δ 1.70 (dt, J = 6, 7 Hz, 2 H), 2.74 (t, J = 7 Hz, 2 H), 2.90 (d, J = 6 Hz, 2 H), 3.92 (app p, J = 6 Hz, 1 H), 4.6 (br s, 1 H), 6.6–7.4 (m, 6 H), 7.49 (dd, J = 2, 8 Hz, 1 H), 8.40 (dd, J = 2, 5 Hz, 1 H).

To a 0 °C solution of 1-(2-pyridyl)-4-(2-hydroxyphenyl)-2-butanol (9.4 g, 39 mmol), triphenylphosphine (10.2 g, 39.0 mmol), and THF (200 mL) was added a solution of diisopropyl azodicarboxylate (7.9 g, 39 mmol) and THF (50 mL) over 20 min and the reaction mixture then stirred at room temperature for 20 h. The reaction was quenched by the addition of water (20 mL) and stirring was continued for an additional 2 h. Methylene chloride (250 mL) was added, and the combined organic layers were washed with water (125 mL), 0.5 M NaOH (50 mL), water (125 mL), and brine (125 mL), and dried ($MgSO_4$). The solvent was removed in vacuo and the residue was purified on silica gel using methylene chloride/methanol (98:2) to afford 2-(2-pyridylmethyl)-3,4-dihydro-2*H*-1-benzopyran (5.1 g, 56%) as a viscous oil: ^1H NMR (60 MHz, $CDCl_3$) δ 2.0 (m, 2 H), 2.7 (m, 2 H), 3.08 (dd, J = 3, 6 Hz, 2 H), 4.35 (ddt, J = 3, 6, 12 Hz, 1 H), 6.6–7.3 (m, 6 H), 7.42 (dd, J = 2, 7 Hz, 1 H), 8.25 (dd, J = 2, 5 Hz, 1 H).

To a 0 °C solution of 2-(2-pyridylmethyl)-3,4-dihydro-2*H*-1-benzopyran (1.4 g, 6.3 mmol) and methylene chloride (20 mL) was added titanium tetrachloride (1.4 mL, 12.6 mmol) followed by α,α-dichloromethyl methyl ether (0.63 mL, 7.0 mmol) dropwise over 5 min. The reaction mixture was stirred for 1 h at 0 °C and then slowly poured into cold 10% $NaHCO_3$ (100 mL). The mixture was extracted with methylene chloride (2 × 50 mL), and dthe combined organic layers were washed with water (50 mL) and brine (50 mL), and dried ($MgSO_4$). The solvent was removed in vacuo to afford 760 mg (48%) of a mixture (2/1) of the title aldehyde [^1H NMR (60 MHz, $CDCl_3$) δ 9.53, s] along with the 8-formyl isomer [^1H NMR (60 MHz, $CDCl_3$) δ 9.95, s].

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Chemical and Biological Studies on a Series of Lipid-Soluble (*trans*-(*R*,*R*)- and -(*S*,*S*)-1,2-Diaminocyclohexane)platinum(II) Complexes Incorporated in Liposomes

Abdul R. Khokhar,* Salaam Al-Baker, Trellis Brown, and Roman Perez-Soler

Department of Medical Oncology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030. *Received June 4, 1990*

