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ORIGINAL ARTICLE

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Intraliposomal conversion of lipophilic *cis*-bis-carboxylato-*trans*-*R*, *R*-1, 2-diaminocyclohexane-platinum (II) complexes into *cis*-bis-dichloro-*trans*-*R*, *R*-1,2-diaminocyclohexane-platinum (II)

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Abstract *Cis*-bis-neodecanoato-*trans*-R,R-1,2-diaminocyclohexane platinum (II) (NDDP) is a lipophilic platinum complex (Pt complex) developed in a liposomal carrier. Prior studies have suggested that NDDP is a prodrug that exerts its biological activity through activation within the liposome bilayers containing dimyristoyl phosphatidylglycerol (DMPG) before in vivo administration. In order to understand the kinetics of the intraliposomal degradation/activation of different liposomal Pt complexes, we studied the effects of their structure, lipid composition, content of acidic phospholipids and size, and the effects of pH, temperature and the presence of residual chloroform on their stability, in vitro cytotoxicity, and in vivo antitumor activity. The following factors were found to enhance the intraliposomal degradation/activation of Pt complexes: (1) the size and spatial configuration of the Pt complex, (2) an acidic pH, (3) a high temperature, (4) the presence and amount of acidic phospholipids, and (5) the presence of residual chloroform. Liposome size did not affect the intraliposomal stability of different Pt complexes. Good inverse relationships between the extent of drug degradation and in vitro cytotoxicity and between the extent of drug degradation and in vivo antitumor potency were observed, thus confirming that the biological activity of these complexes is exerted through the intraliposomal formation of certain active intermediate(s). The only active intermediate that could be identified was *cis*-bis-dichloro-*trans*-R,R-1,2-diaminocyclohexane platinum(II) whose structure was confirmed by ^{1}H, ^{13}C, and ^{195}Pt nuclear magnetic resonance (NMR) spectroscopy.

Key words Lipophilic platinum complex · Liposome · Drug stability

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Introduction

We have previously developed liposomal formulations of lipophilic Pt complexes for in vivo administration [6, 7] and have studied their chemical and biological properties [1, 4, 6]. The general structure of the Pt complexes used is [DACH-Pt-R_2], where DACH is *trans*-R,R-1,2-diaminocyclohexane and R is a lipophilic carboxylato group. The Pt complex is thought to intercalate between the phospholipid molecules of the lipid bilayers of the liposomes. The most remarkable characteristic of these complexes is that they are not cross-resistant with cisplatin, either in vitro or in vivo [1, 6]. The leading formulation, liposomal *cis*-bis-neodecanoato-DACH-platinum(II) (NDDP) uses large liposomes composed of dimyristoylphosphatidyl choline (DMPC) and dimyristoylphosphatidyl glycerol (DMPG) at a 7:3 molar ratio and is now in clinical trials.

Interestingly, liposomal NDDP must undergo a chemical degradation/activation process into active intermediate(s) within the liposomes shortly after liposome preparation in order to exert its antitumor activity [3]. We have previously reported that this chemical reaction depends on the content of DMPG in the lipid bilayer, and based on this finding, we hypothesized that a DMPG-Pt complex might be one of the active intermediates. We have also reported that the structure of the Pt complex has an effect on the intraliposomal drug stability: the compounds with linear

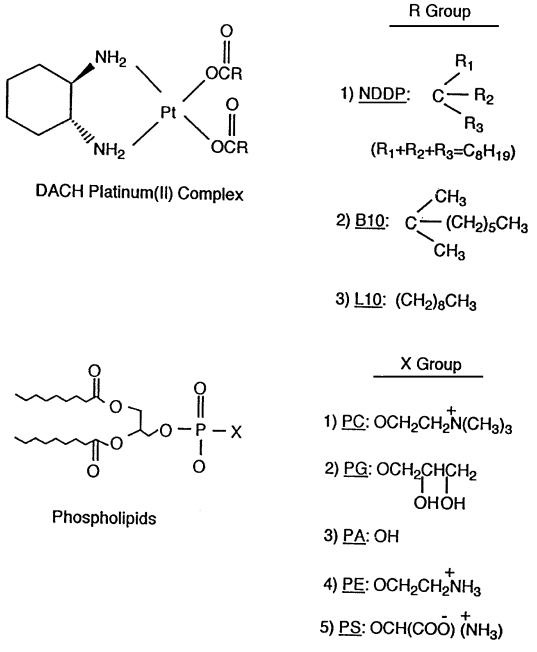


Fig. 1 Chemical structure of NDDP, B10 and L10., and phospholipids

and short carboxylato leaving groups are more stable and less potent than the compounds with branched or longer linear leaving groups [8]. A full characterization of the active intermediate(s) as well as the different factors that influence the degradation/activation process is mandatory for the development of one of these agents as a pharmaceutical product.

