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 indexes, 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 DMPC 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 DMPC 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 antimetabolite 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 N-7 guanine sites. Only high drug concentrations produce adducts involving 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 anitumor 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(3) (Fig. 1) is a lipophilic cisplatin derivative of the 1,2-diaminocyclohexane family which has been formulated in a liposome composed of DMPC and DMPG 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 DMPC 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 DMPC content in the lipid bilayer, the higher its in vivo antitumor potency. We hypothesized that DMPC might alter the pharmacokinetics and/or cellular pharmacology of NDDP or react with NDDP, resulting in more active platinum complexes.

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 or only DMPG or both in a molar ratio of DMPC:DMPG = 7:3 and 3:7 (20-23). DMPC and DMPG 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...
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 × 10^4 cells/well). Cells were then centrifuged. and the DNA pellets were resuspended in 100 µl of 100 mM Tris-HCl (pH 7.4) containing 10 mM EDTA. The concentrations of platinum and alcohol. The samples were stored at -70°C overnight. They were then centrifuged. and the DNA pellets were resuspended in 100 µl of 100 mM Tris-HCl (pH 7.4). Extracted DNA was precipitated with 750 mM NaCl solution and isopropyl alcohol and then DNA was extracted 3 times with phenol (0.3 ml) and chloroform (0.3 ml). Cells were then harvested, centrifuged, and resuspended in 0.1 ml of PBS. The cells were lysed with lysis buffer (0.5% Triton X-100-5 nM EDTA in PBS), and then DNA was extracted 3 times with phenol (0.3 ml) and chloroform (0.3 ml). Extracted DNA was precipitated with 750 mM NaCl solution and isopropyl alcohol. The samples were stored at -70°C overnight. They were then centrifuged. and the DNA pellets were resuspended in 100 µl of 100 mM Tris-HCl buffer (pH 7.4) containing 10 mM EDTA. The concentrations of platinum and DNA were determined by AAS at 265.9 nm and UV absorption spectrometry at 260 nm, respectively.

**DNA Interstrand and DNA-Protein Cross-Link Determination.** DNA interstrand cross-links and DNA-protein cross-links were determined by the alkaline elution technique as described by Kohn et al. (27). The cells (0.5 × 10^6 cells/well) were seeded in 6-well plates and incubated overnight. They were then labeled with 5 µl of [14C]thymidine (Amersham; 56 Ci/mol) for 24 h. After being labeled, the cells were chased for 3 h in fresh medium and exposed to 60 µM of drugs for 1 h. Subsequently, the cells were washed 3 times with PBS and postincubated in fresh medium for 0, 6, and 24 h at 37°C. After being washed once with PBS, the treated and untreated cells were simultaneously irradiated with 600 rads of X-ray at 0°C. Then, the cells were deposited on 2-µm-pore-size polycarbonate filters (Costar, MA) and lysed with 5 ml of lysis buffer containing 2% lauroyl sarcosine, 0.1 M NaCl, and 0.025 M EDTA (pH 9.6). Proteinase K (0.5 mg/ml) was added to accomplish proteolytic digestion of the lysate. The eluting solution (0.1% SDS-0.1 M tetra-n-propyl-ammonium hydroxide-0.02 M EDTA, pH 12.1) was then slowly pumped through the filter at 1.5 ml/h, and the fractions were collected at 2-h intervals for at least 20 h to determine the rate of release of DNA from the filter. Radioactivity of the eluted fractions and filters was determined by liquid scintillation counting. Cross-linking was quantitated in terms of cross-linking coefficients Kc determined by Kc = [(1 - r0) - (1 - r)]^2 - 1 × 600 (rads), where r and r0 are the retention rates of DNA on the filter from the drug-treated and control cells, respectively.

**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 DMPC 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 DMPC 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 DMPC 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.