cis-Bis(neodecanoato)(*trans*-(*R*,*R*)-1,2-diaminocyclohexane)platinum(II) [L-NDDP] is a liposome incorporated lipophilic cisplatin analogue that has shown promising antitumor activity against tumors resistant to cisplatin and liver metastases in mice. L-NDDP is currently under clinical evaluation. However, NDDP is an isomeric mixture of different species having various isomeric neodecanoic moities as liganded leaving groups. A series of new highly lipid-soluble *cis*-bis(neodecanoato)(*trans*-(*R*,*R*)- and -(*S*,*S*)-1,2-diaminocyclohexane)platinum(II) [Pt] complexes, using single isomers of neodecanoic acid, were synthesized and characterized by analytical and spectroscopic techniques (infrared and ^{195}Pt NMR). Multilamellar vesicles (MLVs) composed of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) at a molar ratio of 7:3 were used as carriers of the Pt complexes. The efficiency of incorporation of the liposomal-platinum (L-Pt) preparations was >95% and stability in normal saline at 4 °C was >95% at day 14 in each case. The iv LD_{50} values of all L-Pt preparations tested were in the range of 62.3 to 104 mg/kg. The % T/C obtained after a single ip injection of the optimal dose of L-Pt preparations against L1210 leukemia was in the range of 150 to 253 (160 for cisplatin). When a multiple ip injection schedule was used (on days 1, 5, and 9) the L-Pt preparations of *R*,*R* complexes (1, 7, and 9) were more active than cisplatin at the optimal dose (% T/C = 257 for each vs 220 for cisplatin). The L-Pt preparations of *R*,*R* complexes were also markedly active against L1210 leukemia resistant to cisplatin (% T/C 355, 231, and 185 respectively vs 112 for cisplatin). These studies show that the single isomers of NDDP are comparable to the original isomeric mixture in terms of toxicity and biological activity.

Introduction

Cisplatin is one of the most active antitumor agents.[1,2] The usefulness of cisplatin is, however, compromised by its propensity to cause several severe dose-limiting toxicities including nephrotoxicity, neurotoxicity, and ototoxicity.[3-6] In an attempt to modify the therapeutic index of cisplatin, analogues which are less toxic and non-cross resistant to cisplatin have been synthesized during the last decade. However, the development of some promising analogues has been hampered by their low solubility, formulation problems, and poor stability, which decrease their potential for clinical use.[7]

Another approach to modify the therapeutic index of cisplatin analogues may be the use of drug carriers, among which liposomes are particularly attractive because they are essentially nontoxic, biodegradable lipid vesicles that can alter the distribution and bioavailability of drug.[8,9] The potential use of liposomes as drug carriers has been exploited to improve the therapeutic index of several antimicrobials and anticancer agents. Liposome-incorporated amphotericin B results in an enhancement of the therapeutic index of the drug with lowered toxicity compared to the free drug in the treatment of disseminated candidiasis in both mice[10] and humans.[11] Several investigators

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Table I. Infrared and NMR Data for Platinum Complexes[a]

Pt complex number	complex name	mp, °C	IR[b] ν(C=O), cm^{-1}	IR[b] ν(C–O), cm^{-1}	^{195}Pt,[c] ppm
1	Pt(2,2-dimethyloctanoato)$_2$((*R*,*R*)-dach)	180–182	1594	1385	−1717
2	Pt(2,2-dimethyloctanoato)$_2$((*S*,*S*)-dach)	181–182	1595	1395	−1717
3	Pt(2,2-dimethyl-4-ethylhexanoato)$_2$((*R*,*R*)-dach)	196–198	1591	1385	−1708
4	Pt(2,2-dimethyl-4-ethylhexanoato)$_2$((*S*,*S*)-dach)	196–198	1590	1385	−1701
5	Pt(2-ethyl-2-methylheptanoato)$_2$((*R*,*R*)-dach)	181–182	1595	1379	−1692
6	Pt(2-ethyl-2-methylheptanoato)$_2$((*S*,*S*)-dach)	181–182	1595	1385	−1692
7	Pt(2,2-diethylhexanoato)$_2$((*R*,*R*)-dach)	190–192	1584	1387	−1684
8	Pt(2,2-diethylhexanoato)$_2$((*S*,*S*)-dach)	190–192	1585	1375	−1681
9	Pt(2,2-diethyl-4-methylpentanoato)$_2$((*R*,*R*)-dach)	204–205	1584	1385	−1708
10	Pt(2,2-diethyl-4-methylpentanoato)$_2$((*S*,*S*)-dach)	203–205	1585	1385	−1708

[a] C, H, N, and Pt analyses were obtained for all complexes. Analytical values were within ±0.4%, except 1 and 3 were >0.4% low in Pt. [b] Spectra were recorded as KBr pellets. [c] Chemical shifts are relative to Na_2PtCl_6 (0.00 ppm).

