New Antitumor Platinum(II) Complexes with Both Lipophilicity and Water Miscibility

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Water-miscible platinum(II) complexes with 1,2-diaminocyclohexane as the carrier ligand and bile acids as the leaving ligands were synthesized and tested for antitumor activity against intraperitoneally implanted leukemia L1210 cells in mice. These complexes were water-miscible after appropriate sonication due to the presence of hydrophilic hydroxyl groups in the molecule, even though the complexes were essentially lipophilic. The complexes had high antitumor activity, but their optimal dose levels differed, and the administration route and form affected the antitumor activity. More lipophilic complexes showed higher activity when administered with Lipiodol than in water suspension, while the hydrophilic complexes showed significant activity when administered in water suspension. Intravenous administration of DACHP(cheno)₂ in water suspension resulted in potent antitumor activity, while other complexes showed moderate activity via this route.

Key words: Liposoluble platinum(II) complexes — 1,2-Cyclohexanediammineplatinum(II) — Bile acid derivatives — Micelle-forming platinum(II) complexes — Water-miscible platinum(II) complexes

Cis-dichlorodiammineplatinum(II)¹⁾ (cisplatin) has been used widely for clinical cancer therapy in spite of its severe renal toxicity. Many approaches have been used in attempts to synthesize new platinum complexes having a broader spectrum of antitumor activity, reduced host toxicity, greater solubility, and lack of cross-resistance with cisplatin.²⁻⁷⁾

We found that liposoluble platinum(II) complexes were superior as therapeutic agents to water-soluble platinum complexes in animal experiments.⁸⁾ Although these liposoluble platinum(II) complexes have a strong antitumor activity, their potential value is limited by their insolubility in water; they are only soluble in organic solvents. We, therefore, attempted to obtain antitumor liposoluble platinum(II) complexes with a hydrophilic function using bile acids such as chenodeoxycholic acid, cholic acid, deoxycholic acid, lithocholic acid, and ursodeoxycholic acid as the leaving ligands in 1,2-cyclohexanediammineplatinum(II) (DACHP) complexes.

Bis(chenodeoxycholato) - 1,2 - cyclohexanediammineplatinum(II) [DACHP(cheno)₂], bis(cholato) - 1,2 - cyclohexanediammineplatinum(II) [DACHP(chol)₂], bis(deoxycholato) - 1,2 - cyclohexanediammineplatinum(II)⁹⁾ [DACHP(deoxy)₂], bis(lithocholato) - 1,2 - cyclohexanediammineplatinum(II), [DACHP(litho)₂], and bis(ursodeoxycholato) - 1,2 - cyclohexanediammineplati-

num(II) [DACHP(urso)₂] were synthesized by reaction of the diaqua form of DACHP with two mole equivalents of the corresponding bile acid sodium salt in water in the dark. After work-up, the complexes were obtained as amorphous powders, each of which showed two spots on silica gel TLC due to the stereoisomers (*cis* and *trans*) of the cyclohexane ring. The reaction conditions, yield, and analytical data are shown in Table I. These complexes were miscible with water as expected and the degree of miscibility was different depending on the number and positions of hydroxyl groups. DACHP(chol)₂ gave a clear aqueous solution without sonication. On the other hand, DACHP(cheno)₂, DACHP(litho)₂, and DACHP(urso)₂ gave milky suspensions ("micelle-like") with water after sonication (5-10 min) and DACHP(deoxy)₂ was miscible with water after prolonged sonication (~20 min) at room temperature.

These platinum(II) complexes thus obtained were tested for antitumor activity against intraperitoneally implanted leukemia L1210 in CDF₁ mice. The complexes were dissolved in Lipiodol (Lipiodol Ultra-Fluid; Kodama Co. Ltd., Tokyo) or suspended in distilled water with sonication for 5 to 20 min. Two different administration routes were used to evaluate possible antitumor effects of the complexes. When the test samples were dissolved in Lipiodol, intraperitoneal injection was used. When they were suspended in water, intraperitoneal injection and intravenous injection were used. They were injected on days 1 and 5 after tumor inoculation. The

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Table I. Physico-chemical Properties of Bis(bilato)-1,2-cyclohexanediammineplatinum(II) Complexes

Complex ^{a)}	Reaction time ^{b)} (day)	Yield (%)	TLC R _f ^{c)}	Analysis							
				Calcd.				Found			
				C	H	N	Pt	C	H	N	Pt
DACHP(cheno) ₂	2	95	0.61 0.75*	59.38	8.49	2.56	17.86	59.11	8.65	5.52	17.7
DACHP(chol) ₂	1	88	0.20* 0.41	57.68	8.25	2.49	17.35	57.26	8.43	2.18	18.3
DACHP(deoxy) ₂	3	77	0.55 0.66*	59.38	8.49	2.56	17.86	59.30	8.57	2.39	18.0
DACHP(litho) ₂	3	86	0.87* 0.92	61.16	8.75	2.64	18.40	61.09	8.70	2.68	18.5
DACHP(urso) ₂	2	92	0.66 0.77*	59.38	8.49	2.56	17.86	59.43	8.60	2.64	18.1

a) The dichloro-1,2-cyclohexanediammineplatinum(II) complex used in this experiment was synthesized from a mixture of *cis*-1,2-diaminocyclohexane and *trans*-1,2-diaminocyclohexane (35:65 in molar ratio).

b) The reaction was done at room temperature in the dark for the indicated time (days).

c) The thin-layer chromatography (TLC) was done on pre-coated silica-on-glass plates with a mixture of chloroform-methanol (4:1) by an ascending method. These products showed two spots on silica gel TLC due to the mixture of *cis* and *trans* components. Asterisks show the major components in the complexes.

