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Pharmacokinetics and tissue distribution of liposome-encapsulated *cis*-bis-*N*-decyliminodiacetato-1,2-diaminocyclohexane-platinum (II)*

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Summary. The pharmacokinetics and tissue distribution of a lipophilic analogue of cisplatin, *cis*-bis-*N*-decyl-iminodiacetato-1,2-diaminocyclohexane platinum (II) (*N*-decyl-IDP), were studied after the i.v. administration of the free drug in suspension in phosphate-buffered saline (F-*N*-decyl-IDP) and encapsulated in multilamellar liposomes comprising dimyristoyl phosphatidylcholine and dimyristol phosphatidylglycerol at a molar ratio of 7:3 (L-*N*-decyl-IDP). The encapsulation efficiency and stability at 14 days of L-*N*-decyl-IDP were greater than 95%. The blood clearance of both forms of the drug fit a two-compartment model. The peak blood level of elemental platinum for L-*N*-decyl-IDP was fourfold higher than for the free drug (24.2 versus 6.1 μg/ml). In consequence, a fourfold difference in the volumes of distribution was observed (176 ml/kg for L-*N*-decyl-IDP versus 608 ml/kg for F-*N*-decyl-IDP). Liposome encapsulation reduced the drug clearance by threefold; therefore, the CXT of L-*N*-decyl-IDP was threefold higher than that of F-*N*-decyl-IDP (1308 μg platinum/ml per min versus 395 μg platinum/ml per min). Tissue platinum levels were significantly increased by liposome encapsulation in the lung (33 versus 3.6 μg/g), spleen (38.3 μg/g versus none detected), and liver (16.2 versus 11.7 μg/g), and unchanged in the kidneys. Although only F-*N*-decyl-IDP resulted in detectable levels of platinum in the small bowel (70.5 μg/g), the stool excretion was similar for both forms of the drug. The organ distribution changes secondary to liposome encapsulation may result in an increased antitumor activity of *N*-decyl-IDP in tumors involving the lung, spleen, and liver, and avoidance of gastrointestinal toxicity.

Introduction

Cisplatin (CDDP) is one of the most effective antineoplastic drugs available; however, its use is limited by side effects such as severe nausea and vomiting, nephrotoxicity, and neurotoxicity. New analogues have been synthesized in an attempt to increase the therapeutic index of CDDP. Among the second-generation platinum complexes, the diaminocyclohexane derivatives have been shown to be less nephrotoxic and non-cross-resistant with CDDP [1, 20].

An alternative approach to modify the therapeutic index of CDDP may be the use of drug carriers, of which liposomes are particularly attractive because of their ease of preparation and biodegradability. Liposomes are vesicles comprising two major compartments: an aqueous one, in which hydrophilic drugs can be incorporated; and a large lipid compartment made of multiple phospholipid bilayers that are concentrically arranged and can effectively incorporate hydrophobic drugs. Liposomes, because of their particulate nature, are preferentially concentrated in organs rich in cells of the reticuloendothelial system [7, 12]. Liposome encapsulation of doxorubicin results in an enhancement of the therapeutic index of the drug, as a result of a decrease in cardiotoxicity and increase of antitumor activity against liver metastases in mice [2, 4, 5, 15]. However, most liposomal preparations of antitumor agents reported, including doxorubicin, have a low encapsulation efficiency that hinders their potential for clinical use [3, 5, 8, 13, 16, 22].

We have recently reported on the use of a lipophilic CDDP analogue (*cis*-bis-neodecanoato-1,2-diaminocyclohexane platinum (II)) encapsulated in multilamellar vesicles (MLV) [17]. The MLVs used were composed of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) at a 7:3 molar ratio and the drug to lipid weight ratio used was 1:15. The liposomal preparation had an encapsulation efficiency of greater than 95% and was more effective than CDDP in the treatment and prophylaxis of experimental liver metastases in mice.

Cis-bis-*N*-decyliminodiacetato- 1,2-diaminocyclohexane platinum (II) (*N*-decyl-IDP) is a lipophilic analogue of CDDP, which has been specifically designed and synthesized in our laboratories for liposome encapsulation. The *N*-substituted iminodiacetato-diaminocyclohexane derivatives constitute a promising new family of platinum coordination complexes because they have been shown to be more effective against L1210 leukemia, less nephrotoxic, and less enterotoxic [6, 14] than CDDP. *N*-decyl-IDP can be encapsulated in MLVs with a high efficiency, and in the liposomal form, it is at least as effective as CDDP against L1210 leukemia [18].