have shown that incorporation of doxorubicin into liposomes reduces its cardiac toxicity[12] and increases its antitumor activity against liver metastases in mice.[13-15]

Formulation has been one of the major problems in the clinical development of liposome-incorporated drugs. The problem may be avoided by developing drug analogues with structural features that retain the desired antitumor effect and are more compatible with the drug carrier. Cisplatin has been previously encapsulated in MLVs but with a very low efficiency of incorporation (7.4%) and poor stability.[16] To improve these features, we explored the possibility that lipophilic cisplatin analogues might be better candidates for liposome incorporation. We have previously reported on the synthesis, liposome formulation, and antitumor activity of a lipophilic analogue, *cis*-bis(neodecanoato)(*trans*-(*R*,*R*)-1,2-diaminocyclohexane)platinum(II) [NDDP].[17,18] NDDP incorporated into liposomes was found to have high efficiency of incorporation and good stability. In preclinical studies, L-NDDP was shown to be less nephrotoxic and more effective than cisplatin against liver metastases of M5076 reticulosarcoma and had significant activity against other tumor systems.[19]

A clinical phase I study of L-NDDP has just been completed[20] at M.D. Anderson Cancer Center. The maximum tolerated dose was 312.5 mg/m^2. The primary dose-limiting toxicity was myelosuppression. Other toxicities included nausea, vomiting, fever, diarrhea, and fatigue. No nephrotoxicity was observed. Since the neodecanoic acid used for the synthesis of NDDP is an isomeric mixture of at least 18 isomers, we decided to explore whether similar compounds having single isomers of neodecanoic acid as leaving groups would have biological properties similar to those of NDDP. In addition, the complexes synthesized with a single isomer of neodecanoic acid should be easier to characterize for the purpose of approval by the regulatory agencies. This report describes the synthesis, development of liposomal preparations, and biological activity of a series of highly lipid-soluble (*trans*-(*R*,*R*)- and -(*S*,*S*)-1,2-diaminocyclohexane)platinum(II) ((*trans*-(*R*,*R*)- and -(*S*,*S*)-1,2-dach)platinum(II)) complexes containing different single isomers of neodecanoic acid as leaving groups.

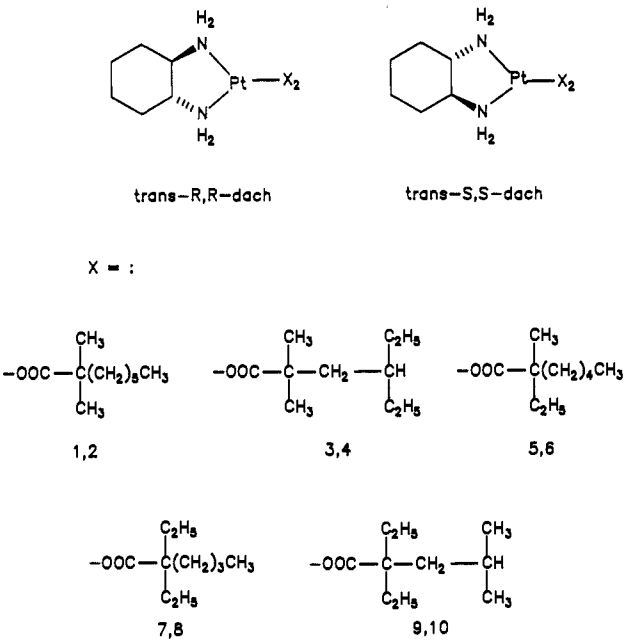

Figure 1. Structure of platinum complexes.

Scheme I

$$[Pt(SO_4)(dach)H_2O)] + 2NaX \rightarrow [Pt(X)_2(dach)] + Na_2SO_4$$

Results and Discussion

Synthesis and Chemical Characterization of Complexes. A series of (*trans*-(*R*,*R*)- and -(*S*,*S*)-1,2-dach)platinum(II) complexes, containing single isomers of neodecanoic acid has been prepared by the reaction of aqua(*R*,*R*)- or (*S*,*S*)-dach)sulfatoplatinum(II) with the sodium salt of corresponding acid, as shown in Scheme I.

