.9
| L-NDDP (30) | 150.5 ± 11.4 | 109.6 ± 15.4 | 58.5 ± 7.6 | 10.4 ± 3.5 | 58.5 ± 9.2 | 31.7 ± 5.0 | 0.53 | 33.5 ± 4.9 | 33.5 ± 4.9
| L-NDDP (60) | 247.3 ± 24.3 | 192.8 ± 15.9 | 74.1 ± 16.6 | 15.2 ± 3.9 | 99.2 ± 9.3 | 37.7 ± 6.3 | 0.51 | 52.0 ± 4.2 | 52.0 ± 4.2
| L-NDDP (120) | 365.6 ± 36.6 | 234.9 ± 24.1 | 93.3 ± 10.6 | 28.6 ± 6.6 | 212.0 ± 18.9 | 50.3 ± 9.7 | 0.90 | 73.0 ± 4.2 | 73.0 ± 4.2

* DNA:protein platination index, the ratio of DNA platination to total cellular accumulation.

Table 3  Cellular accumulation and DNA platination in A2780/PDD cells treated with cisplatin and L-NDDP

A2780/PDD cells were treated for 1 h with cisplatin or L-NDDP composed of DMPC:DMPG (7:3 molar ratio) and postincubated for 0, 6, and 24 h. The cellular accumulation (Pt/protein) and the DNA platination (Pt/DNA) were determined as described in "Materials and Methods."

| Drug (μM) | 0 h | 6 h | 24 h | 0 h | 6 h | 24 h | Pt/DNA* | Pt/protein | % cell kill
|----------|-----|-----|------|-----|-----|------|---------|------------|------------
| Cisplatin (30) | 12.8 ± 3.9 | 10.8 ± 3.3 | 10.0 ± 3.9 | 7.3 ± 3.6 | 13.7 ± 6.2 | 13.7 ± 5.1 | 1.26 | 5.7 ± 1.4 | 5.7 ± 1.4
| Cisplatin (60) | 27.7 ± 5.0 | 20.1 ± 6.3 | 15.8 ± 5.8 | 9.9 ± 5.9 | 19.3 ± 5.1 | 14.4 ± 6.0 | 0.96 | 8.4 ± 3.5 | 8.4 ± 3.5
| Cisplatin (120) | 32.6 ± 9.5 | 24.0 ± 6.9 | 23.9 ± 7.9 | 10.7 ± 5.1 | 26.5 ± 7.5 | 23.5 ± 5.7 | 1.10 | 13.8 ± 5.6 | 13.8 ± 5.6
| L-NDDP (30) | 116.7 ± 19.9 | 96.0 ± 2.8 | 50.7 ± 6.9 | 14.3 ± 6.7 | 34.7 ± 7.8 | 20.8 ± 2.8 | 0.36 | 15.6 ± 4.4 | 15.6 ± 4.4
| L-NDDP (60) | 186.7 ± 17.1 | 105.9 ± 24.3 | 50.3 ± 9.9 | 16.9 ± 3.9 | 58.1 ± 6.9 | 30.4 ± 9.1 | 0.55 | 33.0 ± 1.4 | 33.0 ± 1.4
| L-NDDP (120) | 289.6 ± 37.0 | 159.2 ± 27.8 | 26.5 ± 7.9 | 18.1 ± 5.7 | 82.1 ± 10.1 | 50.3 ± 9.7 | 0.51 | 46.2 ± 4.2 | 46.2 ± 4.2

* DNA:protein platination index, the ratio of DNA platination to total cellular accumulation.

When the data are analyzed at equitoxic concentrations (120 μM cisplatin versus 60 μM L-NDDP), the total cellular accumulation of L-NDDP (192.8) was 2.5-fold higher than that of cisplatin (80.7), while the Pt/DNA levels of L-NDDP (99.2) at 6 h were about 1.5-fold lower than those of cisplatin (152.5). These results might be interpreted as indicating that DNA-platinum lesions induced by cisplatin are less lethal than those of L-NDDP. However, this conclusion cannot be drawn from these experiments alone.

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be drawn because peak DNA/Pt levels occurred at 6 h for cisplatin and 9 h for L-NDDP (Fig. 4).

