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Preclinical toxicity and pharmacology of liposome-entrapped *cis*-bis-neodecanoato-*trans*-R,R-1,2-diaminocyclohexane platinum(II)*

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Summary. Liposome-entrapped *cis*-bis-neodecanoate-*trans*-R,R-1,2-diaminocylohexane platinum(II) (L-NDDP) is a new lipophilic cisplatin derivative formulated in a liposomal carrier currently in phase I clinical trials. The preclinical toxicity and pharmacology of L-NDDP were studied in mice and dogs. At the LD_{50} dose (i.v. bolus) in mice (60.5 mg/kg or 181.5 mg/m^2), a tenfold decrease in the granulocyte and platelet counts was observed in the absence of renal toxic effects. In dogs, the maximum tolerated dose (MTD) of L-NDDP given i.v. over a period of 45–60 min was 150 mg/m^2. This dose produced significant vomiting (6–18 episodes), minimal renal dysfunction, a maximal decrease in granulocyte and platelet counts of from 30% to 70%, and acute and transient elevation of liver enzymes. Higher doses (225 and 300 mg/m^2) resulted in severe gastrointestinal (GI) toxicity in one animal and the death of two others within 48 h. Autopsy results showed multifocal hemorrhages in the lungs, GI tract, kidney, and liver. Three dogs were treated monthly with the MTD up to a cumulative dose of 637.5–712.5 mg/m^2 with excellent tolerance. No cumulative myelosuppression or liver dysfunction was observed, whereas a slight increase in the creatinine baseline level was detected in all three animals. Autopsy results at the end of the study showed mild changes limited to the liver, kidney, and GI tract. Pharmacologic studies showed that the drug was cleared, fitting a two-compartment model with a mean $t_{1/2\alpha}$ of 7.1 min and a $t_{1/2\beta}$ of 87.8 h. These studies show that L-NDDP can safely be given at therapeutic doses to animals and that the dose-limiting toxic effects consists of myelosuppression in mice and a multiorgan hemorrhagic syndrome related to vascular injury in dogs.

Introduction

Liposomes are lipid vesicles that can be used as carriers of hydrophilic or lipophilic drugs to increase their therapeutic index by altering their pharmacokinetics and organ distribution [4, 14, 27]. Multilamellar liposomes are particularly suitable as carriers of lipophilic drugs because most of their inner space is occupied by phospholipid bilayers that can accommodate lipophilic molecules.

The use of liposomes as carriers of different chemotherapeutic agents has been explored for more than a decade [2, 5, 15, 21, 22]. Major problems in the pharmaceutical development of these products were the poor drug-entrapment efficiency and stability of many liposome preparations. However, during the last few years, the development of new drug-loading techniques [16] as well as a better selection of drugs for liposomal entrapment has resulted in pharmaceutically acceptable liposomal preparations. Currently, three liposomal preparations of antitumor agents are undergoing clinical evaluation in the United States.

We have previously reported on several new lipophilic cisplatin analogues designed and synthesized for liposomal entrapment [8, 17, 18]. A special emphasis was put on the diaminocyclohexane derivatives due to their well-known lack of cross-resistance in vivo in the L1210 leukemia model and their reduced nephrotoxicity [1, 25]. The rationale for developing platinum compounds in liposomes was to increase the stability of these compounds, to target organs such as the liver that often show metastatic tumor involvement, and to decrease platinum-related toxic effects such as vomiting, nephrotoxicity, and neurotoxicity. The liposomal preparations of these compounds were shown to have a high entrapment efficiency and stability. Liposome-entrapped *cis*-bis-neodecanoate-*trans*-R,R-1,2-diaminocyclohexane platinum(II) (L-NDDP) was found to be as active as cisplatin against L1210 leukemia, non-cross-resistant with cisplatin against L1210/PDD leukemia, and more effective than cisplatin in inhibiting the growth of microscopic liver metastases of M5076 reticulosarcoma in mice [18]. A clinical phase I study of L-NDDP is being conducted at The University of Texas M. D. Anderson Cancer Center. We present the preclinical toxicity and pharmacology of this new antitumor agent.

Materials and methods

Synthesis of NDDP. NDDP was synthesized and fully characterized as previously reported [18]. Figure 1 shows the chemical structure of NDDP. The compound is a mixture of isomers that have different combinations of aliphatic groups with 2–6 carbons in the neodecanoic moiety.

