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Toxicity and antitumor activity of *cis*-bis-carboxylato(*trans*-R,R-1,2-diaminocyclohexane) platinum(II) complexes entrapped in liposomes*

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Summary. A new series of highly lipid-soluble *cis*-bis-carboxylato(*trans*-R,R-1,2-diaminocyclohexane)platinum(II) complexes were synthesized and characterized by their elemental analysis and by various spectroscopic techniques [infrared (IR), 195pt nuclear magnetic resonance (NMR)]. *cis*-bis-Neopentanoato(*trans*-R,R-1,2-diaminocyclohexane)platinum(II) (NPDP), *cis*-bis-neodecanoato(*trans*-R,R-1,2-diaminocyclohexane)-platinum(II) (NDDP), and *cis*-bis-n-decanoato(*trans*-R,R-1,2-diaminocyclohexane)-platinum(II) (DEDP) complexes were entrapped in multilamellar vesicles composed of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) at a 7:3 molar ratio and tested for toxicity and antitumor activity. The entrapment efficiency of the liposomal platinum (L-Pt) complexes (L-NPDP, L-NDDP, L-DEDP) was >95%, and the stability in 0.9% NaCl solution at 4° C was >95% at day 14 in each case. The LD_{50} values of L-NPDP, L-NDDP, and L-DEDP when injected i.v. were 30, 54, and 150 mg/kg, respectively. L-NPDP, L-NDDP, and L-DEDP had no significant nephrotoxicity [as evidenced by a lack of elevated blood urea nitrogen (BUN) levels]. The percentages of T/C obtained after a single i.p. injection of the optimal dose of L-NPDP, L-NDDP, and L-DEDP tested against L1210 leukemia were 175%, 187%, and 212%, respectively [160% for cisplatin (CDDP)]. When a multiple i.p. injection schedule was used (on days 1, 5, and 9), L-NPDP, L-NDDP, and L-DEDP were more active than CDDP (percentage of T/C: 312%, 312%, 277%, and 220%, respectively). When injected i.v., only L-NDDP showed significant activity against L1210 leukemia i.v. (percentage of T/C: 186%). L-NDDP and L-DEDP were markedly active against L1210 leukemia resistant to CDDP (percentage of T/C: 200% and 145% vs 112% for CDDP). L-NPDP, L-NDDP, and L-DEDP also had good activity against i.p. B16 melanoma when they were injected i.p. on days 1, 5, and 9 (percentage of T/C: 206%, 225%, and 306%, respectively). L-NDDP and L-DEDP were more effective than CDDP in inhibiting the growth of liver metastases of murine M5076 reticulosarcoma, whereas L-NPDP was not active. The results obtained to date suggest that L-NDDP is the best L-Pt-complex candidate for further developmental studies.

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Abbreviations used: CDDP, cis-dichlorodiammineplatinum(II); *DACH, (trans*-R,R-1,2-diaminocyclohexane); *NPDP, cis*-bis-neopentanoato(*trans*-R,R-1,2-diaminocyclohexane)platinum(II); *NDDP, cis*-bis-neodecanoato(*trans*-R,R-1,2-diaminocyclohexane)platinum(II); *DEDP, cis*-bis-n-decanoato(*trans*-R,R-1,2-diaminocyclohexane)platinum(II); *DMPC*, dimyristoyl phosphatidylcholine; *DMPG*, dimyristoyl phosphatidylglycerol; *L-NPDP, L-NDDP, L-DEDP*, liposome-entrapped NPDP, NDDP, DEDP; *IR*, infrared; *NMR*, nuclear magnetic resonance; *L-Pt*, liposomal platinum; *T/C*, median survival time of treated mice divided by median survival time of control mice; LD_{50}, 50% lethal dose; *BUN*, blood urea nitrogen

