Treatment and Prophylaxis of Experimental Liver Metastases of M5076 Reticulosarcoma with cis-Bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II) Encapsulated in Multilamellar Vesicles

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ABSTRACT

cis-Bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II) (NDDP) was encapsulated in multilamellar vesicles composed of dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol at a 7:3 molar ratio. Compared with cisplatin, i.v. administration of an equimolar dose of liposome-encapsulated NDDP (L-NDDP) resulted in 15-fold higher peak platinum levels in the spleen (204.7 versus 13.3 μg/g dry tissue), 5-fold higher in the lungs (116.4 versus 21.0 μg/g dry tissue), 3-fold higher in the liver (71.6 versus 23.9 μg/g dry tissue), and 4-fold higher in the blood (14.8 versus 3.9 μg/ml). At the optimal dose and schedule, L-NDDP administered i.p. in mice bearing peritoneal L1210 leukemia resulted in the percentage of median survival time of treated mice divided by median survival time of control mice (%/C) of 312 versus 225 for cisplatin and free NDDP. When administered i.v., L-NDDP was also more active than cisplatin against L1210 leukemia inoculated i.v. (%/C: 186 versus 142). L-NDDP was markedly active against L1210 leukemia resistant to cisplatin (%/C: 200 versus 112 for cisplatin). In mice bearing liver metastases of M5076 reticulosarcoma, L-NDDP was significantly more effective than cisplatin at equimolar doses (mean survival time, 57 ± 9 (SD) days for L-NDDP versus 42 ± 3 days for cisplatin, P < 0.05). L-NDDP was also effective in preventing liver metastases of M5076 when administered up to 24 h prior to tumor inoculation (mean survival, 28 ± 2 days for L-NDDP versus 22 ± 2 days for cisplatin, P < 0.05). L-NDDP is significantly non-cross-resistant with cisplatin and more effective against phagocytic and nonphagocytic murine tumors.

INTRODUCTION

Liposomes are nontoxic, biodegradable lipid vesicles that can alter the distribution and bioavailability of drugs (1, 2). The potential use of liposomes as drug carriers has been exploited to improve the therapeutic index of several antimicrobials and anticancer agents. Liposomal amphotericin B was shown to be less toxic and more active than free amphotericin B in the treatment of disseminated candidiasis both in mice (3) and humans (4). Several investigators have shown that incorporation of doxorubicin in liposomes reduces its cardiac toxicity (5, 6). Furthermore, Mayhew et al. (7, 8) and Gabizon et al. (9) demonstrated that liposome-encapsulated doxorubicin was more active than free doxorubicin in the treatment of established liver metastases of phagocytic and nonphagocytic tumors.

Formulation has been one of the major problems in the clinical development of liposome-encapsulated drugs. The problem may be avoided by developing drug analogues with structures that retain the desired antitumor effect while promoting a better association with the drug carrier.

Cisplatin is one of the most active antitumor agents (10, 11). However, its use can be limited by nephrotoxicity and neurotoxicity. Recently, less nephrotoxic and non-cross-resistant analogues of cisplatin have become available (12, 13). Liposome encapsulation of some of these platinum complexes may increase their antitumor activity in tumors involving the target organs of liposomes and may modify certain platinum toxicities, such as emesis and neurotoxicity. We report here on the development of a liposomal-platinum formulation containing a lipophilic cisplatin analogue specifically designed for liposome encapsulation, which can be completely incorporated within the phospholipid bilayers. The preparation obtained was found to be stable, significantly non-cross-resistant with cisplatin, and more active than cisplatin against experimental liver metastases of M5076 reticulosarcoma in mice.

MATERIALS AND METHODS

Chemicals and Lipids. Neodecanoic acid was a gift from Exxon Corp. (Houston, TX). The material is an isomeric mixture. Purity (C-10 content) is >95%. Reproducibility between batches (determined by gas chromatography) is >95%. DMPC and DMPG were obtained from Avanti Polar Lipids (Birmingham, AL). Animals. C57BL × DBA/2 F₁ (hereafter called BD2F₁) and C57BL/6 mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA).

Cell Lines. L1210/0 cells were obtained from the Department of Pharmacology, University of Vermont, Burlington, VT, and were kept in vivo as an ascitic tumor in BD2F₁ mice. L1210/PDD cells were obtained from the Division of Cancer Treatment tumor repository, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD. The cell line was grown in vivo in the peritoneal cavity of BD2F₁ mice and was transplanted weekly. Animals bearing L1210/PDD leukemia were treated on day 5 with 5 mg/kg cisplatin.