The characteristic of *N*-decyl-IDP, of forming a fine

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Abbreviations Pt: Elemental platinum; *N*-decyl-IDP: *Cis*-Bis-*N*-decyl-iminodiacetato-1,2-diaminocyclohexane platinum (II); MLV – multilamellar vesicles; L-liposomal; F-Free

suspension in phosphate-buffered saline (PBS), allowed us to compare the pharmacokinetics and tissue distribution of free *N*-decyl-IDP (F-*N*-decyl-IDP) injected i.v. and the liposome incorporated drug (L-*N*-decyl-IDP). We report here on the results of this study.

Materials and methods

Synthesis of N-decyl-IDP. N-decyl-IDP was synthesized as previously reported by Hacker et al. [6], with slight modifications. Briefly, sulfato-diaminocyclohexane platinum (II) H_2O (0.423 g) was dissolved in 10 ml of H_2O. The sodium salt of *N*-decyliminodiacetic acid was prepared in situ by the addition of sodium hydroxide (0.08 g) to *N*-decyliminodiacetic acid (0.273 g) in 50 ml water. The aqueous solution of sodium *N*-decyliminodiacetate was added to the sulfato-diaminocyclohexane-platinum solution and stirred for 30 min at room temperature. The resulting solution was evaporated to dryness in a rotary evaporator at 40 °C under reduced pressure. The yellow solid thus obtained was dissolved in methyl alcohol and filtered through celite. The yellow filtrate was evaporated to dryness in a rotary evaporator under reduced pressure. A yellow crystalline product was obtained, which was further purified from 1-propanol with an 80% yield. The elemental analysis of the product obtained was C38.6%, H6.87%, and N6.82% (theoretical C38.95%, H7.02%, N6.81%). The empirical formula of *N*-decyl-IDP is: $C_{20}H_{39}N_3O_4Pt.2H_2O$ and the infrared spectrum for the complex (as KBr pellet) C=0 1580 cm^{-1} and C-0 1410 cm^{-1}. *N*-decyl-IDP is highly soluble in methanol and chloroform and insoluble in water, but it forms a fine suspension in PBS after sonication in a bath sonicator for 30 to 60 s. The suspension particle size is 0.05 to 0.14 μm in diameter, as determined by negative stain electron microscopy. Figure 1 shows the chemical structure of *N*-decyl-IDP.

Preparation of L-N-decyl-IDP. Multilamellar vesicles containing *N*-decyl-IDP were prepared as follows: chromatographically pure DMPC and DMPG in chloroform solution (25 mg/ml) were purchased from Avanti Polar Lipids, Birmingham, AL. DMPC and DMPG in a 7:3 molar ratio were mixed with a chloroform solution of *N*-decyl-IDP using a lipid to drug weight ratio of 15:1. The chloroform was evaporated in a rotary evaporator (Buchi Brinkman Instruments, Westbury, NY). The dried lipid film obtained was dispersed in normal saline solution (NSS) with vigorous handshaking. The suspension was then centrifuged at 30,000 *g* for 45 min, the supernatant was discarded, and the pellet was resuspended in NSS at the desired concentration. The encapsulation efficiency was measured by determining *N*-decyl-IDP in the supernatant by ultraviolet spectrophotometry at a wavelength of 216 nm, and elemental platinum in the liposome residue and supernatant by flameless atomic absorption spectrophotometry (Varian, Techtron; Mulgrave, Australia). The encapsulation efficiency was consistently greater than 95% by both methods. In vitro stability was assessed by incubating the preparation in NSS for 14 days at 4 °C. The sample was centrifuged at 30,000 *g* for 45 min and the amount of *N*-decyl-IDP was measured in the supernatant. It was found that greater than 95% of the drug was still bound to the liposomes and no vesicle disruption was observed by light microscopy.

Animals. Male Sprague-Dawley rats weighing 480 to 520 g and male CD_1 Swiss mice weighing 28 to 32 g were obtained from the University of Texas Science Park (Bastrop, Tx).