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>5 h</th>
<th>24 h</th>
<th>5 h</th>
<th>24 h</th>
<th>5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cisplatin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A2780/S)</td>
<td>30±2</td>
<td>16.7±3.7</td>
<td>120.7±10.7</td>
<td>75.1±5.9</td>
<td>4.02</td>
<td>4.50</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>
<td>1.30</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>
<td>1.45</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>
<td>1.83</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>
<td>1.81</td>
</tr>
</tbody>
</table>

- All ID50 values expressed as µM.
- RI, resistance index, ratio of ID50 to ID50 in sensitive cells.
- Mean ± SD from at least five separate experiments.
- 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).

Fig. 3 shows the concentration (A) and time (B) course of the cellular accumulation with L-NDDP and cisplatin in A2780/PDD cells. Cellular accumulation of cisplatin in A2780/PDD cells was 3- to 5-fold lower than that in A2780/S cells. In the case of L-NDDP, a slightly lower (30 to 50%) cellular accumulation was observed in A2780/PDD cells compared with that in A2780/S cells. The cellular accumulation of L-NDDP containing DMPC alone in A2780/PDD cells was about 2-fold lower than that of the L-NDDP formulations containing DMPG.

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

<table>
<thead>
<tr>
<th>Drug (μM)</th>
<th>0 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pt/protein (ng/mg)</td>
<td>Pt/DNA (ng/mg)</td>
<td>Pt/DNA* Pt/protein</td>
</tr>
<tr>
<td>Cisplatin (30)</td>
<td>28.8 ± 3.5</td>
<td>21.3 ± 6.1</td>
<td>20.9 ± 5.3</td>
</tr>
<tr>
<td>Cisplatin (60)</td>
<td>59.7 ± 8.8</td>
<td>41.6 ± 8.6</td>
<td>43.1 ± 9.9</td>
</tr>
<tr>
<td>Cisplatin (120)</td>
<td>103.5 ± 9.2</td>
<td>80.7 ± 12.9</td>
<td>51.1 ± 5.7</td>
</tr>
<tr>
<td>L-NDDP (30)</td>
<td>150.5 ± 11.4</td>
<td>109.6 ± 15.4</td>
<td>58.5 ± 7.6</td>
</tr>
<tr>
<td>L-NDDP (60)</td>
<td>247.3 ± 24.3</td>
<td>192.8 ± 15.9</td>
<td>74.1 ± 16.6</td>
</tr>
<tr>
<td>L-NDDP (120)</td>
<td>365.6 ± 36.6</td>
<td>234.9 ± 24.1</td>
<td>93.3 ± 10.6</td>
</tr>
</tbody>
</table>

- DNA:protein platination index, the ratio of DNA platination to total cellular accumulation.
- The percentage of cell kill was obtained by MTT assay following a 1-h drug exposure and 24-h postincubation.
- Mean ± SD from three independent experiments.

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

<table>
<thead>
<tr>
<th>Drug (μM)</th>
<th>0 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pt/protein (ng/mg)</td>
<td>Pt/DNA (ng/mg)</td>
<td>Pt/DNA* Pt/protein</td>
</tr>
<tr>
<td>Cisplatin (30)</td>
<td>12.8 ± 3.9</td>
<td>10.8 ± 3.3</td>
<td>10.0 ± 3.9</td>
</tr>
<tr>
<td>Cisplatin (60)</td>
<td>27.7 ± 5.0</td>
<td>20.1 ± 6.3</td>
<td>15.8 ± 5.8</td>
</tr>
<tr>
<td>Cisplatin (120)</td>
<td>32.6 ± 9.5</td>
<td>24.0 ± 6.9</td>
<td>10.7 ± 5.1</td>
</tr>
<tr>
<td>L-NDDP (30)</td>
<td>116.7 ± 19.9</td>
<td>96.0 ± 2.8</td>
<td>50.7 ± 6.9</td>
</tr>
<tr>
<td>L-NDDP (60)</td>
<td>186.7 ± 17.1</td>
<td>105.9 ± 24.3</td>
<td>50.3 ± 9.9</td>
</tr>
<tr>
<td>L-NDDP (120)</td>
<td>289.6 ± 37.0</td>
<td>159.2 ± 27.8</td>
<td>58.1 ± 9.8</td>
</tr>
</tbody>
</table>

- DNA:protein platination index, the ratio of DNA platination to total cellular accumulation.
- The percentage of cell kill was obtained by MTT assay following a 1-h drug exposure and 24-h postincubation.
- Mean ± SD from three independent experiments.