The presence of the alkyl groups, confers lipophilicity to these complexes. Thus all complexes are highly soluble in chloroform and other organic solvents, but they are completely insoluble in water. This characteristic has allowed us to incorporate such complexes into liposomes so that their antitumor activity could be investigated.

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Table II. Efficacy of Liposomal-Platinum (L-Pt) Preparations against L1210 Leukemia[a]

L-Pt	treatment schedule day	optimal dose mg/kg	% T/C[b]
1	1	25	234
2	1	25	150
3	1	50	212
4	1	50	170
5	1	25	216
6	1	50	210
7	1	25	253
8	1	50	190
9	1	25	225
10	1	50	160
L-NDDP[c]	1	37.5	187
cisplatin	1	10	175

[a] Ip tumor inoculation day 0, ip treatment [b] Mean of three experiments. [c] Liposomal preparation of (trans-(*R*,*R*)-dach)Pt complex containing isomeric mixtures of neodecanoic acid.

Prior to incorporation in liposome and biological evaluation, all platinum complexes were purified by recrystallization and their purity was assessed by thin-layer chromatography. Elemental analysis data clearly establish the composition as one dach, one Pt, and two carboxylate ligands for each of the platinum complexes (Table I). The presence of a band near 1584–1595 cm^{-1} in the IR spectra of these complexes and absence of absorption of the free acid near 1700 cm^{-1} demonstrate that the carboxylate ligand is coordinated to the platinum in each case.[21] The ^{195}Pt NMR spectra for all platinum complexes obtained in chloroform solution are consistent with Pt(II)–ion ligation by two nitrogen and two oxygen donor ligands found in these complexes.[18,22] The platinum complexes exhibit a ^{195}Pt NMR chemical shift in the range of −1681 to −1717 ppm in chloroform solution. This evidence suggests the chemical structure of the platinum complexes as shown in Figure 1.

Efficiency of Incorporation and Stability of Liposomal-Platinum (L-Pt) Preparation. The efficiency of incorporation of L-Pt complexes was >90% as measured by both methods. No precipitate of the free drug could be seen in the liposome pellet by optic microscopy. In previous studies,[23] we could not observe free drug by freeze–fracture electron microscopy. The stability of L-Pt complexes in 0.9% NaCl solution in water at 4 °C was assessed by measuring the amount of free Pt complex in the supernatant at different times. At day 14, the stability was found to be >90% in each case. No evidence of vesicle disruption, morphological distraction, or clumping of unincorporated drug was observed by light microscopy on day 14. Vesicle size ranged from 0.5 to 5 μm in diameter with most vesicles measuring between 1 and 3 μm.

Biological Studies

In Vivo Antitumor Activity. To determine the in vivo oncolytic activity, each L-Pt complex was tested with use of a single ip injection against ip inoculated L1210/0 cells (Table II). All L-Pt complexes had significant antitumor activity (% T/C > 150). Cisplatin was no better, and in many cases was less effective than L-Pt complexes. It is apparent that the (*trans*-(*R*,*R*)-dach)Pt liposomal preparations (L-Pt1,3,5,7,9) were more active than the (*trans*-(*S*,*S*)-dach)Pt preparations (L-Pt2,4,6,8,10). Thus our present study confirms findings of Kidani and Inagaki[24] that trans-(*R*,*R*) isomers are superior to trans-(*S*,*S*) isomer. When the L-Pt 1, 7, and 9 (trans-(*R*,*R*) complexes) were administered on day 1, 5, and 9, only a slight increase in % T/C was observed (Table III).

Table III. Efficacy of Liposomal-Platinum (L-Pt) Preparations against L1210 Leukemia[a]

L-Pt	treatment schedule day	optimal dose, mg/kg	% T/C[b]
1	1, 5, 9	25	257
7	1, 5, 9	25	257
9	1, 5, 9	25	257
L-NDDP[c]	1, 5, 9	25	312
cisplatin	1, 5, 9	5	220

[a] Ip tumor inoculation day 0, ip treatment. [b] Average of two experiments. [c] Liposomal preparation of (trans-(*R*,*R*)-dach)Pt complexes containing isomeric mixture of neodecanoic acid.