Platinum determinations. The pharmacokinetic studies were carried out in rats, and elemental platinum was measured in whole blood by X-ray fluorescence at the Department of Analytical Chemistry, the University of Texas Medical School at Houston, as described previously [21].

In the tissue distribution studies in mice, elemental platinum was measured in solubilized tissue, using a Varian AA 1475 automated, flameless atomic absorption spectrophotometer (FAAS), with a DS15 computer (Varian, Techtron; Mulgrave, Australia). Platinum was determined using a wavelength of 265.9 nm. Platinum concentrations were calculated against standard concentrations of hexachloroplatinic acid in 0.1 *N* HCL. Tissues were solubilized using hyamine hydroxide (methyl benzethonium hydroxide; Sigma Chemical Co., St. Louis, Mo), according to the method of Ziddick et al. [23]. Briefly, up to 200 mg of tissue were mixed with 0.5 ml hyamine hydroxide and incubated overnight at 60 °C to achieve a complete digestion. The solution was then brought to a standard volume with 0.1 *N* HCL and was directly measured by FAAS. Recovery was consistently greater than 80%. The limit of sensitivity of the Varian AA 1475 for platinum was 0.05 μg/ml, which corresponded to 1 μg elemental platinum per g of wet tissue weight after digestion. Values below this limit were reported as not detected.

Pharmacokinetics. Groups of three rats were used for the study. The dose of L-*N*-decyl-IDP and F-*N*-decyl-IDP was 12.5 mg/kg. This dose is the species equivalent of the maximum tolerated dose in the mouse (25 mg/kg). Rats were anesthetized i.m., with 25 mg/kg ketamine (Parke-Davis, Morris Plains, NJ). Repeated doses of 4 mg/kg were administered i.m. for maintenance of anesthesia. The femoral pedicle was dissected and the artery and vein were catheterized with thin polyethylene catheters attached to 27-gauge needles with blunted edges. Drugs were injected as a bolus over 1 min via the vein, and arterial blood samples of 0.5 ml were drawn at 1, 3, 5, 10, 30, 60, 90, 120, and 150 min into heparinized tubes. The catheters were flushed after each sampling with an equal volume of a solution of 1/1000 heparin in NSS. Rats were killed by exsanguination under an overdose of Penthotal (International Medication System Ltd., South El Monte, Calif). Pharmacokinetic parameters were derived from nonlinear regression analysis of the data.

Tissue distribution. CD_1 Swiss mice were injected i.v. via the tail vein with 18.5 mg/kg of L-*N*-decyl-IDP, F-*N*-decyl-IDP or the equimolar dose of CDDP (9.75 mg/kg). These doses were selected based on the maximum tolerated single dose of CDDP in mice (10 mg/kg). At 30 min, 4 h, 24 h, and 72 h, groups of four mice each were killed by exsanguination under anesthesia with methoxyfluoran (Methofane, Pitman-Moore; Washington Crossing, NJ), and the liver, spleen, stomach, small bowel, brain, lung, skin, muscle, kidney, and bone marrow were removed, digested, and assayed for platinum determination.

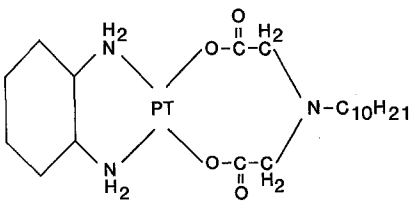

Fig. 1. *Cis*-bis *N*-decyliminodiacetato-1,2-diaminocyclohexane platinum (II) (*N*-decyl-IDP)

Metabolic studies. Groups of three mice each were injected with equimolar doses of L-*N*-decyl-IDP, F-*N*-decyl-IDP, and CDDP as above, and each animal was then placed in a metabolic cage. Urine and stool were collected over 24 h and weight and volumes were measured. Stool pellets were disrupted with a probe sonicator, digested in hyamine hydroxide, and brought to a standard volume with 0.1 *N* HCL. Solid-fiber residues were allowed to sediment, and the clear supernatant was assayed for elemental platinum by FAAS. Urine samples were directly assayed by FAAS without further processing. Results were expressed as micrograms of elemental platinum per 24 h and percent of total injected dose recovered in stool and urine.