Introduction

Liposomes are lipid vesicles that form spontaneously when an aqueous solution is added to a dry lipid film or powder. They are natural carriers of lipophilic molecules that can be used as vehicles for transporting many promising therapeutic agents that have never been developed due to formulation problems [26]. The use of liposomes as carriers of antitumor agents has been extensively explored in animals [17]. Recent studies show that liposomes can reduce certain types of drug-induced toxicity [4–6, 9, 21] or increase antitumor activity as a result of a slow-release mechanism [18, 22], a higher drug uptake by tumor cells, or a more favorable drug distribution throughout the organs [7, 19]. In spite of these promising findings, the use of liposomes as carriers for anticancer drugs in humans has been delayed by the low entrapment efficiency and lack of stability in liposomal preparation, problems inherent in large-scale production, absence of strong evidence that liposome entrapment can overcome tumor drug resistance, and potentially increased toxicity to the reticuloendothelial system.

Since Rosenberg et al. [27] first described the antitumor activity of CDDP[3] in 1969, cisplatin (CDDP) has become an important drug in the treatment of selected human malignant tumors [10, 16]. However, its usefulness is compromised by its propensity to cause several dose-limiting toxicities, including nephrotoxicity, nausea and vomiting, neurotoxicity, ototoxicity, and myelosuppression [11, 14, 30], and its potential to induce resistance in otherwise responsive tumor types [1, 3]. In an attempt to improve its biological properties, CDDP has previously been entrapped in liposomes, but with a very low entrapment efficiency (7.4%) and poor stability [5].

To improve these characteristics, we undertook a long-term research program to develop different liposomal platinum (L-Pt) preparations. We have previously reported on three different platinum complexes designed for liposome entrapment: *cis*-bis-cyclopentenecarboxylato-1,2-diaminocyclohexane-platinum (CPDP) [23], *cis*-bis-*N*-decyliminodiacetato-1,2-diaminocyclohexane-platinum (NDIDP) [15], and *cis*-bis-neodecanoato-1,2-diaminocyclohexane-platinum (NDDP) [25]. Although all of these three complexes showed significant antitumor activity in their liposomal form, CPDP had suboptimal entrapment (<90%) and we could not fully characterize NDIDP. We have recently synthesized two new compounds belonging to this lipid-soluble carboxylato (DACH)-platinum complex series to define further the structural modifications that enhance the entrapment of liposomes and increase the therapeutic index of the L-Pt complex. We report on the synthesis and liposomal preparation of these new platinum complexes and their toxicity and in vivo antitumor activity against four different murine tumor models.

Materials and methods

Chemicals and lipids. K_2PtCl_4 was purchased from Aesar (Johnson Matthey, Inc. Seabrook, NH). *Trans*-R,R-1,2-Diaminocyclohexane (DACH) was purchased from Morton Thiokol, Inc. (Danvers, Mass). Neodecanoic and neopentanoic acids were obtained from Exxon Chemical Co. (Houston, Tex). The n-decanoic acid used in this experiment was purchased from Aldrich Chemical Co. (Milwaukee, Wis). Elemental analysis on the platinum complexes was carried out by Robertson Laboratory, Inc. (Madison, NJ). Infrared (IR) spectra were recorded as a KBr pellet in the range of 250–4000 cm^{-1} using a Beckman 250 MX spectrophotometer, and nuclear magnetic resonance (NMR) spectra were recorded on an IBM NR 200/AF spectrometer. Spectra were obtained in chloroform solution and were externally referenced to and reported relative to Na_2PtCl_6 (0.25 g/3 ml D_2O). The chromatographically pure (thin-layer chromatography) DMPC and DMPG used in this study were obtained from Avanti Polar Lipids (Birmingham, Ala).

Animals. B6D2F1, C57BL/6, and CD1 male mice were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Mass).