For that purpose, we selected NDDP (highly branched structure) and two isomers, B10 (minimally branched structure) and L10 (linear structure) (Fig. 1) and studied the relationship between their biological activity and their intraliposomal stability. We examined the effect of pH, temperature, lipid composition, liposome size, and presence of residual chloroform on the degradation of the Pt complexes, and attempted to identify the active intermediate(s) by tracking experiments using ^{31}P and ^{195}Pt NMR spectroscopy. The degradation/activation of these Pt complexes was greatly dependent on the pH of the suspension, and DACH-dichloroplatinum (DACH-Pt-Cl_2) was the only intermediate that could be identified, thus suggesting that these Pt complexes are prodrugs of DACH-Pt-Cl_2 when incorporated in liposomes suspended in saline.

Materials and methods

Preparation of liposomal Pt complexes.

NDDP, B10, and L10 were synthesized as previously described [1, 2] and recrystallized in acetone. DMPC, DMPG, dioleyl phosphatidyl choline (DOPC), dioleyl phosphatidyl glycerol (DOPG), phosphatidic acid (PA), phosphatidyl ethanolamine (PE), and phosphatidyl serine (PS) were purchased from Avanti Polar Lipids (Alabaster, Ala.).

Multilamellar vesicles containing Pt complexes were prepared by the lyophilization method using the lipids in chloroform solution [1] or as dry powder.

Method 1 Lipids were dissolved in chloroform at the desired molar ratio, and the chloroform was removed in a rotary evaporator. To the dried lipid film, Pt complexes dissolved in *t*-butanol were added and the solutions shaken at 40 °C for 10 min. The solutions were then frozen in a dry-ice/acetone bath, and the *t*-butanol was removed by lyophilization overnight to give lyophilized preliposomal powder.

Method 2 Lipids as dry powder were mixed and dissolved in t-butanol/water(10:1 **v**/**v**). To this solution, Pt complexes dissolved in *t*-butanol were added, and the rest of the procedure was the same as described above.

Saline solution (0.9% aqueous NaCl solution), or phosphate-buffered saline (PBS) was added (1 ml/mg of Pt complex) to reconstitute the lyophilized preliposomal powder, and the suspension was hand-shaken for 10 min to obtain large liposomes. Small liposomes were prepared by sonication of large liposomes for 1 min with an ultrasonic cell disrupter (Laboratory Supplies Co., New York, N.Y.). The size distribution of the different liposomal preparations was determined with a Nicomp Submicron Particle Sizer Model 370 (Nicomp Particle Sizing Systems, Santa Barbara, Calif.).

Intraliposomal stability

The stability of the different Pt complexes incorporated in liposomes was determined as described previously [8] by comparing the HPLC profiles as a function of time. In brief, aliquoted samples of liposome suspension were diluted (7×) with methanol 0, 2, 6 and 24 h after liposome preparation, and each sample was then monitored by HPLC using a chromega-8 bond column (4.6 mm × 25 cm, 8 μm: ES Industries, N.J.) and 10% water/methanol as eluant. The flow rate was 1 ml/min, and the complexes were detected by UV at a wavelength of 224 nm.

Biological activity.

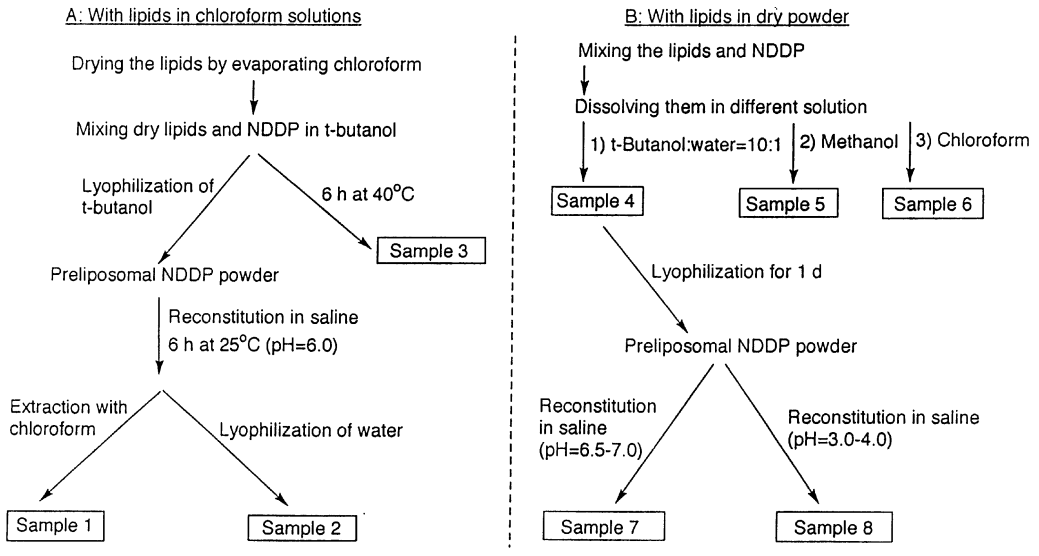
The in vitro cytotoxicity of liposomal Pt complexes against A2780 human ovarian carcinoma cells was assessed by the MTT dye reduction assay. In brief, A2780 cells were seeded in 96-well plates, allowed to attach overnight, and then exposed to various concentrations of drugs for 20 h. After washing the cells with PBS, fresh medium was added for 52 h and the cell survival fractions determined by the MTT assay.

