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Organ distribution and tumor uptake of liposome entrapped *cis-bis*-neodecanoato Trans-R, R-1,2 diaminocyclohexane platinum (II) administered intravenously and into the proper hepatic artery*

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Summary. Blood and tissue levels of elemental platinum (Pt) were measured after the administration of a liposomally entrapped cisplatin analogue, *cis-bis*-neodecanoato-*trans-R,R*-1,2-diaminocyclohexane platinum (II) (L-NDDP). In mice bearing subcutaneous B16 melanoma tumors, Pt tumor levels were not significantly different in animals treated i.v. with an equimolar dose of L-NDDP or cisplatin. In rabbits bearing liver tumors of VX2 carcinoma, i.v. administration of L-NDDP resulted in 2- to 20-fold higher Pt levels in all tissues (including VX2 tumors) except the brain and peripheral nerve than in animals treated with an equimolar dose of cisplatin. Compared with i.v. administration, inoculation of either drug into the proper hepatic artery resulted in a severalfold increase of Pt levels in the VX2 tumors. Blood and other tissue levels were not substantially changed by intraarterial (i.a.) administration. These studies show that (1) multilamellar lipid vesicles can adequately deliver a lipophilic cisplatin analogue (NDDP) to nonphagocytic tumors when administered i.v. and (2) the inoculation of L-NDDP into the proper hepatic artery results in higher Pt tumor levels than with i.v. administration but does not decrease the systemic distribution of the drug.

Introduction

Drug carriers are currently being explored as a way to increase the therapeutic index of antitumor agents. Among drug carriers, liposomes have the advantages of being nontoxic, biodegradable, and relatively easy to make [4, 14]. It is well known that after i.v. injection in man, liposomes concentrate mainly in the liver and spleen, although also in the lungs and bone marrow [5]. Little is known, however, on the affinity or targeting properties of liposomes for tumors that involve these organs. In general, the uptake of liposomes by tumors appears to be dependent on many factors, some of them related to the vesicles, such as size and flexibility, and others to the tissue, such as the type and size of the endothelial fenestrae and the phagocytic activity of the cells [11].

We have previously reported on the development and biological properties of a liposomal formulation of a lipophilic cisplatin analogue, L-NDDP [10]. L-NDDP was more active than cisplatin against liver metastases of M5076 reticulosarcoma and had significant activity against tumor systems resistant to cisplatin [2]. We have also studied the ultrastructural localization of multilamellar vesicles containing NDDP in normal mouse liver and liver metastases of mouse M5076 reticulosarcoma, which is a tumor of the monocyte-macrophage lineage [9]. In these studies, we found that lipid vesicles of up to 2 μm in diameter could cross the liver sinusoids and be taken up by hepatocytes to a larger extent than by Kupffer cells; they were also taken up by M5076 cells of nodular liver metastases.

A logical continuation of these studies was to ascertain the uptake of L-NDDP by tumors that are extrahepatic, such as the subcutaneous B16 melanoma of the mouse, or intrahepatic but not of monocyte-macrophage origin, such as the VX2 carcinoma of the rabbit [1].

The liposomal preparation was given i.v. to mice, and in the studies with VX2 liver tumors in rabbits we compared platinum (Pt) levels in normal tissues and tumors after the administration of the drugs into a peripheral vein and into the proper hepatic artery. The results were compared with those obtained in animals that received an equimolar dose of cisplatin, since a satisfactory free NDDP formulation is not available due to its lack of solubility in nontoxic solvents.

Materials and methods

Materials

K_2PtCl_4 was purchased from Aesar (Johnson Matthey, Inc., Seabrook, NH). *trans-R,R*-1,2-Diaminocyclohexane was purchased from Morton Thiokol, Inc. (Danvers, Mass). Neodecanoic acid was obtained from Exxon Chemical Co, Houston, Tex. Chromatographically pure (thin-layer chromatography) dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) were obtained from Avanti Polar Lipids (Birmingham, Ala). Cisplatin (Platinol) (Bristol Laboratories, Syracuse, NY) was obtained from the hospital pharmacy.