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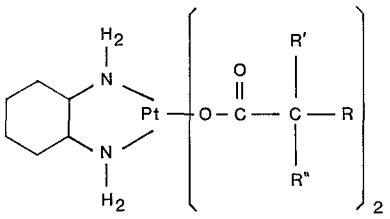

Fig. 1. Chemical structure of NDDP. R, R', and R" can be an aliphatic chain of 2–6 carbons. Combined with the carboxylato group, the neodecanoic moiety has an empirical formula of $C_{10}H_{19}O_2$

Preparation of L-NDDP. For the toxicity studies in mice, L-NDDP was prepared as previously reported [18]. Chloroform solutions of dimyristoyl phosphatidyl choline (DMPC) and dimyristoyl phosphatidyl glycerol (DMPG) at a 7:3 molar ratio were mixed with NDDP at a drug-to-lipid weight ratio of 1:15. The chloroform was evaporated in a rotary evaporator, leaving a dry lipid film containing the lipids and the NDDP. Multilamellar liposomes containing NDDP were formed by the addition of 1 ml 0.9% NaCl solution in water per 1–3 mg NDDP (depending on the desired final concentration) to the dry lipid film, followed by shaking for a few minutes.

For the toxicity studies in dogs, which required large amounts of drug, L-NDDP was prepared as a lyophilized powder containing DMPC, DMPG, and NDDP. To obtain the lyophilized powder, DMPC, DMPG, and NDDP were dissolved in t-butyl alcohol at the above-mentioned ratios; the obtained solution was frozen and lyophilized overnight. The resulting white, flaky powder was stored at 4° C until use; lyophilized L-NDDP is stable at 4° C for at least 4 weeks as assessed by thin-layer chromatography. Residual t-butyl alcohol was measured by gas chromatography/mass spectrometry in three different batches and amounted to $<0.01\%$. To obtain a suspension of liposomes containing NDDP from the lyophilized L-NDDP, the lyophilized powder was reconstituted with 0.9% NaCl solution in water (1 ml/mg NDDP) by hand-shaking the mixture at room temperature for 1 min.

The entrapment efficiency of the liposomal suspensions obtained by both methods was assessed as mentioned above, being close to 100% in both cases. Liposomes were sized in a Coulter counter and channelizer (Coulter Electronics, Hialeah, Fla). Liposome 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 suspensions prepared by the two different methods, except that a slight increase was observed in the number of vesicles measuring 3–5 μm in the suspensions obtained by the hydration of lipid-drug films formed in the rotary evaporator.

Animals. Male CD_1 Swiss mice weighing 20–25 g were purchased from Charles River Laboratories, Inc (Wilmington, Mass) and kept in cages containing six animals each. Mongrel dogs weighing 18–22 kg were obtained from The University of Texas Science Park (Bastrop, Tex). Dogs were housed indoors in individual runs with a floor surface of 15–25 square feet each. Animals were fed daily ad libitum with Wayne Labs dog diet during the study.

Toxicity in mice. Groups of 6–8 CD_1 Swiss mice were given i.v. doses of L-NDDP ranging between 25 and 100 mg/kg in a volume ranging between 0.2 and 1 ml. Animals were observed daily and all deaths were recorded up to day 14. The LD_{10}, LD_{50}, and LD_{90} were obtained by plotting the survival rates against the logarithm of the dose of L-NDDP and by determining from the curve obtained the theoretical drug doses that result in the death of 10%, 50%, and 90% of animals, respectively. This experiment was carried out four times. For the toxicity studies with cisplatin, Platinol (Bristol Laboratories) was obtained from the hospital pharmacy.

The myelotoxicity and nephrotoxicity of L-NDDP were assessed by determining the red blood cell count, total and differential white blood cell counts, platelet count, hemoglobin, and serum blood urea nitrogen (BUN) in groups of 6 mice 96 h after the administration of the LD_{50}. Blood was drawn from the retroorbital plexus using a general anesthetic. Laboratory determinations were carried out at the Veterinary Laboratory, Division of Veterinary Medicine, M. D. Anderson Cancer Center.

Pathologic studies were done in the mice 96 h after the administration of the LD_{50}. Animals were sacrificed and the liver, spleen, lungs, and kidneys were dissected and histologically studied.

Acute and chronic toxicity in dogs. The objectives of the toxicity studies in dogs were to determine the maximum tolerated dose (MTD) of L-NDDP given as a short i.v. infusion, to assess the acute side effects and organ dysfunction secondary to the i.v. infusion of the MTD, and to determine the cumulative side effects and organ dysfunctions secondary to the repeated administration of the MTD at 4- to 6-week intervals.

Six dogs (three males and three females) were used; they were treated according to the dose escalation schedule shown in Table 1. For the first two animals, the starting dose was 75 mg/m^2 (optimal effective dose in the mouse). In subsequent courses the dose was escalated to 112.5, 150, 225, and 300 mg/g^2. Dogs 1–3 were repeatedly treated with monthly doses of L-NDDP, up to cumulative doses of 712.5, 637.5, and 712.5 mg/m^2, respectively.