The in vivo antitumor activity of liposomal Pt complexes was assessed against intraperitoneal L1210 mouse leukemia. Groups of six to eight mice weighing 18–20 g were inoculated with 10^6 cells (0.2 ml, i.p.) on day 0, and treatment (25, 50, 100 and 150 mg/kg) was started on day 1 (0.15–0.5 ml, i.p.). The results are expressed as the median survival of treated animals divided by the median survival of control animals × 100 (%T/C).

Identification of active intermediates.

To characterize the active intermediate(s) in the reaction cascade of liposomal NDDP reconstituted in saline solution, tracking experiments using ^{195}Pt NMR spectroscopy in combination with ^{31}P NMR spectroscopy were performed. The procedures for the preparation of the samples is summarized in Schedule 1. Samples 1–3 were prepared using the lipids DMPC and DMPG purchased as chloroform solutions, while samples 4–8 were prepared using lipids as dry powder. The chloroform was initially evaporated from

Schedule 1 Sample preparation for NMR tracking experiment of liposomal NDDP



samples 1–3 in a rotavapor. The lipid film was dissolved in *t*-butanol containing the NDDP in solution. An aliquot of this solution was kept at 40 °C for 6 h and then lyophilized and extracted with methanol (sample 3). The remaining solution was lyophilized immediately, resulting in a preliposomal powder which was reconstituted with saline to produce the liposome suspension. The liposome suspension was kept at room temperature for 6 h. The Pt compounds and lipids were then extracted with chloroform (sample 1) or the sample was lyophilized to eliminate the water and the powder dissolved in methanol (sample 2) (see Schedule 1A).

Samples 4–6 were prepared by complete evaporation of the solvent after keeping the samples at 40 °C for 6 h, and redissolving them in methanol. Samples 7 and 8 were prepared by lyophilization of water for 1–2 days and redissolution in methanol. All samples were prechecked by HPLC before tracking with NMR. The chemical shifts of the products are expressed in parts per million relative to Na_2PtCl_6 in ^{195}Pt and DMPC in ^{31}P NMR.

DACH-Pt-Cl_2 characterization.

Yellow precipitates from NMR samples were collected and redissolved in DMF-d_7 to characterize them by ^1H and ^{13}C NMR spectroscopy. ^1H NMR (DMF-d_7): 1.13–1.17 (m, 2H), 1.46 ≃ 1.55 (m, 4H), 2.05 ≃ 2.09 (broad, 2H) 2.55 ≃ 2.59 (m, 2H), and 5.07 and 5.63 (broad s, 2 NH2) ppm. ^{13}C NMR (DMF-d_7): 24.9 (C4, C5), 32.3 (C3, C6), and 64.1 (C1, C2) ppm. ^{195}Pt NMR ($CHCl_3$, CH_3OH) 1950 ppm, (DMF) 2250 ppm (strong single peak). Elemental analysis: Calculated C (18.99), H (3.69), N (7.38), Pt (51.50); found C (18.58), H (3.72), N (7.40), Pt (51.30). All these data were confirmed using an authentic sample of DACH-Pt-Cl_2.

Results

Preparation of liposomal Pt complexes

NDDP and its two isomers, B10 and L10, were formulated in liposomes composed of combinations of various lipids including DMPC, DMPG, DOPC, DOPG, PA, PE, and PS. The liposomes were formed by reconstituting preliposomal powders containing the Pt complex and the lipids with unbuffered saline solution or PBS. The entrapment efficiency (%EE) of all liposomal formulations was > 90% and was not significantly affected by the lipid composition, reconstitution solution, or NDDP isomer used. No crystals of free drug were observed in any of these preparations within 24 h as assessed by optical microscopy. The median size of the multilamellar vesicles was 1–2 μm in all preparations. The median size of the small liposomes prepared by ultrasonication of multilamellar vesicles was 50–100 nm.

Intraliposomal stability of Pt complexes

Effect of the spacial configuration of the Pt complex and the pH of the liposome suspension

Figure 2 shows the stability of liposomal Pt complex formulations using saline or PBS as the reconstitution solution and DMPC : DMPG ratios of 7 : 3 and 3 : 7. As observed previously [1], the branched configuration of the leaving group of the Pt complex and the content of DMPG in the lipid bilayers correlated with a higher rate of degradation of the Pt complex. As a result, the complex with a linear leaving group, L10, was highly stable, while the highly branched NDDP was rather unstable, and the minimally branched B10 had an intermediate stability. The use of PBS as the reconstitution solution resulted in a significantly higher stability of the Pt complexes than the use of saline. For example, 6 h after liposome preparation, the percentages of intact NDDP in saline and PBS were 43.7% and 82.1%, whereas the percentages for B10 were 85.0% and 95.9%, and the percentages for L10 were 93.1% and 100%, respectively. The pH of the liposome