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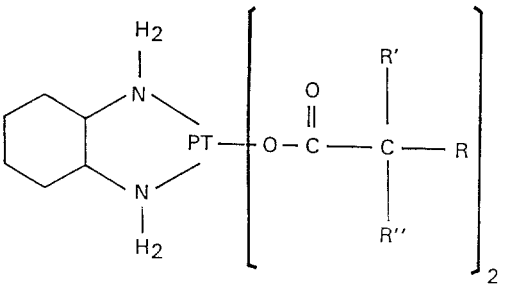

Fig. 1. Chemical structure of NDDP. R, R', and R" can be an aliphatic group of 2 to 4 carbons, resulting in a group having $C_{10}O_2H_{19}$ as its empirical formula

Synthesis of NDDP

Cis-bis-neodecanoato *trans-R,R*-1,2-diaminocyclohexane platinum (II) (NDDP) was synthesized as has previously been extensively described [10]. The complex was characterized by elemental analysis, infrared spectroscopy, and NMR spectrometry. NDDP was found to be more than 95% pure by HPLC analysis using two protein pak 160 columns and 100% methanol. Its chemical structure is shown in Fig. 1.

Preparation of L-NDDP

NDDP was entrapped in multilamellar vesicles composed of DMPC and DMPG at a 7:3 molar ratio as has previously been reported for several lipophilic platinum complexes [3, 6]. The final drug: lipid weight ratio was 1:5, the entrapment efficiency was more than 98%, and the vesicle size ranged from 1 to 5 μm, with most vesicles measuring between 2 and 3 μm in diameter.

Animals

C57BL/6 male mice weighing between 18 and 22 g were purchased from Charles River Lab, Inc. (Wilmington, Mass). New Zealand white male rabbits weighing between 2 and 2.5 kg were purchased from The University of Texas Science Park (Bastrop, Tex).

Tumor lines

B16 melanoma cells were obtained from the DCT tumor repository, National Cancer Institute, Frederick, Md. The B16 melanoma cells were grown subcutaneously in C57BL/6 mice and transplanted every 2 weeks. VX2 carcinoma cells were obtained from the Department of Veterinary Medicine, M. D. Anderson Hospital and Tumor Institute. VX2 cells were grown intramuscularly in New Zealand white rabbits until used for intrahepatic inoculation.

Experimental design
Drug uptake studies in subcutaneous B16 melanoma tumors

Groups of three C57BL/6 mice bearing subcutaneous B16 melanoma tumors were given an equimolar dose of L-NDDP (21.4 mg/kg) or cisplatin (10 mg/kg) i.v. via the tail vein. The animals were sacrificed 30 min, 2 h or 24 h later. Tumors were resected and elemental Pt was measured by X-ray fluorescence [13] at the Department of Analytical Chemistry, University of Texas, Medical School, Houston, Tex. The results were expressed as μg elemental Pt/g dry tissue.

Organ distribution and drug uptake studies in liver VX2 carcinoma tumors

Intramuscular VX2 tumors were resected, immediately cut into small pieces, and kept in suspension in MEM medium. New Zealand white rabbits were anesthestized, a microlaparatomy was carried out, and 0.5 ml of the VX2 tumor fragments suspension was inoculated at a single site in the left hepatic lobe. Liver tumors were allowed to grow for 2 weeks. On day 14, the animals were distributed into four groups. Two animals were given 5 mg/kg cisplatin i.v., and two others received an equimolar dose (10.7 mg/kg) of L-NDDP i.v. The six remaining animals had an intraarterial (i.a.) catheter placed into the proper hepatic artery via a femoral artery under fluoroscopy, for drug inoculation. Three of them were given 5 mg/kg cisplatin and the other three, and equimolar dose (10.7 mg/kg) of L-NDDP. The rate of infusion was in all cases 1 mg/min. Peripheral venous blood samples were obtained 5, 15, 30, 60, and 120 min after the completion of drug infusion. All animals were sacrificed after obtaining the 120-min blood sample. Tissue samples of liver, spleen, lung, kidney, small intestine, brain, peripheral nerve (sciatic nerve), and intrahepatic VX2 tumor were obtained from all animals. Elemental Pt levels in whole blood and tissues except brain and peripheral nerve were measured by X-ray fluorescence. Tissue levels of elemental Pt in brain and peripheral nerve were measured on a Varian (Model 1475/GTA-95) (Mulgrave, Australia) flameless atomic spectrophotometer following solubilization of the tissue as has previously been reported [12], since the levels in these tissues are below the sensitivity limit of the X-ray fluorescence method.