The infusion rate was 4 mg NDDP/min (64 mg L-NDDP/min). No prehydration was given. Animals were allowed to eat and drink ad libitum before and after drug administration. They were observed continuously for

Table 1. L-NDDP toxicity studies in dogs: treatment schedule[a]

Animal number	Dose (mg/m^2)						Cumulative dose (mg/m^2)
	1st	2nd	3rd	4th	5th	6th	
1	75	75	112.5	150	150	150	712.5[b]
2	75	112.5	150	150	150		637.5[b]
3	112.5	150	150	150	150		712.5[c]
4	225[d]						225
5	225[e]						225
6	300[e]						300

[a] Animals were treated with i.v. doses of L-NDDP at 4- to 6-week intervals. The rate of infusion was 4 mg NDDP/min
[b] Animals sacrificed 1 month after the last dose of L-NDDP
[c] Animal sacrificed 1 week after the last dose of L-NDDP
[d] Animal sacrificed 2 months after L-NDDP infusion
[e] Animals expired within 48 h

about 6 h after each dose, and then twice daily, such that the number of episodes of vomiting could be recorded. Food and water intake were recorded daily. Animals were weighed and their rectal temperature was taken two to three times per week. Peripheral blood was drawn two to three times weekly until the end of the study to determine the following values: hemoglobin, hematocrit, red blood cell count, total and differential white cell counts, platelet count, BUN, creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (AP), total serum protein, albumin, glucose, and calcium. All animals were sacrificed and autopsied at the completion of the study: dogs 1 and 2 were sacrificed 1 month after receiving the last dose of L-NDDP; dog 3 was sacrificed 1 week after the last dose of L-NDDP; dog 4 was sacrificed 60 days after the infusion of L-NDDP, having completely recovered from the side effects; dogs 5 and 6 were autopsied after their spontaneous death 2 days after drug administration.

Pharmacology in dogs. Pharmacologic studies were carried out in three dogs after the administration of a dose of 150 mg/m^2. Blood samples were drawn at the end of the infusion, at 5, 10, 15, 20, 30, 40, 60, 90, 120, and 360 min, and on days 1, 2, 5, and 9 from dogs 1 and 2. In dog 3 the late time points were measured at 1, 2, 4, and 8 days. Elemental platinum (Pt) was measured in whole-blood samples by X-ray fluorescence at the Department of Analytical Chemistry at the University of Texas Medical School at Houston [26]. Pharmacokinetic parameters were derived from nonlinear regression analysis of the data.

Results

Toxicity in mice

LD_{10}, LD_{50}, LD_{90}. Subacute toxicity studies were carried out four times. Table 2 shows the LD_{10}, LD_{50}, and LD_{90} doses obtained in the different experiments. The LD_{10} of L-NDDP ranged between 30.0 and 53.1 mg/kg; the LD_{50}, between 50.2 and 75.0 mg/kg (mean, 60.5 mg/kg or 181.5 mg/m^2); and the LD_{90}, between 66.1 and 94.4 mg/kg. For cisplatin (Platinol), the LD_{10} was 21 mg/kg; the LD_{50}, 22 mg/kg; and the LD_{90}, 28 mg/kg. With both drugs most deaths occurred within the 1st week. The optimal doses in mice are 25–37.5 mg/kg for L-NDDP and 6–10 mg/kg for cisplatin.

Table 2. Single-dose i.v. toxicity of L-NDDP in mice

Drug	LD_{10} (mg/kg)	LD_{50} (mg/kg)	LD_{90} (mg/kg)
L-NDDP			
Experiment 1	37.2	64.6	91.2
Experiment 2	33.8	52.5	66.1
Experiment 3	30.0	50.0	75.0
Experiment 4	53.1	75.0	94.4
Mean ± SD	38.5 ± 8.8	60.5 ± 10.0	81.7 ± 11.6
Cisplatin (Platinol)	21.0	22.0	28.0

Renal dysfunction. BUN values were determined in 5 CD_1 Swiss mice 4 days after administration of the predetermined LD_{50}. Table 3 shows the mean BUN values obtained in controls and in animals treated with the LD_{50} of L-NDDP or cisplatin. The BUN value in animals treated with the LD_{50} of L-NDDP was not altered, whereas it was elevated by more than eightfold in animals treated with the LD_{50} of cisplatin.

Bone marrow toxicity. Table 3 shows the mean values for granulocytes, hemoglobin, and platelets in animals treated with the mean LD_{50} of L-NDDP or cisplatin. The granulocyte and platelet counts were reduced by about tenfold in animals treated with the LD_{50} of L-NDDP, whereas they were not significantly altered in animals treated with the LD_{50} of cisplatin. The hemoglobin level was also slightly reduced (by about 20%) in animals treated with the LD_{50} of L-NDDP.