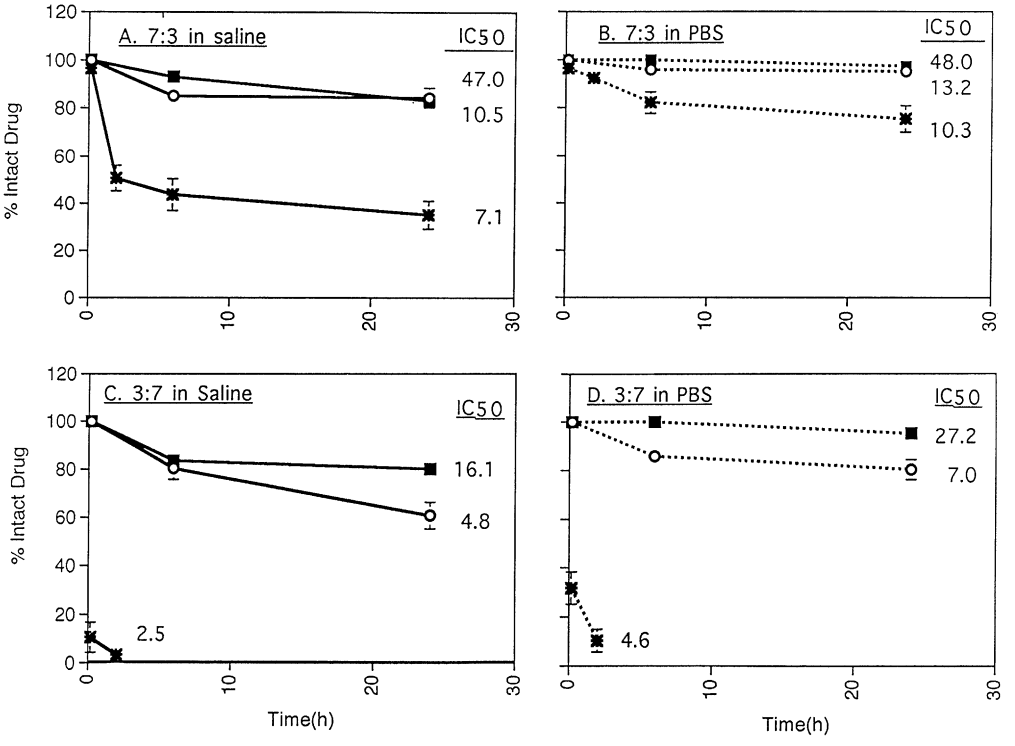


Fig. 2 A–D Effect of structure of Pt complex, DMPG content, and solvent on the intraliposomal stability and cytotoxicity of NDDP (*), B10 (○), and L10 (■). **A** DMPC:DMPG 7:3, saline; **B** DMPC:DMPG 7:3, PBS; **C** DMPC:DMPG 3:7, saline; **D** DMPC:DMPG 3:7, PBS. IC_{50} values are in μg/ml

suspension in saline decreased from 7.0 to 3.8–6.2 depending on the Pt complex, whereas PBS held the pH of the solution to around 6.0–7.0 in all cases. These results indicate that: (1) an acidic pH enhances the intraliposomal degradation of the Pt complexes, and (2) a good neutral buffer system can reduce or stop the intraliposomal degradation of the Pt complexes. To confirm these results, we tested the intraliposomal stability of the Pt complexes in strongly acidic (pH 3.0) or basic (pH 8.0) saline solutions prepared by adding 0.1 *N* HCl or NaOH aqueous solution to pH-7.0 saline. The pH-3.0 saline increased the degradation rate of all Pt complexes, whereas the pH-8.0 saline did not induce any significant Pt complex degradation even at 24 h after liposome preparation. All formulations using a higher amount of DMPG (DMPC:DMPG = 3:7) displayed a higher rate of Pt complex degradation in good correlation with the pH of the liposome suspension, because DMPG is an acidic phospholipid.

Effect of temperature

The intraliposomal stability of the Pt complexes at 40 °C was compared with that at room temperature. Pt complex degradation was temperature-dependent, with the degradation rates being about 30–70% higher at 40 °C than at 25°C, depending on the Pt complex tested.

Effect of lipid composition

Liposomal formulations of NDDP using DMPC:PA, PS:DMPG, and DMPC:PE at a 7:3 molar ratio, and DOPC:DOPG at 1:0, 7:3, 3:7, and 0:1 molar ratios were prepared and tested using saline as the reconstitution solution (Fig. 3). Phospholipid acidity (PA > PG > PS) and decreasing DOPG content (DOPC:DOPG 0:1 > 3:7 > 7:3 > 1:0) enhanced the intraliposomal degradation of NDDP and a good correlation between Pt complex degradation and acidity was again observed.

The same conclusion was drawn in studies with NDDP in liposomes with the same DMPC:DMPG molar ratio (7:3) but different NDDP:total lipid ratios (1:5, 1:10, 1:15, and 1:30) and, therefore, different DMPG contents. Using a 1:5 or 1:10 ratio, 85% of initial NDDP was present at 24 h; in contrast, a 1:15 or 1:30 ratio resulted in an enhanced NDDP degradation, with only 25% of the original NDDP remaining at 24 h, thus suggesting a correlation between the extent of degradation and the absolute amount of DMPG within the lipid bilayers.