Results

Pt levels in subcutaneous B16 melanoma tumors

Table 1 shows the Pt levels achieved in subcutaneous B16 melanoma tumors 30 min, 2 h, and 24 h after the administration of equimolar doses of L-NDDP and cisplatin. No significant difference in the platinum levels between tumors from animals treated with L-NDDP and those from animals treated with cisplatin was observed at any time point.

Blood Pt levels after i.v. and i.a. administration of L-NDDP and cisplatin to New Zealand white rabbits

Figure 2 shows the blood Pt levels at five time points after the i.v. administration of an equimolar dose of L-NDDP or cisplatin to New Zealand rabbits. Although only two animals were eventually included in each group (two tumor-bearing animals died of complications of the microla-

Table 1. Levels of elemental platinum in subcutaneous B16 melanoma tumors[a]

Drug	Elemental platinum μg/g mean ± SD		
	30 min	2 h	24 h
L-NDDP	43.3 ± 24.0	26.4 ± 9.1	17.3 ± 4.6
Cisplatin	40.4 ± 10.8	25.1 ± 4.9	22.6 ± 3.0

[a] Dose of L-NDDP: 21.4 mg/kg (33 μmol/kg). Dose of cisplatin: 10 mg/kg (33 μmol/kg). Route of drug administration: i.v.

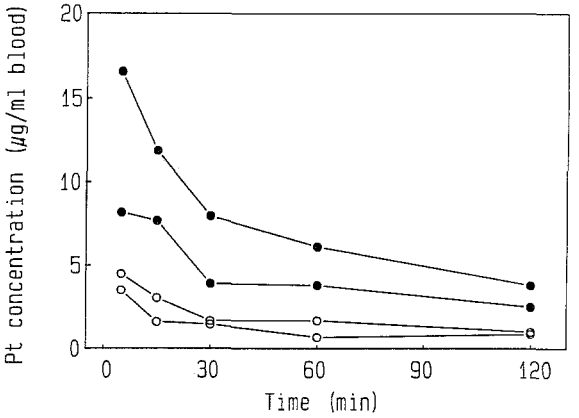

Fig. 2. Blood levels of elemental Pt at different times after the i.v. administration of equimolar doses of L-NDDP (●) and cisplatin (○) to New Zealand white rabbits. Data are shown for each individual rabbit

parotomy), blood Pt levels were consistently 2–3 times higher at all times in the animals that received L-NDDP.

Figure 3 shows the blood Pt levels at five time points after the i.a. administration of an equimolar dose of L-NDDP or cisplatin to New Zealand rabbits. Blood levels were again consistently 2–3 times higher in the animals that received L-NDDP.

Blood Pt levels achieved after i.v. inoculation of L-NDDP or cisplatin were not substantially different from those achieved after i.a administration of the same drugs.

Tissue and VX2 tumor Pt levels after i.v. and i.a. administration of L-NDDP and cisplatin to New Zealand white rabbits

Table 2 shows the Pt levels in normal tissue and VX2 tumors 2 h after the i.v. administration of equimolar doses of L-NDDP or cisplatin to New Zealand rabbits. Although only two animals were included in each group, no major individual differences between the animals in each group were observed. Mean normal tissue Pt levels were 2- to 20-fold higher in liver (73 vs 14 μg/g) spleen (210 vs 11 μg/g), lungs (27 vs 7 μg/g), kidney (128 vs 47 μg/g) and gut (19 vs 10 μg/g) in the animals treated with L-NDDP.

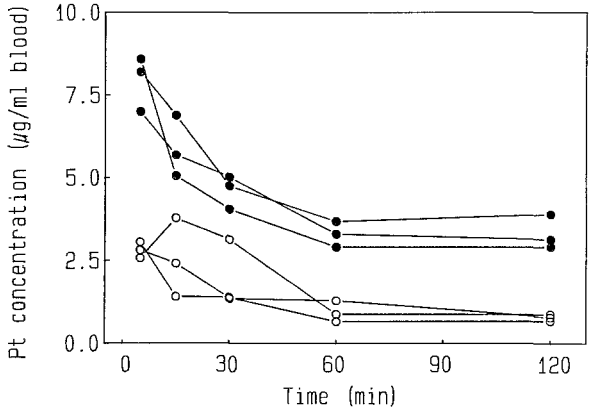

Fig. 3. Blood levels of elemental Pt at different times after the i.a. administration of equimolar doses of L-NDDP (●) and cisplatin (○) to New Zealand white rabbits. Data are shown for each individual rabbit