Pathologic studies. Pathologic studies were carried out in mice 4 days after administration of the LD_{50} of L-NDDP. Five of eight animals that were still alive on day 4 were sacrificed and the liver, spleen, small bowel, kidneys, and lungs of each were resected; histopathologic studies were then done on these organs. Abnormal findings were limited to mild hepatitis in two animals; no lesions were observed in the other organs.

Toxicity in dogs

MTD. The MTD was established at 150 mg/m^2 because this was the highest dose tested that did not result in life-threatening toxic effects or death of the animals. This dose was tolerated with acceptable side effects or with moderate clinically abnormal findings on a total of nine different occasions in three different animals.

Lethal dose. A dose of 225 mg/m^2 was given to two different animals and resulted in the death of one (dog 5) within 48 h. Loose, bloody stools were found in the cage. The other animal (dog 4) had protracted bloody diarrhea for several weeks and a weight loss of >50%, requiring i.v.

Table 3. Renal dysfunction and bone marrow suppression after administration of the LD_{50} of L-NDDP to mice

Drug	LD_{50} (mg/kg)	Blood parameters 4 days after i.v. administration of LD_{50} (mean ± SD)			
		BUN (mg%)	Granulocytes (number/mm^3)	Hgb (g%)	Platelets ($\times 10^3$/mm^3)
CDDP	22	255.0 ± 86	2,138 ± 635	18.1 ± 0.3	1,282 ± 283
L-NDDP	60.5	30.4 ± 2	157 ± 60	12.8 ± 0.4	157 ± 92
Controls	–	31.0 ± 5	1,945 ± 1,064	15.6 ± 0.5	1,286 ± 11

Hgb, hemoglobin

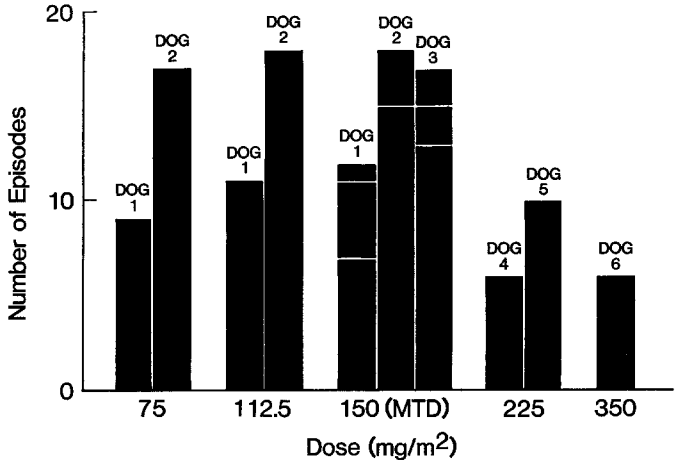

Fig. 2. Number of episodes of vomiting after the administration of L-NDDP to dogs

fluids for approximately 2 weeks. This dog recovered by day 60, when it was sacrificed. Autopsy results showed mild enteritis and lymph node hyperplasia. A dose of 300 mg/m^2 was given to another animal (dog 6), causing his death within 48 h. Again, loose, bloody stools were found in the cage.

The two animals that died after the administration of lethal doses of L-NDDP underwent a complete autopsy. The main findings in dog 5 were diffuse hemorrhagic enterocolitis involving all layers of the gut wall, acute multifocal hemorrhages and thrombosis in the lung, acute renal tubular damage, acute hepatitis, and lymph node necrosis. Dog 6 had extensive multifocal hemorrhages in the lungs, kidneys, liver, and the GI tract.

Description of acute side effects and organ dysfunctions. Vomiting and diarrhea: Vomiting was observed in all animals, including those who received the lower dose of L-NDDP (75 mg/m^2). Figure 2 shows the number of episodes of vomiting observed after each dose in the different animals. No clear relationship between the number of vomiting episodes and the dose of L-NDDP was observed. In all cases, vomiting started about 2 h after completion of the infusion and usually lasted for 2 h. Vomiting 4 h after the infusion was not observed in any case. Loose stools without blood were occasionally observed within 24 h of the infusion of the MTD. Diarrhea was not observed in animals treated with the MTD.

Weight loss: Weight loss after infusion of the MTD of L-NDDP was $<5\%$ in all cases. Loss of appetite occurred for 24–48 h after the infusion, resulting in minimal and transient (a few days) weight loss in some cases.

Renal function: Table 4 shows the baseline and highest values observed for BUN and creatinine after the administration of the MTD (150 mg/m^2) in nine different courses and after one course of 225 mg/m^2. Slight increases in BUN and creatinine levels were observed after each course; these increases were mostly reversible, although a slight increase in the baseline level of creatinine was observed with repeated administration of the MTD.