Cell lines. L1210/0 cells were obtained from the Department of Pharmacology, University of Vermont (Burlington, VT); L1210/CDDP cells and B16 melanoma cells were obtained from the DCT tumor repository, National Cancer Institute (Frederick, Md). The L1210/0 and L1210/CDDP cell lines were grown in vivo in the peritoneal cavity of BDF1 mice and transplanted weekly; animals bearing L1210/CDDP leukemia were treated on day 5 with 5 mg/kg CDDP. The B16 melanoma cells were grown in vivo s.c. in C57BL/6 mice. M5076 is a murine reticulosarcoma that arose in the ovary of a C57BL/6 mouse; these cells display characteristics of the monocyte-macrophage lineage [8], metastasizing predominantly to the liver and spleen, independent of the route of inoculation [29]. M5076 cells 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 maintained in vivo as an ascitic tumor in C57BL/6 mice and transplanted every 3 weeks.

Synthesis of platinum complexes. NDDP was synthesized by using the following multistep procedure. A solution of K_2PtCl_4 was mixed with an equimolar amount of DACH in water and allowed to react at room temperature for 6–8 h with constant stirring. The water-insoluble *cis*-dichloro(DACH)platinum(II) was collected by filtration and washed successively with water, ethanol, and acetone; the final product was dried in a vacuum (yield, 90%). The *cis*-dichloro(DACH)platinum(II) was mixed at room temperature with a slightly less than equimolar amount of Ag_2SO_4 in water for 24 h in a dark environment. The water-soluble sulfato(DACH)platinum(II) was removed from the AgCl precipitate by filtration and evaporated to dryness at 40°–50° C under reduced pressure using a rotary evaporator; the yellow product was dried over P_2O_5 in a vacuum. Finally, the sulfato(DACH)platinum(II). H_2O (4.23 g) was dissolved in 50 ml water, and a solution of sodium neodecanoate (prepared in situ by mixing 4 ml 5 *N* NaOH and 3.44 g neodecanoic acid) was added. A yellow, sticky material formed immediately. The reaction mixture was then stirred at room temperature for 15 min, and 200 ml methanol was added to give a clear yellow solution, which was stirred for an additional 2 h. Methanol was evaporated under reduced pressure, and the final product was extracted from the aqueous solution with 2 × 50 ml dichloromethane, which was dried over anhydrous magnesium sulfate for 30 min, filtered, and then evaporated to dryness to give a solid product. The final product was purified from acetone (yield = 5 g, 75%). NPDP and DEDP were prepared in a similar manner. NPDP, NDDP, and DEDP complexes are highly soluble in chloroform (>25 mg/ml) and other organic solvents, but they are insoluble in water.

Preparation of L-NPDP, -NDDP, and -DEDP. L-NPDP, L-NDDP, and L-DEDP were prepared as previously reported for other platinum complexes [24]. Briefly, chloroform solutions of DMPC and DMPG at a 7:3 molar ratio were mixed with the platinum complex at a drug:lipid weight ratio of 1:15. The chloroform was evaporated in a rotary evaporator, leaving a dry film containing the lipids and the platinum complex. Multilamellar liposomes containing the platinum complex were formed by the addition of 1 ml 0.9% NaCl solution in water for each milligram of platinum complex to the dry lipid film and shaking for a few minutes. To measure the entrapment efficiency, the liposome suspension was centrifuged at 30,000 *g* for 45 min and the amount of platinum complex in the supernatant or elemental platinum in the pellet was determined. The platinum complexes were measured by UV spectrophotometry at a wavelength of 216 nm. Elemental platinum was measured by X-rax fluorescence at the Department of Analytical Chemistry, The University of Texas Medical School, Houston, Texas [28]. The entrapment efficiency was calculated by the following formulas:

$$\text{Entrapment efficiency} = \frac{\text{Total Pt complex added} - \text{Pt complex in supernatant}}{\text{Total Pt complex added}} \times 100 \quad (1)$$

$$\text{Entrapment efficiency} = \frac{\text{Elemental platinum in pellet}}{\text{Elemental platinum added}} \times 100 \quad (2)$$

L-NPDP, L-NDDP, and L-DEDP vesicles were sized in a Coulter counter (Coulter Electronics, Hialeah, Fla).