Table 2. Tissue and VX2 tumor levels of elemental platinum 2 h after the i.v. administration of L-NDDP and cisplatin[a]

Organ	Elemental platinum μg/g tissue			
	L-NDDP		Cisplatin	
	1	2	1	2
Liver	74.4	71.4	17.8	24.0
Spleen	280.9	139.5	12.5	8.6
Lung	18.1	36.4	7.8	6.0
Kidney	96.6	159.1	55.1	38.6
Gut	17.9	19.2	10.1	9.8
Brain	0.032	0.058	0.040	0.061
Peripheral nerve	1.28	2.05	1.39	2.17
VX2 tumor	26.1	24.7	12.8	12.9

[a] Dose of L-NDDP: 10.7 mg/kg (16 μmol/kg). Dose of cisplatin: 5 mg/kg (16 μmol/kg)

Platinum levels in brain and peripheral nerve were comparable (brain: 0.045 for L-NDDP vs 0.050 μg/g for cisplatin; peripheral nerve: 1.66 μg/g for L-NDDP vs 1.77 μg/g for cisplatin).

Table 3 shows the Pt levels in normal tissue and VX2 tumors 2 h after the i.a. inoculation of equimolar doses of L-NDDP or cisplatin to New Zealand white rabbits. Mean normal tissue Pt levels were 2- to 30-fold higher in the animals treated with L-NDDP: liver (75.2 vs 32.5 μg/g), spleen (251.9 vs 8.3 μg/g), lung (23.4 vs 12.0 μg/g), kidney (94.5 vs 30.1 μg/g). Platinum levels were comparable in the gut (17.5 vs 15.1 μg/g), brain (0.034 vs 0.041 μg/g), and peripheral nerve (1.91 vs 2.64 μg/g).

The use of the i.a. route did not markedly alter the normal tissue Pt levels in the animals treated with L-NDDP or cisplatin. All differences were less than 2-fold.

VX2 tumor Pt levels were 2-fold higher in the animals treated by the i.v. route with L-NDDP than in the animals treated with cisplatin (25.4 vs 12.8 μg/g). VX2 tumor Pt levels were markedly increased by inoculating either

Table 3. Tissue and VX2 tumor levels of elemental platinum 2 h after the intrahepatic arterial administration of L-NDDP and cisplatin[a]

Organ	Elemental platinum μg/g tissue mean ± SD[b]	
	L-NDDP	Cisplatin
Liver	75.2 ±16.2	32.5 ±7.9
Spleen	251.9 ±90.8	8.3 ±1.9
Lung	23.4 ± 3.9	12.0 ±4.9
Kidney	94.5 ±14.3	30.1 ±3.9
Gut	17.5 ± 2.2	15.1 ±5.8
Brain	0.034± 0.004	0.041±0.034
Peripheral nerve	1.91 ± 0.60	2.64 ±0.63
VX2 tumor		
animal 1	74.4	164.0
animal 2	146.4	19.5
animal 3	94.8	166.2

[a] Dose of L-NDDP: 10.7 mg/kg (16 μmol/kg). Dose of cisplatin: 5 mg/kg (16 μmol/kg)
[b] Three animals per group

L-NDDP or cisplatin into the proper hepatic artery. VX2 tumor Pt levels were increased 4-fold in the animals that received L-NDDP (105.2 μg/g with the i.a. route vs 25.4 μg/g with the i.v. route). VX2 tumor Pt levels were increased 10-fold in two animals that received cisplatin i.a. (164.0 and 166.2 with the i.a. route vs 12.8 μg/g with the i.v. route). However, in a third animal such an increase was not observed (19.5 vs 12.8 μg/g).

Discussion

This study provides information on three different aspects of the pharmacology of a liposomally entrapped platinum complex (L-NDDP). First, our results show that multilamellar vesicles can deliver this antitumor agent to intrahepatic and extrahepatic to well-established tumors of non-monocyte-macrophage lineage, in amounts at least comparable to those achieved with equimolar doses of cisplatin.

Second, the normal tissue drug distribution observed after inoculation of the liposomal preparation is basically unaltered by using the proper hepatic artery as the route of administration. In previous studies with radiolabeled empty liposomes of the same composition and charge, we have found that systemic liposome uptake was significantly reduced by infusing the liposomes into the hepatic artery [15]. The present study does not confirm this original observation with empty liposomes, the possible explanations being the different animal species (rabbits vs dogs) or the different preparations used (L-NDDP vs empty liposomes).