The results of Pt/protein and Pt/DNA levels obtained in A2780/PDD cells (Table 3) show that the cellular accumulation of L-NDDP was about 7- to 10-fold higher than that of cisplatin at time 0 [this difference was slightly lower (3- to 5-fold) in sensitive cells]. Efflux of cisplatin was minimal as in A2780/PDD cells. In contrast, 60 to 70% of L-NDDP was effluxed by 24 h. As a result, the cellular accumulation of L-NDDP at 6 h was still 5- to 9-fold higher than that of cisplatin. Compared with A2780/S cells, the cellular accumulations of cisplatin and L-NDDP in A2780/PDD cells at 6 h were 2- to 3-, and 1- to 1.5-fold lower, respectively.

Pt/DNA levels with L-NDDP were 2- to 3-fold higher than with cisplatin (in sensitive cells, the difference was a maximum 2-fold). Compared with levels in A2780/S cells, Pt/DNA levels at 6 h in A2780/PDD cells were 2- to 6-fold lower for cisplatin and only about 2-fold lower for L-NDDP, in good correlation with the cellular accumulation. The DNA-protein platination index for cisplatin ranged between 0.96 and 1.26 in A2780/PDD cells (down from 1.09 to 1.92 in sensitive cells) and for L-NDDP, between 0.36 and 0.55 (down from 0.51 to 0.90 in sensitive cells). Therefore, the transfer from drug to nucleus was similarly affected for both drugs in resistant cells compared with sensitive cells, and the increasing difference in Pt/DNA levels between both drugs in resistant cells could be mostly accounted for by the increasing difference in total cellular accumulation. When the data are analyzed at equitoxic concentration (120 μM cisplatin and 30 μM L-NDDP), the cellular accumulation of L-NDDP was about 4-fold higher, whereas levels of Pt/DNA were similar.

To better assess the kinetics of formation of platinum-DNA adducts over time, we exposed both types of cells to 60 μM cisplatin or L-NDDP for 1 h and then measured DNA platination levels at 0, 3, 6, 9, 12, and 24 h postincubation (Fig. 4). Interestingly, Pt/DNA levels induced by L-NDDP reached their peak at 9 to 12 h in both cells, 3 to 6 h later than the cisplatin peaks. Levels at 0 and 24 h were similar for both drugs. The Pt/DNA levels × time were also calculated (Table 4). These results may indicate that L-NDDP is delivered to the nucleus in a more sustained fashion than cisplatin so that high levels are reached at 6 h and even higher levels at 9 h, whereas in the case of cisplatin, levels at 9 h are significantly reduced compared with levels at 6 h. As a result, the Pt/DNA × time levels of L-NDDP are almost 2-fold higher than those of cisplatin in sensitive cells and 3-fold higher in resistant cells. Pt/DNA × time levels of L-NDDP were only slightly reduced in resistant cells compared with sensitive cells.

**DNA Interstrand and DNA-Protein Cross-Link Formation.** To elucidate the type of DNA-platinum interaction that correlates with the cytotoxicity of L-NDDP, DNA interstrand and DNA-protein cross-links were determined by alkaline elution at different postincubation times of cells exposed to either drug.

Results of DNA interstrand cross-linking formation using 60 μM of both drugs are shown in Fig. 5. Cisplatin produced significant DNA interstrand cross-linking in A2780/S cells only at 6 h, but L-NDDP did not induce significant interstrand cross-linking at any time point. In A2780/PDD cells, interstrand cross-linking induced by cisplatin was markedly reduced, and L-NDDP did not induce significant interstrand cross-linking. These results indicate a correlation between interstrand cross-linking and cisplatin cytotoxicity and suggest that this lesion is not involved in the cytotoxicity of L-NDDP.

Fig. 6 shows the results of DNA-protein cross-linking. L-NDDP induced significant DNA-protein cross-linking in both cells, although somewhat less in resistant cells. DNA-protein cross-linking induced by L-NDDP was about twice that induced by cisplatin at equimolar concentrations in sensitive cells. In resistant cells, DNA-protein cross-linking induced by cisplatin was minimal. These results indicate a correlation between DNA-protein cross-links and cytotoxicity for both compounds.