M5076 is a murine reticulosarcoma that arose in the ovary of a C57BL/6 mouse. M5076 cells display characteristics of the monococyte-macrophage lineage (14). M5076 cells metastasize predominantly to the liver and spleen, independently of the route of inoculation (15). M5076 cells were obtained from Dr. Isaiah J. Fidler from the Department of Cell Biology at The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, and were kept in culture in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 15% horse serum. For the in vivo experiments, M5076 cells were kept in vivo as an ascitic tumor in C57BL/6 mice and were transplanted every 3 weeks. In vivo cells tend to result in a higher number of liver metastases when compared with in vitro cells, with no significant change in survival of the animals.

Synthesis of cis-Bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II). Sulfato-trans-R,R-1,2-diaminocyclohexaneplatinum(II) was synthesized as reported (16). NDDP was synthesized through a substitution reaction between sulfato-trans-R,R-1,2-diaminocyclohexaneplatinum(II) and potassium neodecanoate prepared in situ by reaction of neodecanoic acid and potassium hydroxide.

The abbreviations used are: DMPC, dimyristoyl phosphatidylcholine; NDDP, cis-bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II); DMPG, dimyristoyl phosphatidylglycerol; L-NDDP, liposome-encapsulated NDDP; T/C, median survival of treated mice divided by median survival of control mice; MTT, 3(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide.
reacting potassium hydroxide and neodecanoic acid. The resulting material was extracted in chloroform and was dried over anhydrous magnesium sulfate. The magnesium sulfate was separated by filtration, and the filtrate was evaporated to dryness. An off-white solid product was obtained, which was dried in vacuum over P₂O₅. Synthesis yield was 70%. The final product was stored at 0°C. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. The elemental analysis of the product obtained was:

**NDDP**

Calculated: C 47.93, H 8.00, N 4.30  
Found: C 47.75, H 8.16, N 3.98

The compound was found to be 91.7% pure by high-pressure liquid chromatography analysis by using 2 protein-Pak 160 columns in 100% methanol. NDDP (Fig. 1) is highly soluble in chloroform (>25 mg/ml) and methanol but completely insoluble in water solutions.

**Preparation of Liposomal NDDP.** L-NDDP was prepared as reported for other platinum complexes (17, 18). Chloroform solutions of DMPC and DMPG at a 7:3 molar ratio and NDDP were mixed at a 1:15 drug:lipid weight ratio. The chloroform was evaporated in a rotary evaporator, leaving a dry lipid film containing the lipids and the platinum analogue. Multilamellar liposomes containing NDDP were formed by adding 1 ml 0.9% NaCl solution in water per mg NDDP to the dry lipid film and shaking for a few minutes. To measure the encapsulation efficiency we centrifuged the liposome suspension at 30,000 × g for 45 min and measured the amount of NDDP in the supernatant or the elemental platinum in the pellet. NDDP was measured by UV spectrophotometry at a wavelength of 216 nm. Elemental platinum was measured by X-ray fluorescence in the Department of Analytical Chemistry, The University of Texas Medical School at Houston, Houston, TX (19). The encapsulation efficiency was calculated by the following formulas:

Encapsulation efficiency

\[
\text{Encapsulation efficiency} = \frac{\text{Total NDDP added} - \text{NDDP supernatant}}{\text{Total NDDP added}}
\]

Encapsulation efficiency

\[
\text{Encapsulation efficiency} = \frac{\text{Elemental platinum in pellet}}{\text{Elemental platinum added}}
\]

For all preparations, the encapsulation efficiency obtained with both methods was consistently >98%. The encapsulation efficiency was the same for vesicles kept at 4°C, room temperature, or 37°C. In addition, all preparations were checked by light microscopy, and no precipitates of free drug could be seen. In freeze-fracture electron microscopic studies, only multilamellar structures could be identified. The stability of L-NDDP (1 mg NDDP/ml) in a 0.9% NaCl solution in water at 4°C was assessed by measuring the amount of NDDP or elemental platinum in the supernatant at different time points. The stability at 14 days was found to be >95%. The stability of L-NDDP at 37°C was also >95% at 72 h.