Table IV. Efficacy of Liposomal-Platinum (L-Pt) Preparations against L1210/cisplatin Leukemia[a]

L-Pt	treatment schedule day	optimal dose, mg/kg	% T/C[b]
1	1, 5, 9	25	355
7	1, 5, 9	25	231
9	1, 5, 9	25	185
L-NDDP[c]	1, 5, 9	25	200
cisplatin	1, 5, 9	5	112

[a] Ip tumor inoculation day 0, ip treatment. [b] Average of two experiments. [c] Liposomal preparation of (trans-(*R*,*R*)-dach)Pt complex containing isomeric mixture of neodecanoic acid.

Table V. Subacute Toxicity Studies of Liposomal-Platinum (L-Pt) Preparations

L-Pt	LD_{50}, mg/kg
1	94.5
2	87
7	80.8
8	104
9	62.3
10	81
L-NDDP[a]	54
cisplatin	17.5

[a] Liposomal preparation of (trans-(*R*,*R*)-dach)Pt complex containing isomeric mixture of neodecanoic acid.

L-Pt 1, 7, and 9 were also evaluated for in vivo antitumor activity against L1210 cells resistant to cisplatin (Table IV). When administered ip on day 1, 5, and 9 after tumor inoculation, the % T/C obtained with optimal doses (25 mg/kg) of L-Pt 1, 7, and 9 were 355%, 231%, and 185% respectively. The % T/C with L-NDDP (isomeric mixture) was 200. Cisplatin (% T/C 112) had no antitumor activity against L1210/cisplatin leukemia.

These studies show that Pt complexes containing the single isomers of neodecanoic acid are comparable to the original isomeric mixture NDDP in terms of biological activity (Table II–IV).

Toxicological Studies. Preliminary toxicity studies were preformed on selected L-Pt 1, 2, 7–10, and cisplatin. All complexes were less toxic as compared to cisplatin (Table V). The subacute single dose iv LD_{50} of L-Pt 1, 2, and 7–10 was in the range of 62.3 to 104 mg/kg. Most deaths occurred during the first week post-drug administration. No deaths were recorded after day 14. More detailed toxicological studies of such complexes are in progress.

The complexes described in this study can be fully characterized for the purpose of approval of regulatory agencies, thus solving the problem of characterization of

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NDDP, which is a mixture of at least 18 isomers. Although the single isomers of NDDP synthesized have similar biological activity, our studies suggest that there may be a slight difference among them. Because no separation of the different isomers of neodecanoic acid manufactured by Exxon Corp. and used for the synthesis of NDDP has been accomplished, the proportion of each isomer in the mixture is unknown. There is, however, indirect evidence that the 2,2-dimethyloctanoic acid may represent as much as 60–70% of the total mixture. The single isomeric form of NDDP synthesized with this particular isomer of neodecanoic acid resulted in a complex which, in the liposomal form (L-Pt 1), showed enhanced activity against L1210 and L1210/cisplatin resistant compared with the mixture of L-NDDP. Particularly noteworthy was the fact that L-Pt 1 was the best preparation against the cisplatin resistant L1210 leukemia. In addition, L-Pt 1 had an LD_{50} of 94.5 mg/kg and L-NDDP an LD_{50} of 54 mg/kg. On the basis of all these considerations, L-Pt 1 has been selected as the logical candidate for further development. The experiments reported suggested that these compounds may be less toxic when given in a multiple injection schedule on days 1, 5, and 9. The reason for these finding are unknown, but justify exploring different schedules with the compound selected for further development. A second important piece of information derived from these studies is that complexes with an *R,R* configuration are slightly more active than those with *S,S*, while their acute toxicity is similar.