Effect of liposome size

Liposome size did not affect the intraliposomal stability of NDDP: ultrasonication of the original suspension of multilamellar vesicles did not signifi-

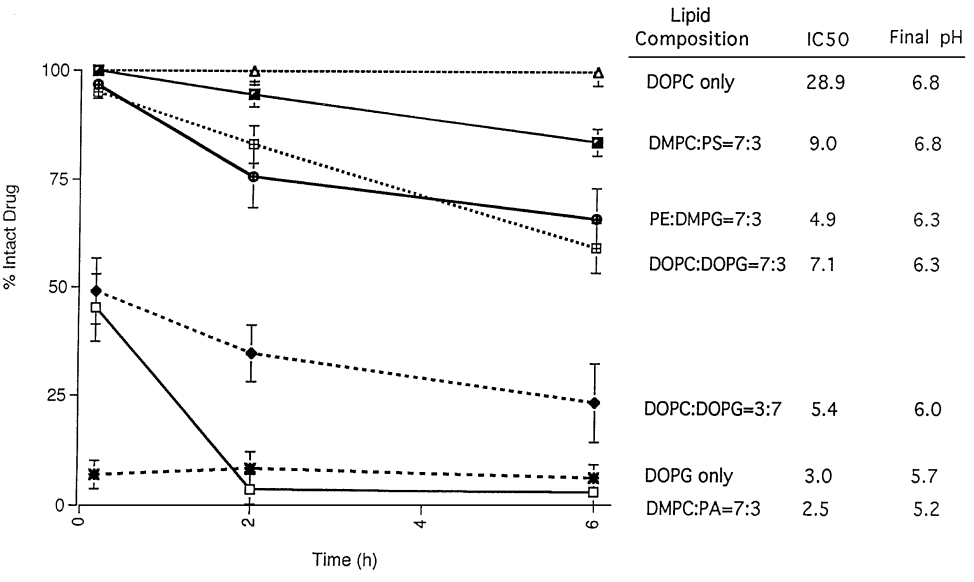


Fig. 3 Effect of lipid composition on the intraliposomal stability of NDDP. All formulations used DMPC and DMPG in a 7:3 molar ratio. Saline solution (0.4% NaCl, starting pH 7.0) was used as reconstituting solution for liposomes and the final pH was checked at 6 h after liposome preparation

cantly change the stability of NDDP regardless of the lipid composition used.

Correlation between in vitro cytotoxicity and intraliposomal stability

We studied the in vitro cytotoxicity of different liposomal Pt complex preparations against A2780 cells with the MTT assay and correlated the results with the intraliposomal stability of the Pt complex. The results are shown in Fig. 2. The IC_{50} values correlated fairly well with drug stability: the more stable Pt complexes were less toxic, i.e. had higher IC_{50} values. When about 20%, 50%, and 90% of the original Pt complex remained at 6 h, the IC_{50} was approximately 3–5, 7–10, and 20–50 μg/ml, respectively. These results indicate that intraliposomal degradation of the Pt complex is required for its cytotoxic effect to be exerted and is, therefore, an intraliposomal activation step.

Identification of active intermediate(s) of NDDP

No new peaks corresponding to the degradation products were observed by the HPLC method developed for NDDP, either because they eluted with the phospholipids or they did not show UV absorbance. Attempts to separate any new peaks from the lipid peaks have been so far unsuccessful.

To keep track of the reaction cascade of liposomal NDDP, we tried to apply the NMR tracking technique used by other researchers [2, 3, 9–11] to characterize the degraded/activated products of NDDP

Formulations prepared using lipids in chloroform

^{195}Pt NMR of sample 1 (Schedule 1A; saline, chloroform extraction; Fig. 4A) and sample 2 (Schedule 1A; saline, lyophilization, Fig. 4B) showed the NDDP peak at 1750 ppm and a new peak corresponding to DACH-Pt-Cl_2 at 1950 ppm. Prolonging the reaction time, lowering the pH, increasing the temperature, and increasing the amount of DMPG in the liposomes enhanced the degradation/activation of NDDP, increasing the intensity of the peak at 1950 ppm. However, ^{31}P NMR showed no new peaks, except those corresponding to DMPC (2 ppm) and DMPG (3 ppm), indicating that the new product shown by ^{195}Pt NMR was not a DMPG-incorporating Pt complex.

These spectra results were similar to those for sample 3 (Schedule 1A; *t*-butanol, lyophilization; Fig. 4C), in which liposomal NDDP was not exposed to saline, thus indicating that the presence of chloroform can also act as a donor of chloride to form DACH-Pt-Cl_2. After storing the yellowish NMR samples at 4 °C overnight, yellow crystals slowly precipitated. The precipitates were filtered, dried, and the structure of the compound was proved to be DACH-Pt-Cl_2 by ^{1}H COSY (Fig. 5A), ^{13}C (Figure 5B), and ^{195}Pt NMR. A yellow precipitate was also observed 2–3 weeks after leaving the original liposomal NDDP suspension at room temperature. NDDP in liposomes composed of only DMPC did not give any new peaks by either ^{195}Pt or ^{31}P NMR, which correlates with its completely preserved stability in the absence of DMPG.