Bone marrow function: Table 5 shows the baseline and lowest values observed for granulocytes, hemoglobin, and platelets after administration of the MTD (150 mg/m^2) in nine different courses and after one course of 225 mg/m^2.

Table 4. Renal dysfunction secondary to the administration of L-NDDP to dogs[a]

Dose and course	BUN		Creatinine	
	baseline mg%	highest mg% (day)	baseline mg%	highest mg% (day)
Dog 1				
150 mg/m^2				
Course 1	24	30 (5)	1.1	1.6 (1)
Course 2	24	32 (7)	1.4	1.5 (21)
Course 3	26	28 (12)	1.3	1.6 (1)
Dog 2				
150 mg/m^2				
Course 1	20	32 (13)	1.1	1.4 (24)
Course 2	25	36 (15)	1.2	1.7 (8)
Course 3	27	34 (3)	1.7	1.7 (17)
Dog 3				
150 mg/m^2				
Course 1	25	40 (26)	1.1	1.8 (26)
Course 2	28	28 (9)	1.4	1.7 (2)
Course 3	27	31 (27)	1.4	1.6 (15)
Dog 4				
225 mg/m^2				
Course 1	22	26 (3)	1.2	1.6 (1)

[a] Normal values: BUN, 9–25 mg%; creatinine, 0.5–1.5 mg%

Hemoglobin levels decreased by 10%–20% after each course. Absolute granulocyte counts decreased by 50%–80% after each course, although they never fell below 1,000/mm^3. The degree of granulocytopenia tended to increase with repeated administration of the MTD to the same animal. Platelet counts also decreased by 30%–70% after each course, although counts below 150,000/mm^3 were not observed. The degree of thrombocytopenia did not change with repeated administration of the MTD to the same animal. In most cases, myelosuppression changes were detected between days 10 and 14, usually lasting for less than 1 week, and were completely reversible.

Liver function: An acute, transient, dose-related, and completely reversible elevation of liver enzymes (SGOT, SGPT, and AP) was observed in all animals. Enzyme values returned to normal limits within 10 days. Table 6 shows the liver enzyme abnormalities observed 1 and 5 days after the administration of a dose of 150 mg/m^2 in five different courses.

Other chemical parameters: No significant changes in values for electrolytes and glucose and total serum proteins were observed. Prothrombin time was measured in two animals and was found to be within normal limits after administration of the MTD.

Description of chronic or cumulative side effects and organ dysfunctions. Weight loss: No weight loss was observed in the three animals treated repeatedly up to a cumulative dose of >600 mg/m^2 (prestudy weights: 21.3, 20.5, and 21.5 kg; poststudy weights: 24.4, 20.4, and 21.8 kg, respectively). At the end of the study, all animals had good appetites and no manifestations of GI dysfunction, such as vomiting or diarrhea, were seen.

Renal function: As shown in Table 4, the baseline BUN and creatinine values in three animals showed a slight tendency to increase with repeated administration of

Table 5. Myelosuppression secondary to the administration of L-NDDP to dogs

Dose and course	Hemoglobin		Granulocytes		Platelets	
	baseline g%	nadir g% (day)	baseline $\times 10^3/mm^3$	nadir $\times 10^3/mm^3$ (day)	baseline $\times 10^3/mm^3$	nadir $\times 10^3/mm^3$ (day)
Dog 1 150 mg/m²						
Course 1	16.8	13.8 (22)	12.8	7.4 (8)	395	315 (29)
Course 2	15.2	14.7 (14)	11.0	2.5 (14)	460	205 (25)
Course 3	14.7	14.1 (8)	10.5	2.3 (12)	430	300 (8)
Dog 2 150 mg/m²						
Course 1	17.9	15.5 (17)	7.4	4.9 (10)	350	210 (10)
Course 2	17.4	14.9 (15)	8.0	3.7 (8)	350	230 (12)
Course 3	17.4	14.2 (10)	7.4	2.0 (10)	530	150 (10)
Dog 3 150 mg/m²						
Course 1	17.0	16.0 (19)	5.3	2.7 (15)	220	215 (12)
Course 2	16.7	16.9 (9)	6.4	2.3 (16)	340	220 (20)
Course 3	18.6	15.1 (22)	8.2	1.2 (12)	280	205 (12)
Dog 4 225 mg/m²						
Course 1	17.6	11.9 (14)	6.2	2.8 (13)	480	270 (38)

Table 6. Enzyme abnormalities 1 and 5 days after the administration of the MTD of L-NDDP to dogs