Statistical analysis. Statistical differences between the different groups of animals in the pharmacokinetic parameters and the tissue levels of platinum were determined by Student's *t*-test.

Results

Pharmacokinetics

The blood platinum disappearance curves for L-*N*-decyl-IDP and F-*N*-decyl-IDP were biphasic and fit a two-compartment model (Fig. 2). The volume of distribution was 176 ml/kg for L-*N*-decyl-IDP (34% of body weight) and 608 ml/kg for F-*N*-decyl-IDP (120% of body weight) ($P < 0.02$) (Table 1). The peak blood platinum levels for L-*N*-decyl-IDP were 4 times higher than for F-*N*-decyl-IDP (24.2 versus 6.1 μg Pt/ml; $P < 0.02$). The half-life of the initial rapid phase of distribution ($t_{1/2\alpha}$) of L-*N*-decyl-IDP was similar to that of F-*N*-decyl-IDP (4.74 versus 3.0 min; $P = 0.10$). However, the $t_{1/2\beta}$ of L-*N*-decyl-IDP was half that of F-*N*-decyl-IDP (73.4 versus 131.5 min; $P < 0.02$). The clearance rate was 3 times lower for L-*N*-decyl-IDP than for F-*N*-decyl-IDP (3.4 versus 10.8 ml/kg per min; $P < 0.02$). Consequently, the CXT of L-*N*-decyl-IDP was threefold higher than that of F-*N*-decyl-IDP (1308 versus 395 μg Pt/ml per min $P < 0.02$).

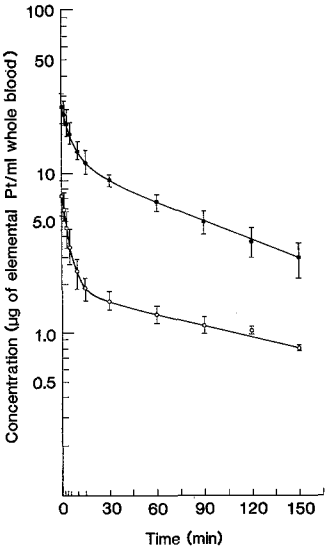

Fig 2. Blood clearance of L-*N*-decyl-IDP (-●-) and F-*N*-decyl-IDP (-○-). Each point represents mean ± SD ($N=3$). Dose 12.5 mg/kg for both forms of the analogue

Tissue distribution

The administration of L-*N*-decyl-IDP resulted in higher peak tissue levels of platinum in the spleen (38.3 μg/g wet weight versus none detected), lung (33 versus 3.6 μg/g; $P < 0.02$), and liver (16.2 versus 11.7 μg/g; $P < 0.02$) than with F-*N*-decyl-IDP (Table 2). No platinum was detected in the stomach, small bowel, or skin after the administration of L-*N*-decyl-IDP. In contrast, F-*N*-decyl-IDP resulted in platinum levels that were low in the stomach and high in the small bowel (70.5 μg/g). Kidney platinum levels were similar, with both forms of the drug (10.4 μg/g for L-*N*-decyl-IDP versus 10.5 μg/g for F-*N*-decyl-IDP). Platinum levels were undetectable in most tissues 24 h after the administration of both L-*N*-decyl-IDP and F-*N*-decyl-IDP; however, detectable levels in the liver were still present at 72 h. In the case of CDDP, although the peak tissue levels of platinum were lower than those obtained with L-*N*-decyl-IDP, platinum was still within the detectable range in the liver, skin, and kidney by 72 h. No platinum level was detected in brain, muscle or bone marrow with either of the three drugs used.

Metabolic studies

The combined urinary and fecal excretion of elemental platinum at 24 h accounted for 54% of the injected dose of L-*N*-decyl-IDP, 65% of the injected dose of F-*N*-decyl-

Table 1. Blood pharmacokinetics of L-*N*-decyl-IDP in rats[a]

Drug	Dose (mg/kg)	Vd[b] (ml/kg)	Peak blood level (μg Pt/ml)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	Clearance (ml/kg/min)	CXT (μg Pt/ml/min)
L-*N*-decyl-IDP	12.5	176 ± 29.7	24.2 ± 4.3	4.7 ± 0.9	73.4 ± 12.6	3.4 ± 0.6	1308 ± 204.0
F-*N*-decyl-IDP	12.5	608 ± 97.6[c]	6.1 ± 1.6[c]	3.0 ± 0.5	131.5 ± 15.0[c]	10.8 ± 1.4[c]	395 ± 37.3[c]