Formulations prepared using lipids as dry powder

We performed the same tracking experiment with formulations prepared using lipids as dry powder (samples

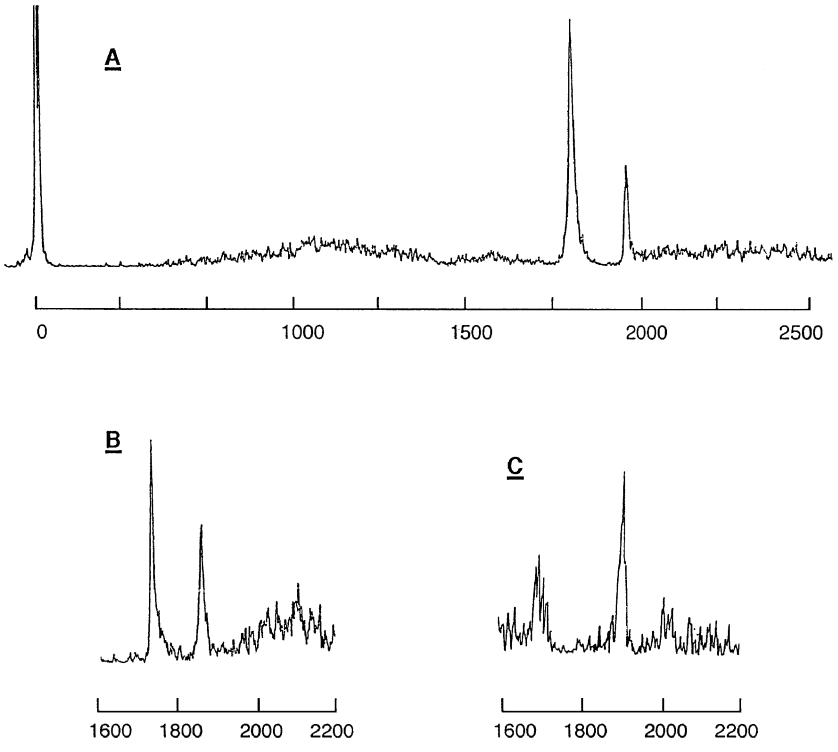


Fig. 4 A–C ^{195}Pt NMR of liposomal NDDP suspension. **A** ^{195}Pt NMR of sample 1 in chloroform prepared by extraction with $CHCl_3$ from liposomal NDDP suspension reconstituted in saline and kept for 6 h at room temperature. **B** ^{195}Pt NMR of sample 2 in methanol prepared by reconstitution of liposomal NDDP in saline for 6 h, lyophilization of water for 2 days, and redissolution of mixtures in methanol. **C** ^{195}Pt NMR of sample 3 in methanol prepared by evaporation of *t*-butanol and redissolution of mixtures in methanol

4–8) instead of as chloroform solutions to eliminate the influence of the presence of residual chloroform on the degradation of NDDP (see Schedule 1B). In samples 4 and 5 (solvent *t*-butanol + water and methanol, respectively) containing lipids and NDDP, no reactions occurred, whereas sample 6 (chloroform solution of lipids and NDDP) showed the presence of DACH-Pt-Cl_2 by ^{195}Pt NMR. The results with these samples, which were incubated at 40 °C for 6 h, confirm that the presence of chloroform can induce the degradation of NDDP into DACH-Pt-Cl_2. Sample 7 was prepared by reconstitution of preliposomal NDDP powder in saline of pH 6.5–7.0 for 6 h at room temperature. No significant degradation ($< 5\%$) was observed, whereas when reconstituted in acidic saline of pH 3.0–4.0 (sample 8), a 60–95% degradation of NDDP occurred in 10 min yielding DACH-Pt-Cl_2 as determined by ^{195}Pt NMR and HPLC (Table 1).

Relationship between Pt complex stability and in vivo antitumor activity

Preparations containing residual chlorofom

These studies were done with liposomal Pt complex suspensions prepared using lipids dissolved in chloroform. In in vivo antitumor activity studies against L1210 leukemia, an inverse relationship between drug stability and antitumor potency was observed (Table 2). The optimal doses of liposomal NDDP were 50 mg/kg in saline and 100 mg/kg in PBS (%T/C = 211 and 200, respectively). For liposomal B10 and L10, the optimal dose was 100 mg in saline (%T/C = 228 and 178), but no significant antitumor activity was observed when both drugs were reconstituted in PBS. In conclusion, the most potent liposomal Pt complex preparations were those with the lowest stability of the Pt complex. However, all formulations had similar antitumor activity when administered at the optimal dose.