Dog number	SGOT[a] (IU/ml)			SGPT[b] (IU/ml)			AP[c] (IU/ml)		
	bas	1 day	5 days	bas	1 day	5 days	bas	1 day	5 days
1	38	149	30	35	67	61	16	281	109
	37	462	34	29	55	41	20	201	80
2	29	397	26	14	126	43	23	150	62
3	25	290	34	29	242	89	47	237	105

[a] Serum glutamic oxaloacetic transaminase: normal range, 11–35 IU/l
[b] Serum glutamic pyruvic transaminase: normal range, 10–44 IU/l
[c] Alkaline phosphatase: normal range, 4–60 IU/l
bas, baseline value

the MTD. Increases in baseline BUN values were minimal (from 24, 20, and 25 to 26, 27, and 27, respectively). Increases in baseline creatinine values were more significant (from 1.1 in all three animals to 1.3, 1.7, and 1.4).

Bone marrow function: As shown in Table 5, in three animals no cumulative decrease in the baseline values for hemoglobin, granulocytes, and platelets was observed with repeated administration of the MTD.

Liver function: No chronic elevation of liver enzymes was observed; all enzyme levels were within normal limits at the completion of the study.

Neurotoxicity: No evident signs of chronic neurologic dysfunction were observed in the three animals that received a cumulative dose of >600 mg/m² L-NDDP. Special studies to detect subclinical neurologic dysfunction were not carried out.

Other effects: A persistent eosinophilia was observed in dog 1. Autopsy results showed that the dog was occultly infected with the canine heartworm *Dirofilaria immitis* and had verminous arteritis in the lungs.

Table 7. Autopsy findings in dogs treated monthly with the MTD of L-NDDP

Organ	Pathologic findings		
	Dog 1[a]	Dog 1[b]	Dog 1[c]
Lungs	dirofilariasis	normal	normal
Liver	normal	hepatitis (mild to moderate)	hepatitis (mild)
GI tract	normal	colitis (mild)	colitis (moderate)
Kidney	normal	tubular damage (mild)	normal
Lymph nodes	hyperplasia	hyperplasia	hyperplasia
Heart	normal	normal	normal
Brain	normal	normal	normal

[a] Cumulative dose, 712.5 mg/m²
[b] Cumulative dose, 637.5 mg/m²
[c] Cumulative dose, 712.5 mg/m²

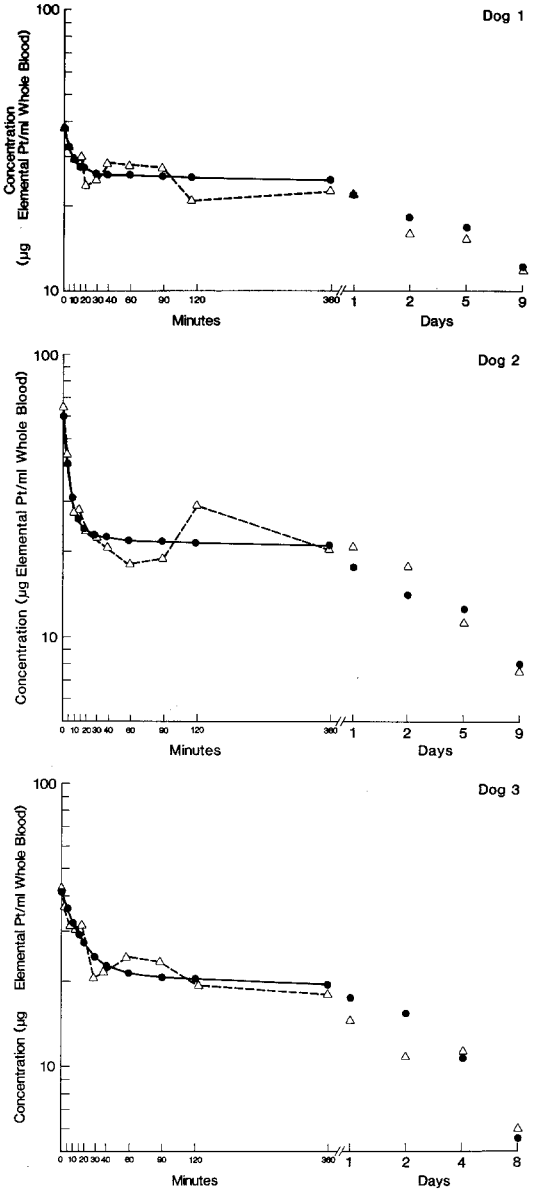

Fig. 3. Clearance of L-NDDP from blood in three dogs after the administration of a dose of 150 mg/m^2. The drug was infused over a period of 45–60 min. Time 0 represents the time of completion of the infusion (△, actual Pt values obtained; ●, Pt values obtained by computer)

Pathologic studies. Table 7 shows the main findings at autopsy for the animals repeatedly treated with the MTD at monthly intervals. Mild changes in the GI tract, liver, and kidneys were common. Lymph node hyperplasia was found in all three animals. The other organs did not show significant abnormalities.