L-NDDP vesicles were sized in a Coulter Counter (Coulter Electronics, Hialeah, FL). The size ranged from 0.5 to 5 μm, with most vesicles measuring between 1 and 3 μm. The pattern of vesicle size distribution was virtually identical in all batches of L-NDDP prepared.

**Tissue Distribution Studies.** Groups of 3 C57BL/6 mice were given injections of an equimolar dose of L-NDDP or cisplatin i.v. (18.5 mg/kg L-NDDP and 10 mg/kg cisplatin). Animals were sacrificed at 30 min, 4 h, and 24 h under anesthesia. Blood was collected from the retroorbital plexus in heparinized tubes, and the liver, spleen, lungs, and kidneys were resected. Whole blood and tissue levels of elemental platinum were measured by X-ray fluorescence. Results were expressed as μg of platinum/ml of blood and μg of platinum/g of dry tissue.

**In Vitro Cytotoxicity against M5076 Reticulosarcoma Cells.** Cytotoxicity was assessed with the MTT reduction assay, as reported but with slight modifications (20). The MTT dye was obtained from Sigma Chemical Co., St. Louis, MO. M5076 cells (2 × 10⁶ cells/well) were plated in 96-well microassay culture plates. Cisplatin or L-NDDP in a 0.9% NaCl solution in water was added to the wells to achieve a final drug concentration ranging from 1 to 10 μg/ml (8 wells were used for each concentration). The same volume of a 0.9% NaCl solution in water was added to control wells. Wells containing RPMI 1640 plus 15% horse serum without cells were used as blanks. The plate was incubated at 37°C in a 5% CO₂ incubator for 24 h. When incubation was complete, 15 μl of a stock solution of MTT dye in a 0.9% NaCl solution in water were added to each well to achieve a final dye concentration of 0.5 mg/ml. The plate was incubated at 37°C in a 5% CO₂ incubator for 4 h. To each well 165 μl of acidified isopropyl alcohol (0.04 N HCl in isopropanol) were added to solubilize the MTT formazan. Complete solubilization of the dye was achieved by repeated pipeting of the well contents with a multichannel pipet. The plate was kept at room temperature for 10 min, and the optical density of each well was measured with a microplate spectrophotometer at a wavelength of 600 nm. The percentage of cell viability was calculated by the following equation:

\[
\% \text{ of viability} = \frac{\text{Mean optical density of treated wells}}{\text{Mean optical density of control wells}}
\]

The percentage of viability values obtained were plotted against the drug concentrations used on semilogarithmic paper, and the drug concentrations resulting in a 50% cell viability and a 10% cell viability were calculated from the curve.

**In Vitro Antitumor Activity against L1210/0 and L1210/PDD Leukemia.** Two different routes of tumor inoculation and treatment were used in the experiments performed with the L1210/0 line. BDF2; mice, weighing 20–25 g, were inoculated with 10⁵ cells i.v. on day 0. Treatment was started on day 1, using the same route used for tumor inoculation. Two different treatment schedules were used: single dose on day 1 or triple dose on days 1, 5, and 9. Free NDDP was administered in suspension in hydroxypropylcellulose in the experiments in which the i.p. route was used. Cisplatin and L-NDDP were given at the maximum nontoxic dose defined as the dose that produces no lethality and less than 10% weight loss. For the single i.p. or i.v. injection, the maximum nontoxic doses of cisplatin and L-NDDP are 10 mg/kg and 37.5 mg/kg, respectively.

In the therapeutic experiments performed with the L1210/PDD line, 10⁶ cells were inoculated i.p. on day 0. Treatment was given i.p. starting on day 1, according to 2 schedules: single dose on day 1, or triple dose on days 1, 5, and 9.

**Treatment and Prophylaxis of Liver Metastases of M5076 Reticulosarcoma.** In the therapeutic experiments, groups of 6 to 8 C57BL/6 mice were inoculated i.v. on day 0 with 2 × 10⁶ M5076 cells obtained from the peritoneal cavity of tumor-bearing animals. Treatment was administered i.v. and was started on day 4. Three treatment schedules were used: 2 doses on days 4 and 8, 3 doses on days 4, 8, and 12, or 4 doses on days 4, 11, 18, and 25. The antitumor activity of both drugs was compared at maximum nontoxic doses and at equimolar doses. Animals were sacrificed at different time points, depending on the tumor inoculum and the treatment schedule used. The livers were dissected and placed in Bouin’s solution, and 2 independent investigators counted the number of tumor nodules on the liver surface. The remaining animals were used to record survival times.