[a] All data calculated as micrograms of elemental platinum injection per kg body weight. All values are expressed as mean ± SD ($N = 3$)
[b] Volume of distribution at time 0
[c] $P < 0.02$

Table 2. Tissue distribution of platinum preparations in mice

Drug	Liver	Spleen	Stomach	Small bowel	Lung	Skin	Kidney
			30 min				
L-*N*-decyl-IDP	16.2±1.5[a,b]	38.3±5.0	ND	ND	33.0±7.0[b]	ND	10.4±1.9
F-*N*-decyl-IDP	11.7±1.9[c]	ND	3.5±1.5	70.5±51[c]	3.6±0.5	4.0±0.7	10.5±1.1[c]
CDDP	6.6±1.3	2.5±3.0	1.9±0.3	3.4±0.9	3.4±0.6	3.7±2.2	7.4±1.7
			4 h				
L-*N*-decyl-IDP	5.4±0.7	25.8±7.3	ND	ND	5.2±1.2	ND	3.8±0.9
F-*N*-decyl-IDP	3.9±0.6	ND	ND	ND	ND	5.6±1.3	4.0±0.6
CDDP	4.9±0.8	ND	ND	ND	ND	2.4±1.0	3.6±0.8
			24 h				
L-*N*-decyl-IDP	3.9±1.2	ND	ND	ND	ND	ND	ND
F-*N*-decyl-IDP	2.6±0.5	ND	ND	ND	ND	ND	ND
CDDP	3.5±0.5	ND	1.4±0.2	ND	1.9±0.1	2.3±1.5	4.7±0.6
			72 h				
L-*N*-decyl-IDP	1.9±1.2	ND	ND	ND	ND	ND	ND
F-*N*-decyl-IDP	2.1±1.4	ND	ND	ND	ND	ND	ND
CDDP	3.7±0.5	ND	ND	ND	ND	2.4±0.6	1.9±0.3

[a] μg Pt/g wet tissue mean ± SD ($N = 4$)
[b] $P < 0.02$ (L-*N*-decyl-IDP and F-*N*-decyl-IDP)
[c] $P < 0.02$ (F-*N*-decyl-IDP and CDDP)
ND, None detected (< 1 μg Pt/g wet weight); L-*N*-decyl-IDP and F-*N*-decyl-IDP dose, 18.5 mg/kg; CDDP dose, 9.75 mg/kg

IDP, and 86% of the injected dose of CDDP. The stool and urine excretion for L-*N*-decyl-IDP and F-*N*-decyl-IDP were not significantly different (stool, 33.6 μg Pt/24 h versus 33.8 μg Pt/24 h, $P > 0.05$; urine, 74.8 μg Pt/24 h versus 98.3 μg Pt/24 h $P > 0.05$). In contrast, CDDP was almost exclusively excreted in the urine (170.5 μg Pt/24 h) (Table 3).

Discussion

Liposome encapsulation altered the pharmacokinetics and tissue distribution of *N*-decyl-IDP. The blood clearance curves of L-*N*-decyl-IDP and F-*N*-decyl-IDP were biphasic and similar to that of the parent compound, CDDP [10]. The volume of distribution (Vd) of L-*N*-decyl-IDP was only 34% of body weight, which probably reflected a nonuniform distribution of the drug caused by the well-known preferential localization of liposomes in organs rich in RES cells. In contrast, the Vd of F-*N*-decyl-IDP was larger than the body weight (120%), probably as a result of a more uniform distribution and/or a faster excretion of the free drug. A Vd similar to that of F-*N*-decyl-IDP has been reported for CDDP in the rat by Litterst [11]. Liposome encapsulation did not modify the duration of the distribution phase, but shortened the elimination phase ($t_{1/2\beta}$) and resulted in a threefold decrease of the blood clearance rate. The net result of the decreased clearance of L-*N*-decyl-IDP was a CXT threefold higher than that of F-*N*-decyl-IDP.