Subacute toxicity studies. Groups of 6–8 CD1 Swiss mice weighing 20–25 g received different i.v. doses of the L-Pt complexes. The animals were observed, and deaths were recorded on a daily basis. The LD_{50} dose was calculated from the curve obtained by plotting the logarithm of the dose and the percentage of survival on day 15.

Renal function studies. Groups of 6 CD1 Swiss mice weighing 20–25 g received an i.v. subacute LD_{50} of the L-Pt complexes. Animals were sacrificed 96 h later, and the serum BUN was measured.

In vivo antitumor activity against L1210/0 and L1210/CDDP leukemia. Two different routes of tumor inoculation and treatment were used in the experiments carried out with the L1210/0 line. Groups of 6 B6D2F1 mice weighing 20–25 g were inoculated with 10^6 (i.p.) or 10^5 cells (i.v.) on day 0. Treatment was started on day 1 via the same route used for tumor inoculation. Two different treatment schedules were used: a single dose on day 1 or once-daily doses on days 1, 5 and 9. In the therapeutic experiments carried out using the L1210/CDDP cell line, 10^6 cells were inoculated i.p. on day 0. Treatment was given (i.p.) on days 1, 5, and 9. The results were expressed as the median survival of treated animals divided by the median survival of control animals $\times 100$ (% T/C).

In vivo antitumor activity against B16 melanoma. Suspensions of B16 melanoma cells were obtained by homogenizing s.c. B16 melanoma tumors with cold phosphate-buffered solution (approx. 10 ml/g tumor). C57BL/6 mice were inoculated i.p. with 0.1 ml B16 melanoma cell suspension on day 0. Treatment was given i.p. on day 1 or on days 1, 5, and 9. The results were expressed as % T/C.

Treatment of liver metastases of M5076 reticulosarcoma. Groups of 6–8 C57BL/6 mice were inoculated i.v. on day 0 with 2×10^4 M5076 cells obtained from the peritoneal cavity of tumor-bearing animals. Drugs were injected i.v. on days 4, 8, and 12 in volumes ranging from 0.2 to 0.5 ml. Differences in survival were analyzed for statistical significance with Student's *t*-test.The results were expressed as % T/C.

The optimal doses of the various drugs were used in all antitumor activity studies. For L-NPDP and L-NDDP these ranged between 12.5 and 25 mg/kg; for L-DEDP, between 50 and 100 mg/kg; and for CDDP, between 5 and 10 mg/kg. In each case the results presented are the mean of two experiments. The free-platinum complexes could not be used as controls in the non-entrapped form due to their lack of aqueous solubility.

Results

Characterization of platinum complexes

The platinum complexes were submitted for elemental analysis prior to biological evaluation. Analytical data (Table 1)

Table 1. Analytical data of lipophilic platinum complexes

Complex	Elemental analysis								IR data (cm^{-1})	^{195}PtNMR/ppm[a]
	Observed (%)				Calculated (%)					
	Carbon	Hydrogen	Nitrogen	Platinum	Carbon	Hydrogen	Nitrogen	Platinum	(C = O)	
NPDP	36.39	6.50	5.22	36.78	36.29	6.43	5.29	36.86	1605	−1716
NDDP	47.80	7.85	4.29	29.86	47.93	8.00	4.30	29.95	1598	−1716.5
DEDP	47.63	8.01	4.19	29.26	47.93	8.00	4.30	29.95	1607	−1719