In the prophylactic experiments, C57BL/6 mice were treated i.v. on day −1, −2, or −3. On day 0, animals were inoculated i.v. with 2 × 10⁶
M5076 cells obtained from the peritoneal cavity of tumor-bearing animals. Some animals were sacrificed on day 21, the livers were dissected and placed in Bouin's solution, and the number of tumor nodules on the liver surface was counted by 2 independent investigators. The remaining animals were used to record the survival times.

Statistical Analysis. Differences in the number of liver tumor nodules and survival times were analyzed for statistical significance with the Mann-Whitney and Student's t tests, respectively.

RESULTS

Tissue Distribution Studies. Peak platinum levels were observed at 30 min in most organs and blood both in the animals treated with L-NDDP and those treated with cisplatin. Peak liver platinum levels were observed at 4 h in the L-NDDP-treated mice (Table 1). Compared with the animals treated with cisplatin, those given injections of L-NDDP had 3- to 4-fold higher platinum levels at 30 min in the blood (14.8 versus 3.9 μg/ml), 2-fold higher in the liver (68.0 versus 33.3 μg/g dry tissue), 5-fold higher in the lung (116.4 versus 21.0 μg/g dry tissue), similarly in the kidneys (34.6 versus 41.7 μg/g dry tissue), and 15-fold higher in the spleen (204.7 versus 13.3 μg/g dry tissue). At 4 h, animals treated with L-NDDP still showed 3-fold higher platinum levels in blood (6.1 versus 1.9 μg/ml). Between 30 min and 4 h, the difference in liver platinum levels increased from 2- to 3-fold (76.6 versus 23.9 μg/g dry tissue), the difference in lung levels was reduced from 5- to 3-fold (41.9 versus 12.9 μg/g dry tissue), no significant change was observed in kidney platinum levels, and the difference in spleen levels increased from 15- to 20-fold (139.7 versus 7.3 μg/g dry tissue). At 24 h, platinum levels were similar in the blood, lung, and kidney in both groups of animals. Between 4 h and 24 h, the difference in platinum levels was reduced from 3- to 2-fold in the liver (40.44 versus 16.4 μg/g dry tissue) and from 20- to 3-fold in the spleen (28.7 versus 9.30 μg/g dry tissue) (Table 1).

In Vitro Cytotoxicity against M5076 Cells. The 50% cell viability of cisplatin was 3.7 ± 0.6 μM (mean ± SD of 3 different experiments), and that of L-NDDP was 5.6 ± 2.9 μM (3.5 ± 1.8 μM). The 10% cell viability of cisplatin was 11.3 ± 2.7 μM and that of L-NDDP was 13.4 ± 1.8 μM.

Antitumor Activity against L1210/0 and L1210/PDD Leukemia. L-NDDP at a dose of 37.5 mg/kg (60 μmol/kg) on day 1 resulted in a %T/C of 187 (mean of 3 experiments) (Table 2). Free NDDP at the same dose resulted in a %T/C of 137. The optimal nontoxic dose of cisplatin (10 mg/kg = 33 μmol/kg) resulted in a mean %T/C of 178. In the triple-dose experiments, 25 mg/kg (40 μmol/kg) of L-NDDP given on days 1, 5, and 9 resulted in a mean %T/C of 312, whereas the mean %T/C obtained with free NDDP at the same dose was 225, and with cisplatin, 7.5 mg/kg (25 μmol/kg), given on days 1, 5, and 9, it was 225.

Treatment and Prophylaxis of Liver Metastases of M5076 Reticulosarcoma. L-NDDP was more effective than cisplatin in inhibiting the growth of established liver metastases of M5076 reticulosarcoma and in prolonging survival (Table 3). In experiment 1, treatment consisted of 2 equitoxic doses given on days 4 and 8. L-NDDP was more effective than cisplatin in inhibiting the growth of liver metastases (median number of liver metastases on day 30, 4 versus >200, P < 0.05) and in prolonging survival (mean survival time, 46 versus 39 days, P < 0.05). In experiments 2 and 3, a third dose was given on day 12. L-NDDP was more effective than cisplatin in inhibiting the growth of liver metastases (median number of liver metastases, 0 versus 175, P < 0.05) and in prolonging survival (mean survival time, 57 versus 42 days, P < 0.05). In experiment 4, the antitumor effect of four equimolar doses of L-NDDP and cisplatin was compared. L-NDDP was significantly more effective than cisplatin in prolonging the life span of tumor-bearing animals (mean survival time, 48 days versus 36 days, P < 0.05).