Pharmacology in dogs

Figure 3 shows the blood levels of elemental platinum (Pt) obtained from three dogs at different time points, plotted on semilogarithmic paper. In all three animals, an initial rapid disappearance phase was observed: in dogs 1 and 3, there was a second rise in Pt levels, with a second peak at 40–60 min and a subsequent fall by 120 min; in dog 2, a similar second rise occurred, with the second peak at 60–90 min.

Figure 3 and Table 8 show the blood-disappearance curves and pharmacokinetic parameters obtained by nonlinear regression analysis of the Pt levels after administration of the MTD of L-NDDP (150 mg/m^2) to three dogs. The blood-disappearance curve of L-NDDP fitted a two-compartment model, with a $t_{1/2\alpha}$ of 7.1 min and a $t_{1/2\beta}$ of 87.8 h. The mean volume of distribution at time 0 was 563 ml/kg, which represents 56% of the total body weight. This low volume of distribution is in accordance with the preferential localization of liposome-entrapped drugs in certain organs such as the liver, lung, and spleen. The mean blood clearance of L-NDDP in dogs was 0.15 ml/kg per minute, and the mean C × T, 17.7 mg Pt/ml per minute.

Discussion

This study was designed to obtain preclinical information on the toxicity of a new lipophilic platinum compound (L-NDDP) designed for liposome entrapment. The toxicity of L-NDDP was not compared with that of free NDDP because we have previously shown that NDDP is a liposome-dependent drug that can only be satisfactorily formulated when entrapped in liposomes and that requires the liposomal carrier to exert its biological activity [20].

In mice, the major toxic effect observed was bone marrow suppression. At the LD_{50} dose (60.5 mg/kg or 181.5 mg/m^2), L-NDDP caused a tenfold decrease in the granulocyte and platelet counts in the absence of nephrotoxicity. However, the LD_{50} dose of cisplatin caused an

Table 8. Blood pharmacokinetics of L-NDDP in dogs[a]

	Dose (mg/kg)	Vd[b] (ml/kg)	Peak blood level (μg Pt/ml)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	Clearance (ml/kg per min)	C × T (mg Pt/ml per min)
Dog 1 (21 kg)	7.2 (150 mg/m^2)	632	3.8	5.2	95.2	0.11	21.4
Dog 2 (21 kg)	7.4 (150 mg/m^2)	405	6.1	4.6	72.2	0.18	14.0
Dog 3 (23 kg)	8.2 (150 mg/m^2)	654	4.2	11.5	96.2	0.15	17.8
Mean ± SD	7.6 ± 0.5	564 ± 138	4.7 ± 1.3	7.1 ± 3.8	87.8 ± 13.5	0.15 ± 0.03	17.7 ± 3.8

[a] Data calculated as mg elemental Pt/kg body weight
[b] Volume of distribution time 0

eightfold elevation of the BUN value but did not alter the peripheral blood counts.

Myelosuppression was found to be more severe in mice than in dogs. There is no clear explanation for this finding. Apart from the species, the two main differences between the studies were the drug infusion time (bolus in mice, 45–60 min in dogs) and type of L-NDDP preparation used (from a dried lipid-drug film in mice, from lyophilized lipid-drug powder in dogs). The infusion time might have been crucial for determining the distribution and bone marrow uptake of the drug, although no data supporting this hypothesis are available. However, it is unlikely that the type of L-NDDP preparation used played an important role, as the characteristics of both liposomal suspensions were similar.

At the MTD in dogs (150 mg/m^2), L-NDDP caused vomiting, weight loss of $<5\%$, short-lived and moderate myelosuppression (granulocyte counts of $<1{,}000/mm^3$ and platelet counts of $<150{,}000/mm^3$ were not observed), transient elevation of liver enzymes, and very mild renal dysfunction. All of these abnormalities were completely reversible, with the possible exception of a slight, cumulative renal dysfunction that might in part have been secondary to the dehydration associated with vomiting. No other chronic toxic effects were detected in animals that repeatedly received the MTD at monthly intervals, up to a cumulative dose of between 637.5 and 712.5 mg/m^2.

In dogs, the major manifestation of lethal toxicity of L-NDDP was an acute hemorrhagic syndrome mainly affecting the GI tract and lungs. The dog that received 300 mg/m^2 died within 48 h; autopsy results showed a diffuse hemorrhagic process that involved several organs, including the GI tract and lungs. One of two dogs that received 225 mg/m^2 also died within 48 h, and that animal's autopsy results showed diffuse hemorrhagic enterocolitis, focal hemorrhages in lungs and other organs, and lymph node necrosis. The other animal had very severe GI toxic effects, with protracted diarrhea, melenic stools, and massive weight loss, and required the administration of i.v. fluids. However, this animal had only mild renal dysfunction and myelosuppression and recovered fully by day 60.