Experimental Section

Abbreviations. Abbreviations are as follows: L-NDDP, liposome-incorporated *cis*-bis(neodecanoato)(*trans*-*R,R*)-1,2-diaminocyclohexane)platinum(II); NDDP, *cis*-bis(neodecanoato)(*trans*-*R,R*)-1,2-diaminocyclohexane)platinum(II); MLVs, multilamellar vesicles; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; L-Pt, liposomal-platinum preparations; iv, intravenously; LD_{50}, 50% lethal dose; % T/C, median survival of treated mice divided by median survival of control mice × 100; dach, *trans*-(*R,R*)- or *trans*-(*S,S*)-1,2-diaminocyclohexane; ip, intraperitoneally.

Chemistry. *trans*-(*R,R*)- and *trans*-(*S,S*)-dach were purchased from Morton Thiokol, Inc. (Danvers, MA). K_2PtCl_4 was purchased from Aesar (Johnson Matthey, Inc. Seabrook, NH). All isomers of neodecanoic acid such as 2,2-dimethyloctanoic, 2,2-dimethyl-4-ethylhexanoic, 2-ethyl-2-methylheptanoic, 2-2-diethylhexanoic, and 2-2-diethyl-4-methylpentanoic were obtained from The Liposome Co., Inc. (Princeton, NJ). Thin-layer chromatography (TLC) was performed on precoated silica gel plates with 254-nm fluorescent indicator in a solvent system consisting of ethyl acetate–methanol (9:1). The plates were visualized under ultraviolet light or as yellow-brown spots after exposure to iodine vapor. Melting points were determined on an electrothermal-digital apparatus and are uncorrected. Microanalysis on the platinum complexes were performed by Robertson Laboratory, Inc. (Madison, NJ). ^{195}Pt NMR spectra were recorded on IBM NR200/AF spectrometer. Spectra were obtained in chloroform solution and were externally referenced to and reported relative to Na_2PtCl_6 (0.25 g per 3 mL of H_2O). Infrared spectra were recorded as a KBr pellet in the range of 250–4000 cm^{-1} with use of a Beckman 250 MX spectrophotometer.

Synthesis of Platinum Complexes. Pt(2,2-dimethyloctanoato)$_2$((*R,R*)-dach) (Pt 1) was synthesized by using the following multistep procedure. A solution of K_2PtCl_4 was mixed with an equimolar amount of dach in water and allowed to react at room temperature for 6–8 h with constant stirring. The water insoluble dichloro(dach)platinum(II)[25] was collected by filtration and washed successively with water, ethanol, and acetone. The final product was dried in a vacuum (yield 90%). The dichloro(dach)platinum(II) was mixed at room temperature with a slightly less than equimolar amount of Ag_2SO_4 in water for 24 h in the dark. The water soluble aqua(dach)sulfatoplatinum(II) was removed from the AgCl precipitate by filtration and was evaporated to dryness at 40–50 °C under reduced pressure using a rotary evaporator. The yellow product was dried over P_2O_5 in a vacuum. Finally, the aqua(dach)sulfatoplatinum(II) complex (0.846 g) was dissolved in 50 mL of water, and a solution of sodium 2,2-dimethyloctanoate (prepared in situ by mixing 0.8 mL of 5 N NaOH and 0.688 g of 2,2-dimethyloctanoic acid) was added. A yellow sticky material was formed immediately. The reaction mixture was stirred at room temperature for 15 min, 100 mL of methanol was added to obtain a clear yellow solution, and the solution was stirred for an additional 12 h. Methanol was evaporated under reduced pressure, and the aqueous solution was extracted with 2 × 50 mL of dichloromethane. The extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated to give a solid. Crystallization from acetone gave the final product (yield = 1.0 g, 77%).