L-NDDP prevented the growth of liver metastases of M5076 reticulosarcoma and prolonged survival when administered before tumor inoculation, whereas cisplatin at the maximum nontoxic dose had no prophylactic effect (Table 4). In experiment 1, L-NDDP administered on day −1 resulted in a 4-fold reduction in the number of liver metastases compared with cisplatin (median number of liver metastases, 45 versus >200, P < 0.05). In experiment 2, the duration of the prophylactic effect prior to tumor inoculation was investigated. A significant reduction in the number of liver metastases was observed in animals treated with L-NDDP up to 48 h before tumor inoculation, compared with animals pretreated with cisplatin or with control animals (median number of liver metastases, 16 for L-NDDP on day −1, 82 for L-NDDP on day −2, >200 for cisplatin on day −1, and >200 for controls, P < 0.05). Animals treated 72 h before tumor inoculation also had fewer liver
TREATMENT OF EXPERIMENTAL LIVER METASTASES WITH L-NDDP

Table 3 Treatment of experimental liver metastases of M5076 reticulosarcoma

C57BL/6 mice were inoculated i.v. with 2 x 10⁶ M5076 cells on day 0. Treatment was administered i.v. Mice were sacrificed on day 30 (experiment 1) and day 45 (experiment 2).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Preparation</th>
<th>Dose (mg/kg)</th>
<th>Schedule day</th>
<th>No. of liver metastases median (range)</th>
<th>P*</th>
<th>Survival days, mean ± SD</th>
<th>P*</th>
</tr>
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<td>Cisplatin</td>
<td>7.5 (25)</td>
<td>4, 8</td>
<td>&gt;200 all (1-26)</td>
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<td>39 ± 3</td>
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<td>L-NDDP</td>
<td>25 (40)</td>
<td>4, 8</td>
<td>Dead</td>
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<td>Control</td>
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<td>4, 8</td>
<td>Dead</td>
<td></td>
<td>29 ± 3</td>
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<tr>
<td>2</td>
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<td>4, 8, 12</td>
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<td>42 ± 3</td>
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<td>29 ± 3</td>
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<td>3</td>
<td>Cisplatin</td>
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<td>4, 8, 12</td>
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<td>36 ± 2</td>
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<td>4, 8, 12</td>
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<td>48 ± 5</td>
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<td></td>
<td>Control</td>
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<td>4, 8, 12</td>
<td>95 (63-140)</td>
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<td>4, 11, 18, 25</td>
<td>&gt;200 all</td>
<td>&lt;0.05</td>
<td>147 (110-200)</td>
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<td>4, 11, 18, 25</td>
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Table 4 Prophylaxis of experimental liver metastases of M5076 reticulosarcoma

Tumor cell inoculum i.v., 2 x 10⁶ M5076 cells. The animals were sacrificed on day 21.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Preparation</th>
<th>Dose (mg/kg)</th>
<th>Schedule day</th>
<th>No. of liver metastases median (range)</th>
<th>P*</th>
<th>Survival days, mean ± SD</th>
<th>P*</th>
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<td>16 (10-66)</td>
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<td>82 (18-112)</td>
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<td>-1</td>
<td>147 (110-200)</td>
<td>&lt;0.05</td>
<td>36 ± 2</td>
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<td>8 (20-52)</td>
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<td>48 ± 5</td>
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<tr>
<td></td>
<td>Control</td>
<td>25 (40)</td>
<td>-1</td>
<td>95 (63-140)</td>
<td>NS</td>
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<td>3*</td>
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<td>-1</td>
<td></td>
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DISCUSSION

The results indicate that L-NDDP is partially not cross-resistant with cisplatin and more active than cisplatin against L1210 leukemia and experimental liver metastases of M5076 reticulosarcoma. NDDP was designed and synthesized to obtain a highly lipophilic molecule with the favorable activity and toxicity characteristics of the diaminocyclohexane analogues (12, 13). The lipophilicity of the compound synthesized resulted in a liposomal preparation with an entrapment efficiency and long-term stability of nearly 100%. These characteristics were not dependent on the lipids used (data not shown). DMPC and DMPG at a 7:3 molar ratio was the lipid combination selected for further study because we have shown before that it is nontoxic in humans (4).