**DISCUSSION**

The results of our study indicate that (a) L-NDDP is not cross-resistant to cisplatin against A2780/PDD ovarian carcinoma cells, (b) the cellular accumulation of L-NDDP is similar in both cells and several-fold higher than that of cisplatin, (c) the cytotoxicity of both drugs against both cells correlates with the extent of DNA platination over time, (d) the lack of cross-resistance of L-NDDP is associated with its ability to induce similar DNA platination over time in both cells, and (e) DNA interstrand cross-links formation does not play a role in the cytotoxicity of L-NDDP.

The cell lines used in our study have been extensively characterized (28-33). The resistance of A2780/PDD cells has been found to be associated with a markedly reduced cellular accumulation of cisplatin (31, 33) and an increased ability to repair the DNA adducts caused by cisplatin (30-33). In our studies, we found that the cellular accumulation of cisplatin in resistant cells was about 30% of that in sensitive cells, which is in agreement with what has been reported previously (33). In sensitive cells, DNA platination by cisplatin reached a peak at 6 h and then declined quickly. In resistant cells, no DNA platination peak was observed, just a baseline value at all time points. It is not possible to discern from our studies whether the lack of DNA platination peak was due either to reduced delivery of cisplatin to the nucleus because of the markedly reduced cellular accumulation, or to...
about 50% higher than that of cisplatin at equimolar concentrations, in correlation with the 2-fold higher potency of L-NDDP. Therefore, our data do not suggest that DACH-Pt adducts are more cytotoxic than (NH₃)₂-Pt adducts as suggested by Schmidt and Chaney (33).

In sensitive cells, the peaks of DNA platination were observed at 6 and 9 h for cisplatin and L-NDDP, respectively. Formation of DNA adducts by L-NDDP was parallel with that of cisplatin until 6 h. The main difference between both drugs was observed from 6 to 9 h; i.e., the extent of DNA platination induced by cisplatin decreased quickly during this period, while the extent of DNA platination induced by L-NDDP slightly increased. The DNA platination curve of L-NDDP after 9 h was parallel to that of cisplatin after 6 h. It would appear that the sustained DNA platination by L-NDDP may be explained by a continuous delivery of L-NDDP to the nucleus, which would be able to replace the platinum-DNA adducts repaired and/or by a decreased ability of the cells to remove the platinum-DNA adducts induced by L-NDDP, or a combination of both. In regard to the first possibility, it is reasonable to hypothesize that, because of its high lipophilicity, most intracellular L-NDDP may remain bound to lipidic cytoplasmic structures and then may be slowly released and transferred to the nucleus, the cytoplasm acting as a depot system.

In resistant cells, the DNA platination curve of L-NDDP was similar to that in sensitive cells, with the peak of platination at 9 h, except for a slightly decreased slope in the initial 9 h. No peak was observed in the DNA platination curve of cisplatin in resistant cells. As a result, the extent of DNA platination over time in the case of L-NDDP was only about 20% lower in resistant cells (in the case of cisplatin it was 50% lower) compared with sensitive cells. This suggests that the delivery of L-NDDP to the nucleus and the repair of DNA adducts induced by L-NDDP are only slightly affected in resistant cells compared with sensitive cells.

Another important observation was that L-NDDP did not induce significant interstrand cross-linking in either cell line, thus suggesting that this lesion does not play a role in the cytotoxicity of L-NDDP, while in the case of cisplatin, induction of interstrand cross-linking correlated with cytotoxicity. This finding suggests significant differences in the way both drugs interact with DNA, and such different interaction may also explain differences in DNA adduct repair for both drugs.

In conclusion, our study suggests that L-NDDP is able to overcome cisplatin resistance, probably by a combination of the following factors: increased cellular drug accumulation; more prolonged drug delivery to the nucleus; and decreased ability of resistant cells to repair DNA adducts induced by L-NDDP compared with those induced by cisplatin. The favorable toxicity profile of L-NDDP in humans (21) and the confirmation in a different cell system of its previously reported ability to overcome resistance (20) justify continuing the development of this agent for the treatment of cisplatin-resistant tumors.

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