Tables 2 and 3 show the total cellular Pt (expressed as ng of Pt per mg of protein) and DNA-bound Pt (expressed as ng of Pt per mg of DNA) values for each drug concentration at different postincubation times in A2780/S and A2780/PDD cells, respectively. The DNA platination levels measured include any type of monofunctional or bifunctional platinum-DNA adducts including DNA intrastrand cross-links, DNA interstrand cross-links, and DNA-protein cross-links. The tables also include the percentage of drug killing to allow for comparisons in DNA platination between cell cultures in which a similar cytotoxic effect was observed.

The results of Pt/Protein and Pt/DNA levels on A2780/S cells showed that the total cellular accumulation of L-NDDP was 3- to 5-fold higher than that of cisplatin at 0 and 6 h. Drug efflux during the initial 6 h was similar for both drugs in both cells (about 30%). Between 6 and 24 h, cisplatin was not significantly effluxed, but the efflux of L-NDDP by 24 h was about 60%. From these results, it would appear that L-NDDP is more easily transported across the cell membrane in both directions compared with cisplatin. Pt/DNA levels increased from 0 to 6 h and then decreased by 24 h for both drugs. Pt/DNA levels reached with L-NDDP were, in general, similar to or more than 2-fold higher than those achieved with cisplatin. For example, at 60 μM, cisplatin achieved Pt/DNA levels of 80 ng/mg of DNA from a total cellular accumulation (Pt/Protein) of 41.6 ng/mg of protein, whereas 60 μM L-NDDP resulted in Pt/DNA levels of 99.2 ng/mg of DNA from a total cellular accumulation of 192.8 ng/mg of protein. These results indicate that a higher proportion of intracellular cisplatin reacts with DNA compared with L-NDDP. Therefore, although the total cellular accumulation of L-NDDP is much higher, the impact of such increased accumulation on DNA platination is smaller. A DNA:protein platination index, which is an indication of DNA platination relative to total cellular accumulation, was calculated. In A2780/S cells this index ranged between 1.09 and 1.92 for cisplatin and between 0.51 and 0.90 for L-NDDP at 6 h postincubation.

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, that conclusion cannot...
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 severalfold 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

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**Table 4** DNA platination × time in A2780/S and A2780/PDD cells treated with cisplatin and L-NDDP

<table>
<thead>
<tr>
<th>Drug (60 µM)</th>
<th>A2780/S</th>
<th>A2780/PDD</th>
<th>A2780/S</th>
<th>A2780/PDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>960</td>
<td>480</td>
<td>2.0</td>
<td>1.28</td>
</tr>
<tr>
<td>L-NDDP</td>
<td>1575</td>
<td>1230</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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![Graph showing DNA interstrand and DNA-Protein Cross-Link Formation](image)
an increased detoxification of cisplatin in the cytoplasm, or to a rapid removal of the DNA adducts formed.

The lack of cross-resistance of L-NDDP is interesting in view of the fact that tetraplatin, also a diancyclohexaneplatinum(II) compound like NDDP, has been found to be cross-resistant with cisplatin in this cell line, while other diancyclohexaneplatinum(II) compounds are able to partially overcome drug resistance (33). This raises the possibility that the liposomal carrier or the high lipophilicity of NDDP plays a more important role than the diancyclohexane moiety in overcoming the resistance to cisplatin. Because the focus of the studies presented was the comparison between cisplatin and L-NDDP, no conclusions can be drawn on the contribution of the carrier or the high lipophilicity of NDDP. Nevertheless, 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 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.

ACKNOWLEDGMENTS

We wish to thank Dr. Zahid Siddik for supplying the A2780/S and A2780/PDD cells, Dr. Timothy Madden for assisting in the platinum determinations, and Kevin Flynn for editorial assistance.

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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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