Tissue distribution studies showed that L-NDDP resulted in spleen, lung, liver, and blood platinum levels that were severalfold higher than those achieved with cisplatin when equimolar doses of both drugs were administered i.v. Except for the lung, these changes in drug distribution are comparable to those other investigators have found with liposomal doxorubicin, even though small unilamellar vesicles were used in those studies (size range, 0.05-0.2 μm) (8, 9, 21). Tissue distribution studies of free NDDP could not be carried out because the drug is not soluble in nontoxic solvents. The role played by liposome encapsulation was studied by using another lipophilic cisplatin analogue that can also be administered as free drug i.v. These studies showed that liposome encapsulation increases by severalfold the drug levels in the liver, spleen, and lung (22).

In the i.p./i.p. L1210/0 leukemia model, at equitoxic doses,
L-NDDP was more active than cisplatin and free NDDP. Because the slow local drug release achieved through i.p. administration might enhance the antitumor activity of anticancer agents encapsulated in liposomes, we studied the antitumor activity of L-NDDP and cisplatin administered i.v. against L1210/0 leukemia inoculated i.v. Free NDDP was not tested because it is not soluble in nontoxic solvents and it was less effective than L-NDDP against i.p. L1210/0 leukemia. In this system, too, L-NDDP was more active than cisplatin when a triple-dose schedule was used.

L-NDDP was more effective than cisplatin in inhibiting the growth of established liver metastases of M5076 reticulosarcoma and in prolonging survival both at maximum nontoxic doses and equimolar doses. This effect was not due to an increased sensitivity of the M5076 cells to L-NDDP because at equimolar concentrations cisplatin was slightly superior to L-NDDP in the in vitro cytotoxicity studies. The preferential distribution of L-NDDP to the liver and its reduced toxicity compared with cisplatin (maximum nontoxic molar dose of L-NDDP was 60–80% higher than that of cisplatin) are the most likely mechanisms involved in this increased antitumestatic activity. Another possible mechanism might be direct phagocytosis of liposomes by M5076 cells. However, in studies with liposomal doxorubicin, Mayhew et al. (7, 8) and Gabizon et al. (9) showed that liposome encapsulation of doxorubicin enhances its antitumor activity against established liver metastases of not only phagocytic but also nonphagocytic tumors.

L-NDDP also had significant prophylactic activity against liver metastases of M5076 reticulosarcoma when administered up to 48 h before tumor inoculation, whereas cisplatin was inactive. The mechanisms involved in the prophylactic activity are not clearly understood but may involve any of the following: a slow and prolonged drug release from arrested liposomes in the tissues with sinusoidal capillaries or delayed excretion of active platinum metabolites by macrophages. Studies on the uptake, release, and metabolism of L-NDDP by mouse peritoneal macrophages and Kupffer cells and a study of the plasma antitumor activity at several time points after the i.v. administration of L-NDDP are under way in our laboratory.

Our study suggests that the selection of analogues that are more suited for liposome encapsulation may be a way of overcoming the formulation problems involved in the development of liposome-encapsulated drugs. In addition, by using this approach, the potential of liposome-encapsulated anticancer drugs will not depend solely on the pharmacological changes brought about by the liposomal carrier but also on the activity and toxicity advantages of the analogues selected (i.e., lack of cross-resistance and reduced nephrotoxicity). L-NDDP is an example of an antitumor agent that was designed to be formulated in a liposomal carrier and has shown remarkable biological properties. At present, a lyophilized formulation of L-NDDP is being developed and preclinical toxicology studies are in progress.

ACKNOWLEDGMENTS

We want to thank Trellis Brown and Karen Francis for excellent technical assistance and Pam Ansley and Jennie Schreyer for typing the manuscript.

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Multilamellar Vesicles-1,2-diaminocyclohexaneplatinum(II) Encapsulated in trans-R,R-Bis-neodecanoato-cis-M5076 Reticulosarcoma with Treatment and Prophylaxis of Experimental Liver Metastases of M5076 Reticulosarcoma with cis-Bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II) Encapsulated in Multilamellar Vesicles

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