[a] Chemical shifts are relative to Na_2PtCl_6

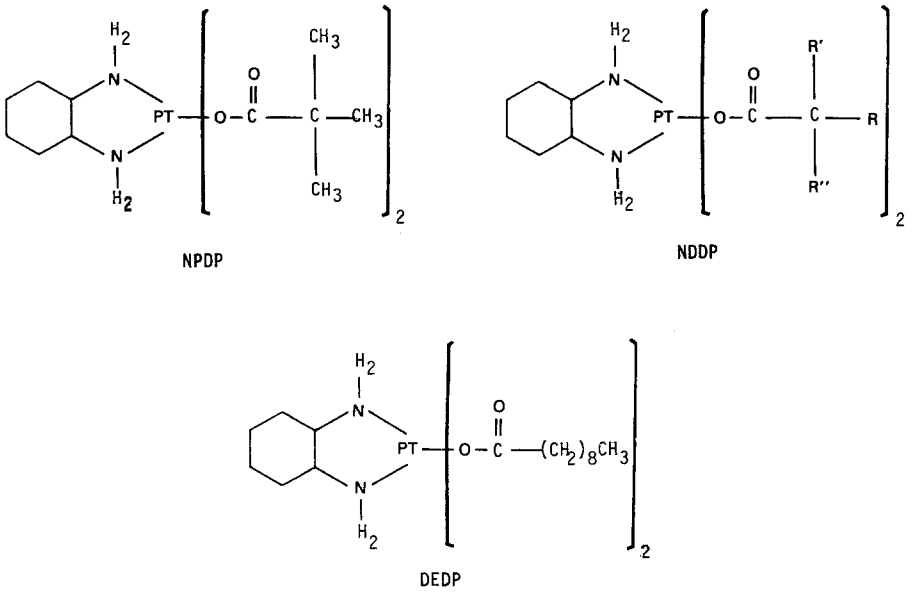

Fig. 1. Chemical structures of NPDP, NDDP (OOC-C-R,R′,R″; where R, R′, and R″ can be $-CH_3$, $-C_2H_5$, or $-C_3H_7$, to give a radical with an empirical formula of $C_{10}H_{19}O_2$), and DEDP

clearly establish their formulation as 1 DACH:1 Pt and 2 carboxylate ligands for the NPDP, NDDP, and DEDP complexes. The presence of a sharp, strong band near 1600–1607 cm^{-1} in the IR spectrum of each of these complexes and the absence of absorption of the free acid near 1700 cm^{-1} demonstrate that the carboxylate ligand is coordinated to the platinum in each case [12]. The ^{195}Pt NMR spectra for all platinum complexes obtained in chloroform solution are consistent with Pt(II)-ion ligation by two nitrogen and two oxygen donor ligands found in these complexes [20]. The platinum complexes exhibit the ^{195}Pt NMR chemical shift in the range of -1716 to -1719 in chloroform solution. This evidence suggests the chemical structure shown in Fig. 1.

Entrapment efficiency, stability, and size distribution of L-NPDP, L-NDDP, and L-DEDP

The entrapment efficiency of L-NPDP, L-NDDP, and L-DEDP was >95%. In addition, no precipitation of free drug could be seen. The stability of L-NPDP, L-NDDP, and L-DEDP in a 0.9% NaCl solution in water at 4° C was assessed by measuring the amount of NPDP, NDDP, and DEDP in the supernatant or elemental platinum in the pellet at various times. At day 14, the stability was found to be >95% in each case, and no evidence of vesicle disruption, morphologic distraction, or clumping of non-entrapped drug was observed by light microscopy. The vesicles ranged in size from 0.5 to 5 μm, with most vesicles measuring between 1 and 3 μm.

Subacute toxicity

Table 2 shows the LD_{50} for the three L-Pt complexes tested and CDDP. All L-Pt complexes were less toxic than CDDP. For L-NPDP, the LD_{50} was 30 mg/kg, with most deaths occurring within the first 24 h after drug administration. For L-NDDP, it was 54 mg/kg, with most deaths occurring between 3 and 10 days after drug administration, and for L-DEDP it was 150 mg/kg.

Nephrotoxicity. Table 2 also shows the mean serum BUN values observed 96 h after administration of the LD_{50} of the three L-Pt complexes and CDDP. At this time, renal dysfunction was only observed with CDDP.