Pt(2,2-dimethyloctanoato)$_2$((*S,S*)-dach) (Pt 2), Pt(2,2-dimethyl-4-ethylhexanoato)$_2$((*R,R*)-dach) (Pt 3), Pt(2,2-dimethyl-4-ethylhexanoato)$_2$((*S,S*)-dach) (Pt 4), Pt(2-ethyl-2-methylheptanoato)$_2$((*R,R*)-dach) (Pt 5), Pt(2-ethyl-2-methylheptanoato)$_2$((*S,S*-dach) (Pt 6), Pt(2,2-diethylhexanoato)$_2$((*R,R*-dach) (Pt 7), Pt(2,2-diethylhexanoato)$_2$((*S,S*)-dach) (Pt 8), Pt-(2,2-diethyl-4-methylpentanoato)$_2$((*R,R*)-dach) (Pt 9), and Pt-(2,2-diethyl-4-methlpentanoato)$_2$((*S,S*)-dach) (Pt 10) were prepared in a similar manner as Pt1 complex mentioned above. All Pt complexes are highly soluble in chloroform (>25 mg/mL) and other organic solvents, but are completely insoluble in water.

Liposomal-Platinum (L-Pt) Preparation. The chromatographically pure (by thin-layer chromatography) DMPC and DMPG used in the study were obtained from Avanti Polar Lipids (Birmingham, AL). Pt complexes were prepared as reported for other platinum complexes.[19] Briefly, chloroform solutions of DMPC and DMPG at a 7:3 molar ratio were mixed with the platinum complex at a drug:lipid weight ratio of 1:15. The chloroform was evaporated in a rotary evaporator, leaving a dry film containing the lipids and the platinum complex. Multilamellar liposomes containing the platinum complex were formed by adding 1 mL of 0.9% NaCl aqueous solution for each milligram of platinum complex to the dry lipid film and shaking for a few minutes. To measure the efficiency of incorporation, the liposome suspension was centrifuged at 30000*g* for 45 min, and the amount of the platinum complex in the supernatant or the elemental platinum in the pellet was determined. Quantitation of the platinum complexes was measured by UV spectrophotometry at a wavelength of 216 nm. Elemental platinum was measured by X-ray fluorescence in the Department of Analytical Chemistry at The University of Texas Medical School at Houston, TX.[26]

The efficiency was calculated by the following formulas

$$\text{efficiency of incorporation} = [(\text{total Pt complex added} - \text{Pt complex in supernatant})/\text{total Pt complex added}] \times 100 \quad (1)$$

$$\text{efficiency of incorporation} = (\text{elemental platinum in pellet}/\text{elemental platinum added}) \times 100 \quad (2)$$

L-Pt complex vesicles were sized in a Coulter Counter (Coulter Electronics, Hialeah, FL).

All the preparations were regularly checked for the presence of free drug crystals in the pellet by optic microscopy.

Biological Studies. L1210/0 cells were obtained from the Department of Pharmacology, University of Vermont, Burlington, VT; L1210/cisplatin cells were obtained from the DCT tumor repository, National Cancer Institute, Frederick, MD. L1210/0 and L1210/cisplatin cell lines were grown in vivo in the peritoneal cavity of BDF1 mice and transplanted weekly; animals bearing L1210/0 cisplatin leukemia were treated on day 5 with 5 mg/kg cisplatin.

CD1 Swiss mice, 6–8 weeks old and weighing 22–25 g, were purchased from The University of Texas Cancer Center, Science

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Park (Bastrop, TX). BDF1 mice weighing 18–20 g were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA).

In vivo antitumor activity was assessed against L1210/0 and L1210/cisplatin leukemia. Groups of six B6D2F1 mice weighing 20–25 g were inoculated with 10^6 cells (ip) on day 0. Treatment started on day 1 using the same route used for tumor inoculation. Two different treatment schedules were used: a single dose on day 1 or once daily doses on days 1, 5, and 9. The following doses were administered 12.5, 25, and 50 mg/kg. The optimal dose was defined as the dose that resulted in a higher % T/C without causing toxic deaths. Toxic deaths were defined as those deaths occurring during the first 10 days in animals without ascites or liver involvement by tumor at autopsy.

In the experiments performed using the L1210/cisplatin cell line, 10^6 cells were inoculated ip on day 0. Treatment was given (ip) on days 1, 5, and 9. Results were expressed as median survival of treatment animals divided by median survival of control animals multiplied by 100 (% T/C). All experiments were designed to be terminated on day 60. Long term survivors were rarely seen. They were not excluded from the calculations of % T/C since we used the median survival time rather than the mean survival time.