Preparations not containing residual chloroform

These studies were done with liposomal Pt complex suspensions prepared with dry lipids. The relationship between NDDP stability and antitumor activity was again studied using the in vivo L1210 leukemia model. Table 3 shows the results with formulations reconstituted with saline solutions of different pH values and administered at different time-points after reconstitution. The optimal doses of liposomal NDDP reconstituted with saline solutions of pH 3.0, 5.0 and 7.0 were 25, 50 and 100 mg/kg, respectively. Therefore, the lower the pH value, the higher the potency of the preparation, in good correlation with the increased intraliposomal drug degradation/activation. At the optimal doses, the %T/C obtained were 214, 271 and 271, respectively. Delaying the time of drug administration increased the antitumor activity of the formulations, in good correla-

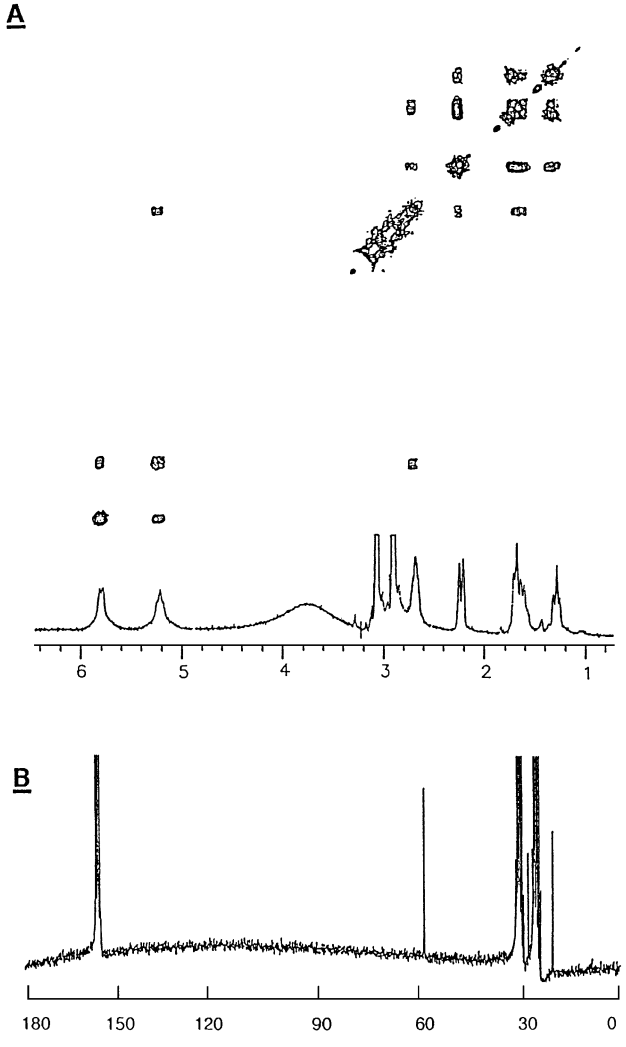


Fig. 5 A ^{1}H-^{1}H correlated spectroscopy of DACH-Pt-Cl_2 in DMF-d_7. **B** ^{13}C NMR of DACH-Pt-Cl_2 in DMF-d_7

Table 1 Effect of various pH values of saline on intraliposomal stability of NDDP prepared with lipids as dry powder

pH of saline	NDDP stability (%)			
	Time after reconstitution			
	10 min	1 h	2 h	6 h
3.0	43	–	15	7
5.0	80	72	67	38
7.0	100	–	95	92

Error within ± 5%

tion with the increased drug activation with time. However, antitumor activity did not correlate perfectly with the calculated amount of activated Pt species formed intraliposomally during the activation process. For example, 100 mg/kg at pH 7.0 gave a similar %T/C value to 50 mg/kg at pH 5.0, although only 10% of NDDP at pH 7.0 (10 mg/kg) and about 70% at pH 5.0

Table 2 In vivo antitumor activity of liposomal Pt complexes against L 1210 leukemia. The DMPC : DMPG ratio was 7:3 for all preparations. The starting pH value of the saline and PBS was 7.0. The tumors were inoculated i.p. on day 0, followed by drug injection i.p. on day 1

Pt complex	Dose of Pt complex (mg/kg)	%T/C	
		Reconstitution solution	
		Saline	PBS
NDDP	25	157	114
	50	211	171
	100	Toxic	200
	150	Toxic	150
B10	25	114	114
	50	128	114
	100	228	142
	150	163	163
L10	25	100	114
	50	157	100
	100	178	100
	150	123	100
Cisplatin	10	142	

Table 3 Antitumor activity of liposomal NDDP against L1210 leukemia. Values are the mean of two-separate experiment

Drug	pH of saline	Dose of Pt complex (mg/kg)	%T/C		
			Time of drug administration		
			10 min	2 h	6 h
NDDP	3.0	12.5			157
		25	150	186	240
		50			Toxic
NDDP	5.0	12.5			186
		25			200
		50	133	216	270
		100			Toxic
NDDP	7.0	25			186
		50			200
		100	163	216	257
Cisplatin		10			150

(35 mg/kg) were transformed into the active Pt species under those conditions. Further in vivo activation must, therefore, have occurred to cause these discrepancies.