Table II. Effects of Bis(bilato)-1,2-cyclohexanediammineplatinum(II) Complexes on the Survival Time of CDF₁ Mice Bearing L1210 Leukemia^{a)}

Complex	Administration ^{b)}		Dose ^{c)}		MST ^{d,e)} (day)	T/C ^{f)} (%)	60-day survivors /total ^{g)}
	Route	Vehicle	mg/kg×2	μmol/kg×2			
DACHP(cheno) ₂	ip	L	53	49	19.0	190	1/6
	ip	W	88	81	>120	>1200	4/6
	iv	W	158	145	>41	>410	3/6
DACHP(chol) ₂	ip	L	284	253	11.0*	122	0/6
	ip	W	792	705	>97	>970	3/6
	iv	W	163	145	10.0*	111	0/6
DACHP(deoxy) ₂	ip	L	264	242	>120	>1200	5/6
	ip	W	285	261	>120	>1200	4/6
	iv	W	37	34	15.0*	167	1/6
DACHP(litho) ₂	ip	L	264	249	>87	>870	3/6
	ip	W	264	249	>120	>1200	5/6
	iv	W	36	35	14.5*	161	0/6
DACHP(urso) ₂	ip	L	53	49	25.5*	283	2/6
	ip	W	88	81	>120	>1200	5/6
	iv	W	66	60	16.0*	178	0/6

a) L1210 cells (10⁴) were transplanted intraperitoneally on day zero.

b) The complexes were administered intraperitoneally (ip) or intravenously (iv) with Lipiodol (L) (injection volume, 0.3 ml) or distilled water as a suspension (W) (injection volume, 0.2 ml for ip and 0.1 ml for iv).

c) The complexes were administered on days 1 and 5 after tumor inoculation.

d) Median survival time of the control group was 10 days and that of the control of the asterisked groups was 9 days.

e) The survival of mice was observed for 120 days for ip-L, ip-W, and 60 days for iv-W.

f) The ratio of the treated (T) vs. the control (C) in median survival time.

g) Sixty-day survivors per total mice in number.

best results with each administration route and vehicle are summarized in Table II.

Of the complexes tested, the most liposoluble complex, DACHP(deoxy)₂, showed the highest antitumor activity (T/C > 1200%) with 5 out of 6 mice tested being 60-day survivors when the Lipiodol solution was administered

intraperitoneally. DACHP(litho)₂ also showed a strong activity (T/C > 870%) with 3 of 6 mice tested being 60-day survivors. DACHP(cheno)₂ and DACHP(urso)₂ showed a moderate activity; the T/C values were 190% and 283%, respectively. The most hydrophilic complex, DACHP(chol)₂, had only marginal antitumor activity

under these experimental conditions. These results showed that intraperitoneal administration of more liposoluble complexes in Lipiodol solution resulted in higher activity than could be obtained with less lipophilic (hydrophilic) complexes. These findings suggest that the complexes are retained in the Lipiodol phase and an active principle is gradually released from Lipiodol. On the other hand, intraperitoneal administration of these complexes in water suspension showed potent antitumor activity; the T/C values of DACHP(cheno)₂, DACHP(chol)₂, DACHP(deoxy)₂, DACHP(litho)₂, and DACHP(urso)₂ were >1200%, >970%, >1200%, 1200%, and >1200%, respectively, with a very high rate of 60-day survivors [DACHP(cheno)₂ 4/6, DACHP(chol)₂ 3/6, DACHP(deoxy)₂ 4/6, DACHP(litho)₂ 5/6, DACHP(urso)₂ 5/6] in contrast with Lipiodol solution [DACHP(cheno)₂ 1/6, DACHP(chol)₂ 0/6, DACHP(deoxy)₂ 5/6, DACHP(litho)₂ 3/6, DACHP(urso)₂ 2/6]. A possible reason for the dependence on administration route is that the antitumor activity in Lipiodol is influenced by the partition coefficient of the complex into Lipiodol, whereas this is not a factor for the suspension form. Furthermore, the antitumor activity of the complexes was tested by intravenous administration. In this case, DACHP(cheno)₂ showed the highest activity (T/C>410%) with a 60-day survival rate of 3 of 6 mice

tested, and DACHP(deoxy)₂ showed some activity (T/C, 167%) with one 60-day survivor out of 6 mice tested. Other complexes showed moderate antitumor activity; the T/C values of DACHP(chol)₂, DACHP(litho)₂, and DACHP(urso)₂ were 111%, 161%, and 178%, respectively, with no 60-day survivors. It is noteworthy that there is great variation in the antitumor effect of these complexes depending on their administration route and form (solution or suspension in Lipiodol and suspension in water). The carrier ligand is the same and the carbon skeletons of the leaving ligands in the complexes are the same, with only small differences in the number of hydroxyl groups, their attachment positions, and their configuration in cholanic acid. Therefore, the leaving ligand is clearly important for antitumor activity as well as the carrier ligand in these platinum complexes.

In conclusion, bile acid derivatives of 1,2-cyclohexanediammineplatinum(II) show antitumor activity in an experimental tumor system when they are satisfactorily miscible with water.

A part of this work was supported by a Grant-in-Aid for Cancer Research (01010024) from the Ministry of Education, Science and Culture, Japan.

(Received February 26, 1990/Accepted April 25, 1990)

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