Two possible explanations for the hemorrhagic diathesis could be disseminated intravascular coagulation (DIC) and diffuse vascular damage. DIC appears unlikely to be the cause, as no thrombocytopenia was detected in these two animals at the time of death. Prothrombin time was subsequently measured in other dogs before and after two courses of 150 mg/m^2 and no abnormalities were detected. In addition, this particular phospholipid combination (DMPC and DMPG at a molar ratio of 7:3) has been extensively tested in humans, and no coagulation abnormalities or bleeding complications were reported [11, 12]. Therefore, vascular damage appears more likely to be the mechanism. In previous electron microscopic studies [19], we observed swelling of endothelial cells after i.v. injection of therapeutic doses of L-NDDP into mice. In cats, pulmonary edema associated with microangiopathy of alveolar capillaries is the main form of lethal toxicity of cisplatin [7].

At lethal doses in dogs, cisplatin causes hemorrhagic enterocolitis, renal tubular necrosis, pulmonary edema, and lymph node necrosis [3, 24, 28]. At therapeutic doses in dogs, it causes nephrotoxicity, bone marrow suppression, and vomiting; lymph node hyperplasia has also been reported [3, 24, 28]. The MTD of L-NDDP was found to cause vomiting, minimal nephrotoxicity, moderate myelosuppression, lymph node hyperplasia, and a transient elevation of liver enzymes. The different chemical structure, the liposomal carrier, or both must account for these differences. Some structural features of NDDP (diaminocyclohexane group, neodecanoato groups) have been associated with a decreased nephrotoxic potential [6, 25]; however, toxicity studies in baboons using another diaminocyclohexane platinum compound showed very significant nephrotoxicity [13]. Liposomal entrapment of certain drugs, such as amphotericin B, has been reported to reduce drug-related nephrotoxicity [11, 12]. It is therefore likely that the combination of both factors accounts for the decreased nephrotoxic potential of L-NDDP.

As to the drug's liver toxicity, we previously reported that the liposomal entrapment of NDDP results in levels of elemental platinum in liver tissue that are about 3–4 times higher than those observed after the administration of nonentrapped NDDP [20]. Electron microscopic studies and measurement of platinum levels in different mouse liver-cell compartments after administration of L-NDDP have shown that the parenchymal cells contain 3 times more platinum than the Kupffer's cells [9, 19]. This suggests that the transient hepatotoxicity is most likely related to liposomal entrapment of the drug. However, in the present study, hepatotoxicity was in all cases completely reversible. The severity of vomiting and bone marrow suppression appeared to be similar to that reported for cisplatin and carboplatin in previous studies, although a direct comparison is not available [23, 24].

The results of the pharmacologic studies suggest that L-NDDP is initially rapidly cleared from the blood, with a subsequent, long elimination phase (half-life of 3–4 days), and that it distributes preferentially to certain organs. These observations are in accordance with those of our previous studies on liposome-entrapped platinum compounds in rodents [8]. A second peak in blood platinum levels was observed 40–90 min after the initial rapid-disappearance phase. This finding might be relevant, as the second peak was found to occur approximately at the onset of vomiting (data not shown). In our previous organ distribution studies in mice [8], we observed high levels of liposome-entrapped drug in the lungs shortly after i.v. administration, followed by complete clearance by 4 h, suggesting an initial sequestration of the vesicles in the pulmonary microvasculature with subsequent clearing. Further evidence for this hypothesis was provided by our previous electron microscopic studies showing the presence of liposomal vesicles in the lung capillaries (but not in the alveolar space) up to 2 h after the i.v. administration of L-NDDP [19]. The extremely long elimination phase might be due to a slow release of the drug from the tissues into the bloodstream, with significant drug binding to the serum proteins and lipoproteins [10]. Further studies to clarify this issue will be carried out in conjunction with the ongoing phase I clinical trial.

In summary, our study shows that therapeutic doses of L-NDDP can be given to animals with acceptable tolerance and without cumulative side effects. No unpredictable, irreversible, or life-threatening acute reactions were observed. Some of the side effects and toxicities observed at the MTD, such as vomiting, myelosuppression, and renal dysfunction, are classically associated with the admin-

istration of platinum compounds [23, 24]. Others, such as the mild hepatotoxicity, are most likely related to the liposomal carrier. The dose-limiting toxic effects appeared to be myelosuppression in mice and GI toxicity secondary to diffuse vasculopathy in dogs.

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