Peripheral blood levels of elemental Pt were similar after i.v. and i.a. administration of L-NDDP. The same phenomenon was also observed with cisplatin. Comparison of animals that received equimolar doses of either L-NDDP or cisplatin reveals that blood levels of elemental Pt were 2- to 3-fold higher in those treated with L-NDDP using either route of administration, which is in accordance with results of our previous studies in mice and rats using the i.v. route [3].

L-NDDP resulted in higher Pt levels than cisplatin in most normal tissues in rabbits, when given either i.v. or i.a. at equimolar doses. Differences were most substantial in the spleen (20- to 25-fold), liver (5-fold when given i.v., 2-fold when given i.a.), and lungs and kidneys (3-fold). Differences were less striking in the small bowel. We have previously reported similar differences in mice [7]. Intraarterial inoculation did not result in significant changes in normal tissue distribution for either L-NDDP or cisplatin. In VX2 liver tumors, Pt levels were about 2-fold higher than with cisplatin when both drugs were given i.v. However, Pt tumor levels were increased by 3- to 6-fold in the animals treated i.a. with L-NDDP and more than 10-fold in two of three rabbits treated i.a. with cisplatin. The net result was that tumor Pt levels after i.a. administration were comparable in animals treated with either L-NDDP or cisplatin. Surprisingly enough, whereas tumor Pt levels were increased by severalfold after i.a. infusion, normal liver levels were unchanged in the animals treated with L-NDDP and increased less than 2-fold in the animals treated with cisplatin. This probably indicates that the tumor blood supply is predominantly provided by the hepatic artery, whereas the parenchymal blood supply is predominantly provided by the portal vein.

From our data, we cannot ascertain whether the delivery of NDDP to the different tissues is predominantly that of NDDP in intact liposomes (direct delivery), of NDDP bound to a second carrier such as serum proteins, or of metabolites. Elemental Pt levels in the tissues do not necessarily correlate with biological activity, since the latter depends on the levels of bioavailable platinum complex. However, methods for measuring the different species of platinum complexes in tissues are not available. Therefore, the only way to assess the bioavailability of the drug delivered is by studying the antitumor effect. These studies are currently in progress.

Finally, a third piece of information generated by this study relates to the Pt levels in brain and peripheral nerve. Differences in the levels achieved in both organs between rabbits that were given an equimolar dose of L-NDDP or cisplatin were less than 2-fold in all cases. This indicates that NDDP, although lipophilic, cannot significantly cross the blood-brain barrier, one of the likely reasons being that most of it remains entrapped in the vesicles. In spite of the limited predictive biological value of the Pt determinations, these results suggest that an increased neurotoxicity or an increased antitumor effect in the brain are unlikely to occur with the use of L-NDDP instead of cisplatin.

In conclusion, multilamellar vesicles can deliver a lipophilic cisplatin analogue in greater amounts than cisplatin to most organs, including liver tumors of a colon carcinoma in rabbits. In the brain and peripheral nerve, Pt levels were not significantly different. Any differences in neurotoxicity that may be observed between both agents in future clinical studies are more likely to be related to the different chemical structures of the platinum complexes rather than to the different forms of drug delivery. In the kidney, the Pt levels achieved by the liposomal preparation are also higher than those achieved with cisplatin. However, we have previously shown that L-NDDP is not nephrotoxic in mice and dogs [8].

The use of the intrahepatic arterial route results in markedly higher intrahepatic tumor Pt levels with L-NDDP as well as cisplatin but no significant decrease in the amount of drug delivered systemically. Therefore, the intraarterial route does not appear to offer any additional advantage in terms of systemic protection for this liposomal anticancer agent compared with standard agents (nonentrapped agents). The 3-fold higher Pt levels achieved in the normal liver compared with those achieved in the established liver tumors of VX2 carcinoma when L-NDDP is administered i.v. suggest that the most advantageous clinical setting for the i.v. use of this liposomally entrapped agent may be the prophylaxis of liver metastases, that is, the eradiaction of microscopic nests of cells that do not have vascularity of their own. In this situation, microenvironmental drug levels that are the result of uptake and release of the drug by macrophages and/or parenchymal cells may exert higher antitumor activity than nonliposomally entrapped agents.

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