The optimal doses of the different drugs were used in all antitumor activity studies. For L-Pt complexes the optimal doses for single injection on day 1 are in the range of 25–50 mg/kg, and for cisplatin, is 10 mg/kg. Results presented are the mean of three experiments in each case.

For days 1, 5, and 9 the optimal doses for L-Pt 1, 7, and 9 are 25 mg/kg and for cisplatin is 5 mg/kg. Results presented are the average of two experiments in each case.

The free platinum complexes could not be used as controls in the non-liposomal form due to their lack of aqueous solubility.

Subacute Toxicity Studies. Groups of six to eight CD1 Swiss mice weighing 20–25 g each received iv doses of the L-Pt complexes. Animals were observed, and deaths were recorded on a daily basis. The LD_{50} dose was calculated from the curve obtained by plotting the logarithm of the dose and the % survival on day 15.

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Preparation and Anti-HIV Activities of Aurintricarboxylic Acid Fractions and Analogues: Direct Correlation of Antiviral Potency with Molecular Weight

Mark Cushman,*,† Pinglang Wang,† Steve H. Chang,† Carl Wild,† Erik De Clercq,‡ Dominique Schols,‡ Mark E. Goldman,§ and Julie A. Bowen§

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium, and Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486.
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Aurintricarboxylic acid (ATA) was fractionated by a combination of dialysis, ultrafiltration, and gel permeation chromatography. The number average and weight average molecular weights of the ATA fractions were determined by the universal calibration method. The sulfonic acid analogue of ATA was prepared and separated in high and low molecular weight fractions. The phosphonic acid analogue of ATA was also synthesized. All of the ATA fractions were tested for prevention of the cytopathic effect of HIV-1 and HIV-2 in MT-4 cell culture as well as against HIV-1 in CEM cell culture. The abilities of the fractions and analogues to inhibit syncytium formation between HIV-1- and HIV-2-infected HUT-78 cells and uninfected MOLT-4 cells were evaluated. In addition, the fractions and analogues were tested for cytotoxicity in mock-infected MT-4 cells, prevention of the binding of the OKT4A monoclonal antibody to the CD4 receptor, inhibition of the binding of anti-gp120 monoclonal antibody to gp120, inhibition of attachment of HIV-1 virions to MT-4 cells, and inhibition of HIV-1 reverse transcriptase. In all of these assays except cytotoxicity, there was a correlation of potency with molecular weight. The higher the molecular weight, the higher the activity. Several of the lower molecular weight fractions of ATA, which bound to gp120 but not to CD4, prevented HIV-1 and HIV-2 cytopathicity. A similar profile was observed for the phosphonic acid analogue of ATA and the lower molecular weight fraction of the sulfonic acid analogue. The results on the ATA fractions indicate that the binding of ATA to gp120 in the absence of CD4 binding is sufficient for anti-HIV activity. The active compounds bind more avidly to gp120 than to CD4. The anti-HIV activity of the ATA fractions is due to inhibition of virus binding due to an interference with the gp120-CD4 interaction.

Aurintricarboxylic acid (ATA) is a heterogeneous mixture of polymers that forms when salicylic acid is treated with formaldehyde, sulfuric acid, and sodium nitrite.[1-3] ATA is often incorrectly represented as a triphenylmethane dye 1 rather than as a polymer as schematically portrayed in structure 2. Recent interest in ATA has resulted from the finding that ATA inhibits the cytopathic effect of HIV-1 in ATH8, MT-4, and HUT-78 cell cultures.[4,5] ATA selectively prevents the binding of the OKT4A/Leu-3a monoclonal antibody to the CD4 receptor and it inhibits the attachment of HIV-1 particles to MT-4 cells.[6] ATA also prevents the staining of membrane-bound gp120 by a monoclonal antibody against it.[7]

The inhibition of protein nucleic acid interactions by ATA in cell-free systems has been known for a long time. The evidence indicates that ATA binds to the nucleotide

† Purdue University.
‡ Katholieke Universiteit Leuven.
§ Merck Sharp and Dohme Research Laboratories.

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