Table 3. Urinary and fecal excretion of platinum preparations in mice

Drug	Fecal excretion (μg Pt/24 h)	Urinary excretion (μg Pt/24 h)
L-*N*-decyl-IDP	33.6± 9.0[a] (17%)[b]	74.8±20.0 (37%)
F-*N*-decyl-IDP	33.8±28.3 (17%)	98.3±15.0 (49%)
CDDP	1.4± 0.9 (0.7%)	170.5±77.0 (85%)

[a] Mean ± SD ($N = 3$)
[b] Percent of injected dose

Tissue levels of elemental platinum achieved with L-*N*-decyl-IDP were much higher than those achieved with F-*N*-decyl-IDP in the lung and spleen, slightly higher in the liver, lower (none detected) in the gastrointestinal tract, and similar in the kidney. A possible explanation for the only slightly higher platinum levels achieved in the liver with L-*N*-decyl-IDP may be the binding of the lipophilic drug to serum lipoproteins with subsequent sharing of the normal blood clearance pathway through the liver. In the lung, the high peak platinum levels and subsequent fast clearance observed with L-*N*-decyl-IDP are probably due to the arrest of an important fraction of liposomes in the pulmonary microvasculature, as reported previously [19]. Although both forms of the drug resulted in high peak platinum levels in the kidneys, the risk of nephrotoxicity is probably low since the diaminocyclohexane and the iminodiacetato-diaminocyclohexane analogues are much less nephrotoxic than CDDP [6, 20].

Although the differences in organ distribution between F-*N*-decyl-IDP and CDDP were less striking, peak platinum levels with F-*N*-decyl-IDP were significantly higher in the small bowel, liver, and kidney; similar in the lung and skin; and lower in the spleen. The high levels of platinum in the small bowel with F-*N*-decyl-IDP suggest the biliary route as a possible way of excretion.

The metabolic studies showed that 17% of the injected doses of L-*N*-decyl-IDP and F-*N*-decyl-IDP were excreted

in the stool. F-*N*-decyl-IDP probably reached the stool from the small bowel. In the case of L-*N*-decyl-IDP, delivery to the lumen by macrophages present in the large bowel wall, as described by Lee et al. [9], might play an important role.

In summary, in this study, we have shown that the encapsulation of an analogue of CDDP in MLVs results in marked changes in the pharmacokinetics and organ distribution of the drug. The enhanced delivery of platinum to the lung, spleen, and liver, in conjunction with the low or absent gastrointestinal tract platinum levels achieved with the liposomal form of the drug, suggest that liposome encapsulation may be of potential value in the treatment of tumors involving those organ sites and, at the same time, avoid gastrointestinal toxicity. We are currently investigating the bioavailability of lipophilic CDDP analogues encapsulated in liposomes by testing the antitumor activity in animal models of experimental tumor metastases, studying the in vitro macrophage drug metabolism, and by ultrastructural localization.