Antitumor activity

Table 3 shows the results obtained after administration of the optimal doses of the three L-Pt complexes and CDDP tested against L1210 leukemias in vivo. When given as a single i.p. dose, the L-Pt complexes were as active as CDDP against L1210/0 leukemia (% T/C: 175% for L-NPDP,187% for L-NDDP, 212% for L-DEDP, and 160% for CDDP), but they were significantly more active than CDDP when given on a three-injection schedule (%T/C: 312%, 277%, and 220%, respectively). When drugs were injected i.v., only L-NDDP showed significant antitumor activity (%T/C: 186%; neither L-NPDP, L-DEDP, nor CDDP was active. In the L1210/CDDP model CDDP was inactive, as expected, whereas L-NDDP and L-DEDP showed significant antitumor activity when injected i.p. (%T/C: 200% and 145%, respectively).

Table 4 shows the results obtained when these drugs were given against B16 melanoma. In these experiments, the i.p. route was used for tumor inoculation and drug administration. When given as a single dose, L-NPDP was as active as CDDP (%T/C: 156%), L-NDDP was less active (%T/C: 125%), and L-DEDP was more active (%T/C: 231%). When given on a three-dose schedule, all three L-Pt complexes were moderately more active than CDDP (%T/C: 206%, 225%, 306%, and 182%, respectively).

Table 5 shows the results of tests using the L-Pt complexes and CDDP against liver metastases of M5076 reticulosarcoma. In this model, both tumor cells and treat-

Table 2. Acute nephrotoxicity of L-Pt complexes

L-Pt complexes	LD_{50} (mg/kg)	(BUN[a] mg/100 ml; mean ± SD)
L-NPDP	30	27 ±1.41
L-NDDP	54	30.4±0.2
L-DEDP	150	24 ±1.41
CDDP	17	78.3±8.0

[a] Measured 4 days after i.v. injection of LD_{50}; normal values = 31±5.5 mg/100 ml

Table 3. Efficacy of L-Pt complexes against L1210 leukemia

L-Pt complexes	Schedule (days)	Route	Optimal dose (mg/kg)	L1210/0[a] T/C (%)	L1210/CDDP[b] T/C (%)
L-NPDP	1[a]	i.p.	25	175	ND
	1, 5, 9[a]	i.p.	25	312	ND
	1, 5, 9[b]	i.v.	25	128	ND
L-NDDP	1[a]	i.p.	37.5	187	ND
	1, 5, 9[a]	i.p.	25	312	200
	1, 5, 9[b]	i.v.	25	186	ND
L-DEDP	1[a]	i.p.	100	212	ND
	1, 5, 9[a]	i.p.	50	277	145
	1, 5, 9[b]	i.v.	50	128	ND
CDDP	1[a]	i.p.	10	160	ND
	1, 5, 9[a]	i.p.	5	220	112
	1, 5, 9[b]	i.v.	7.5	128	ND

[a] Tumor inoculation, 1×10^6 cells i.p. on day 0; treatment, i.p.; median survival of control animals, 7–9 days
[b] Tumor inoculation, 1×10^5 cells i.p. on day 0; treatment, i.v.; median survival of control animals, 6–8 days
ND, not done

Table 4. Efficacy of L-Pt complexes against i.p. inoculated B16 melanoma[a]

L-Pt complexes	Schedule (days)	Optimal dose (mg/kg)	%T/C
L-NPDP	1	25	156
	1, 5, 9	25	206
L-NDDP	1	25	125
	1, 5, 9	12.5	225
L-DEDP	1	75	231
	1, 5, 9	50	306
CDDP	1	10	156
	1, 5, 9	5	182

[a] Tumor inoculation, 1×10^6 cells i.p. on day 0; treatment, i.p.; median survival of control animals, 16 days

Table 5. Antitumor activity of L-Pt complexes against liver metastases of M5076 reticulosarcoma[a]

L-Pt complexes	Optimal dose (mg/kg)	Schedule (days)	%T/C
L-NPDP	12.5	4, 8, 12	110
L-NDDP	12.5	4, 8, 12	196*
L-DEDP	50	4, 8, 12	187
CDDP	7.5	4, 8, 12	144

[a] C57BL/6 mice were inoculated i.v. with 2×10^4 M5076 cells on day 0; treatment, i.v.; Median survival of control animals, 21–24 days

* $P < 0.05$ compared with animals treated with CDDP (Student's *t*-test)

ment were given i.v. L-NPDP was inactive (%T/C: 110%), whereas L-NDDP and L-DEDP were moderately more active than CDDP (%T/C: 196%, 187%, and 144%, respectively).