Discussion

Our results indicate that NDDP and its isomers are prodrugs of DACH-Pt-Cl_2 when entrapped in liposomes containing acidic phospholipids and in the presence of sodium chloride or residual chloroform as donors of chloride. The rate of transformation of NDDP into DACH-Pt-Cl_2 was directly related to the pH of the liposome suspension. The studies performed

suggest that DMPG and other acidic phospholipids enhanced the reaction by providing an acidic milieu within the liposome membranes. No evidence could be generated to support a direct reaction between NDDP and DMPG to form a DACH-Pt-DMPG complex as one of the active intermediates of NDDP, as we had previously hypothesized, nor the formation of DACH-Pt hydrated species.

DACH-Pt-Cl_2 is the leading compound of the DACH family of Pt complexes. However, it was never developed because of a lack of solubility in water. We initially considered this compound for liposome entrapment, but determined it to be an inappropriate drug for liposome formulation because it is insoluble in most organic solvents. DACH-Pt-Cl_2 has good solubility only in dimethylformamide (DMF), which has a very high boiling point and, therefore, cannot be used to prepare liposomes using the standard evaporation methods, and it is not soluble in any of the organic solvents used for the lyophilization procedures. Our studies indicate that DACH-Pt-Cl_2 can be generated within the liposome membranes under the conditions described, and that the drug remains liposome-bound without leaking out and crystallizing for at least 24 h. In contrast, DACH-Pt-Cl_2 precipitates quickly when formed from NDDP by the addition of HCl.

This is the first report of a liposome formulation in which the compound is synthesized in situ from an entrapped precursor with the liposome preventing its spontaneous precipitation. The results are encouraging because they suggest a possible avenue for the development of a much-needed delivery system for this very interesting compound.

Future studies will focus on controlling the degradation/activation that takes place within the liposomes so that all reconstituted vials contain the same amount of the active species, DACH-Pt-Cl_2. This control is essential for the continued pharmaceutical development of this agent. A potential strategy is to use a two-step reconstitution procedure by which an acidic saline solution is used first to induce the fast transformation of $> 80\%$ of NDDP into DACH-Pt-Cl_2, followed after a predetermined period of time by the addition of a buffer solution to bring the pH to > 7.0 to stop the reaction.

References

1. Han I, ling Y-H, Al-Baker S, Khokhar AR, Perez-Soler R (1993) Cellular pharmacology of liposomal cis-bis-neodecanoato-trans-R,R-1,2-diaminocyclohexaneplatinum(II) in A2780/S and A2780/PDD cells. Cancer Res 53:4913–4919
2. Hollis LS, Miller AV, Amundsen AR, Schurig JE, Stern EW (1990) cis-Diamineplatinum(II) complexes containing phosphono carboxylate ligands as antitumor agents. J Med Chem 33:105–111.
3. Ismail IM, Sadler PJ (1983) ^{195}Pt- and ^{15}N-NMR studies of antitumor complexes. In: Lippard SJ (ed) Platinum, gold, and other metal chemotherapeutic agents. American Chemical Society, Washington DC, Vol 209, pp 171–189
4. Khokhar AR, Al-Baker S, Brown T, Perez-Soler R (1991) Chemical and biological studies on a series of lipid-soluble (trans-(R,R)- and -(S,S)-1, 2-diaminocyclohexane)platinum(II). J Med Chem 34:325–329
5. Perez-Soler R, Khokhar AR (1992) Lipophilic cisplatin analogues entrapped in liposomes: role of intraliposomal drug activation in biological activity. Cancer Res 52:6341–6347
6. Perez-Soler R, Yang LY, Drewinko B, Lauterzstain J, Khokhar AR (1988) Increased cytotoxicity and reversal of resistance of cis-diamminedichloroplatinum(II) with entrapment of cis-bis-neodecanoato-trans-R,R-1, 2-diaminocyclohexaneplatinum(II) in multilamellar lipid vesicles. Cancer Res 48:4509–4512
7. Perez-Soler R, Khokhar AR, Lautersztain J, Al-Baker S, Francis K, Macias-Kiger D, Lopez-Berestein B (1990) Clinical development of liposomal platinum. J Liposome Res 1:437–449
8. Perez-Soler R, Han I, Al-Baker S, Khokhar AR (1994) Lipophilic platinum complexes entrapped in liposomes: improved stability and preserved antitumor activity with complexes containing linear alkyl carboxylato leaving groups. Cancer Chemother Pharmacol 33:378–384
9. Qu Y, Farrell NJ (1990) Effect of diamine linker on the chemistry of bis(platinum) complexes. A comparison of the aqueous solution behavior of 1,4-butanediamine and 2,5-dimethyl-2,5-hexanediamine complexes. J Inorg Biochem 40:255–264
10. Qu Y, Farrell NJ (1991) Interaction of bis(platinum) complexes with the mononucleotide 5′-guanosine monophosphate. Effect of diamine linker and the nature of the bis(platinum) complex on product formation. J Am Chem Soc 113:4851–4857
11. Slavin LL, Bose RN (1990) Phosphonato complexes of platinum(II): kinetics of formation and phosphorus-^{31}NMR characterization studies. J Inorg Biochem 40:339–347