References

1. Burchenal JH, Kalaher K, O'Toole T, Chisholm J (1979) Lack of cross-resistance between certain platinum coordination compounds in mouse leukemia. Cancer Res 37: 3455
2. Forssen EA, Tokes ZA (1981) Use of anionic liposomes for the reduction of chronic doxorubicin-induced cardiotoxicity. Proc Natl Acad Sci USA 78: 1873
3. Freise J, Mueller WH, Lagerstedt P, Schmoll HJ (1982) Pharmacokinetics of liposome-encapsulated cisplatin in rats. Arch Int Pharmacodynamie Ther 258: 182
4. Gabizon A, Dagan A, Goren D, Barowholz Y, Fuks Z (1982) Liposomes as *in vivo* carriers of Adriamycin: reduction of cardiac uptake and preserved antitumor activity in mice. Cancer Res 42: 4734
5. Gabizon A, Goren P, Fuks Z, Barowholz Y, Dagan A, Meshover A (1983) Enhancement of Adriamycin delivery to liver metastatic cells with increased tumoricidal effect using liposomes as drug carriers. Cancer Res 43: 4730
6. Hacker MP, Khokhar AR, Krakoff IH, Brwon DB, McCormack JJ (1986) Water-soluble N substituted iminodiacetate diaminocyclohexane platinum (II) complexes as potential antitumor agents. Cancer Res (in press)
7. Kasi LP, Lopez-Berestein G, Mehta K, Rosenblum M, Glenn HJ, Haynie TP, Mavligit GM, Hersh EM (1984) Distribution and pharmacology of ^{99m}Tc-labeled multilamellar liposomes in rats and mice. Int J Nucl Med 11: 35
8. Layton D, Trouet A (1980) A comparison of the therapeutic effects of free and liposomally encapsulated vincristine in leukemic mice. Eur J Cancer 16: 945
9. Lee SH, Starkey PM, Gordon S (1985) Quantitation analysis of total macrophage content in adult mouse tissues. J Exp Med 161: 475
10. Litterst CL, LeRoy AF, Guarino AM (1979) Disposition and distribution of platinum following peritoneal administration of cis-dichlorodiammine platinum to animals. Cancer Treat Rep 63: 1485
11. Litterst CL, Torres IJ, Guarino AH (1979) Plasma levels and organ distribution of platinum in the rat, dog and dogfish shark following single intravenous administration of cis-dichlorodiamine platinum (II). Wadley Med Bull 7: 170
12. Lopez-Berestein G, Kasi, LP, Rosenblum MG, Haynie T, Jahns M, Glenn H, Mehta R, Mavligit GM, Hersh EM (1984) Clinical pharmacology of ^{99m}Tc-labeled liposomes in patients with cancer. Cancer Res 44: 375
13. Mayhew E, Rustum YN, Szoka F, Papahadjopoulos D (1979) Role of cholesterol in enhancing the antitumor activity of cytosine arabinoside entrapped in liposomes. Cancer Treat Rep 63: 1923
14. Newman RA, Khokhar AR, Sunderland BA, Travis BL, Bulger ER (1986) A comparison in rodents of renal and intestinal toxicity of cisplatin and a new water-soluble antitumor platinum complex: N-methyl-iminodiacetatodiaminocyclohexane platinum (II). J Pharmacol Toxicol (in press)
15. Olson F, Mayhew E, Haslow D, Rustum Y, Szoka F (1982) Characterization, toxicity and therapeutic efficacy of Adriamycin encapsulated in liposomes. Eur J Cancer Clin Oncol 18: 167
16. Patel KL, Baldeschweiller JD (1984) Mouse Lewis lung carcinoma and hepatoma. Ascites treatment by combination of liposome chemotherapy and non-specific immunotherapy. Int J Cancer 34: 717
17. Perez-Soler R, Khokhar AR, Lopez-Berestein G (1986) Treatment and prophylaxis of experimental liver metastases with cis-bis-neodecanoate-1,2-diaminocyclohexane platinum (II) encapsulated in liposomes (Abstract 1602). Proceedings American Association of Cancer Research, vol. 27, March 1986, p 403
18. Perez-Soler R, Khokhar AR, Lopez-Berestein G (1986) Development of lipophilic cisplatin analogues encapsulated in liposomes. In: Gregoriadis G (ed) Liposomes as drug carriers: trends and progress. John Wiley & Sons, New York (in press)
19. Poste G, Bucana C, Raz A, Bugelski P, Kirsh R, Fidler IJ (1982) Analysis of the fate of systemically administered liposomes and implications for their use in drug delivery. Cancer Res 42: 1412
20. Prestayko AW, Bradner WT, Huftalen JB, Rose WC, Schurig JE, Cleare MI, Hydes PC, Crooke ST (1979) Antileukemic (L1210) activity and toxicity of *cis*-dichlorodiammine platinum (II) analogs. Cancer Treat Rep 63: 1503
21. Seifert WE, Stewart DJ, Benjamin RS, Caprioli RM (1983) Energy dispersive x-ray fluorescence determination of platinum in plasma, urine and cerebrospinal fluid of patients administered *cis*-dichlorodiammine platinum (II). Cancer Chemother Pharmacol 11: 120
22. Todd JA, Levine, AM, Tokes ZA (1980) Liposome encapsulated methotrexate interactions with human chronic lymphocytic leukemia cells. J Natl Cancer Inst 64: 715
23. Ziddick ZH, Boxall FE, Calvent AH, Harrap KR (1985) Use of a solubilizing procedure in the determination of platinum (Pt) in rodent and human tissues following carboplatin (JMS, CBDCA) administration (Abstract 1383). Proceedings of the American Association of Cancer Research 26: 351

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