Discussion

In the past, several investigators have tried, with very poor results, to entrap CDDP in liposomes. This study presents chemical and biological data on three different L-Pt complexes. These complexes, used for liposome entrapment, are lipophilic analogs of CDDP with branched and linear aliphatic leaving groups. They were synthesized in an attempt to enhance their association with the phospholipid constituents of the liposomal bilayers; the very high entrapment efficiency and stability obtained suggest that such a goal was achieved.

During the last decade, many investigators have studied the structure-activity relationship in platinum coordination complexes. One of the structural modifications that is widely accepted as having resulted in an increased therapeutic index is the attachment of a cyclohexanediamine group [2, 13]. For this reason, we used 1,2-diaminocyclohexane derivatives in our effort to synthesize the new platinum complexes. In spite of their structural similarities, the L-Pt complexes differed substantially in their biological activity. L-NPDP and CDDP had similar LD_{50} values, whereas those of L-NDDP and L-DEDP were 3- and almost 10-fold higher, respectively, than that of CDDP. All three L-Pt complexes were significantly less nephrotoxic than CDDP.

When given i.p., the L-Pt complexes were moderately more active than CDDP against i.p. inoculated L1210 leukemia. When injected i.v. against i.v. inoculated L1210 leukemia, only one of the L-Pt complexes was active (L-NDDP). Neither CDDP nor the other two L-Pt complexes showed activity at the doses tested. L-NDDP and L-DEDP were definitely not cross-resistant to CDDP in the L1210/CDDP tumor model. When the L-Pt complexes were given against B16 melanoma that had been inoculated i.p., they were at least as active as CDDP. Finally, L-NPDP was inactive against liver metastases of M5076 reticulosarcoma, whereas L-NDDP and L-DEDP appeared to be more active than CDDP. In summary, L-NDDP was found to have significant activity in all five tumor systems tested (L1210/i.p./i.p., L1210/i.v./i.v., L1210/CDDP, B16, and M5076). L-DEDP was active against four of these systems and was most active against B16 melanoma. L-NPDP was tested in four tumor systems but showed significant activity in only two. CDDP was active in three of the systems.

The significant differences in toxicity and potency between L-NDDP and L-DEDP constitute an interesting finding, since the molecular weight of both compounds is the same and they differ only in that the leaving group is branched in NDDP and linear in DEDP. We believe that these differences may be related to the tightness of association of the complexes with the phospholipid bilayers in the bloodstream and with the rate of hydrolysis of the complex. For example, the branched leaving groups might enhance a tighter association of the complex with the bilayer, which would protect the complex from hydrolysis by keeping it in a lipid environment. Studies to confirm this hypothesis are now under way.

The role of liposome entrapment in determination of the toxic and antitumor activities of the L-Pt complexes and CDDP is unknown at this point because we have thus far been unable to obtain satisfactory free-drug formulations due to problems with solubility in these compounds. Differences between the three platinum complexes must be attributed to their different chemical structures, as the composition and size of the liposomes used were identical.

In summary, this study shows that L-Pt complexes with a high level of entrapment and good stability, which are relatively free of nephrotoxicity and more active than CDDP yet cross-resistant to the latter, can be obtained using lipophilic 1,2-diaminocyclohexane-platinum(II) analogs. Research on the structural requirements for liposome entrapment and structure-activity relationship studies will continue synthesizing and testing a full series of lipophilic CDDP analogs using linear and branched aliphatic leaving groups of different lengths.

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