The Synthesis and Antitumor Properties of a Series of Water Soluble Carboxylato-(1,2-diaminocyclohexane) Platinum(II) Complexes

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Abstract

Water soluble carboxylato-(1,2-diaminocyclohexane)–platinum(II) complexes have been synthesized and their mode of coordination characterized by elemental analysis and infrared spectra. Preliminary *in vitro* and *in vivo* screening tests for antitumor activity of these complexes against L1210 murine leukemia were performed. The results indicate that this class of complexes have good *in vivo* efficacy that can be greatly increased multiple drug administration.

Introduction

Since Rosenberg *et al.* [1] first described the antitumor activity of *cis*-dichlorodiammine–platinum(II) (DDP) in 1969, DDP has become established as an important agent in the treatment of selected human malignant tumors [2, 3]. The usefulness of this transition metal complex is limited, however, by its limited spectrum of responsive tumors [4], rather severe host toxicities such as nausea and vomiting, nephrotoxicity and neurotoxicity [5–7] and the potential to induce resistance in otherwise responsive tumor types [8, 9]. In an attempt to circumvent these shortcomings of DDP, many new platinum complexes have been synthesized.

One of the most promising second generation platinum complexes was *cis*-dichloro-(1,2-diaminocyclohexane)–platinum(II). The biological properties of this complex which included excellent antitumor activity, little or no nephrotoxicity and lack of cross resistance with DDP [10] were, unfortunately, negated by its virtual lack of water solubility. Our group [11–13], as well as others [14, 15], has undertaken a platinum complex synthesis program specifically directed towards enhancing the water solubility of *cis*-dichloro-(1,2-diaminocyclohexane)–platinum(II) while maintaining the many favorable biological attributes. To this end we replaced, in a systematic manner, the chloride ions with specific organic anionic ligands.

The present work describes the synthesis and biological characteristics of a class of water soluble 1,2-diaminocyclohexane–platinum(II) complexes in which the chloride ions have been substituted with a series of carboxylate ligands. The chemical structures of these compounds are depicted in Table I.

Materials and Methods

Synthesis and Chemical Characterization

A solution of K_2PtCl_4 was mixed with an equimolar amount of 1,2-diaminocyclohexane (DACH) in water and allowed to react at room temperature for 6 to 8 h with constant stirring. The water insoluble *cis*-dichloro-(DACH)–platinum(II) was collected by filtration and washed successively with water, ethanol and acetone. After drying *in vacuo* the *cis*-dichloro-(DACH)–platinum(II) was stirred at room temperature with an equimolar amount of Ag_2SO_4 in water for 24 h in the dark. The water soluble *cis*-sulfato-(DACH)–platinum(II) was removed from the AgCl precipitate by filtration and evaporated to dryness at 45–50 °C under reduced pressure using a rotary evaporator. The yellow-brown product was dried over P_2O_5 under vacuum. Finally, the sulfato-(DACH)–platinum(II) (0.423 g) was dissolved in 20 ml of water and an appropriate amount of barium cyclopropanecarboxylate (prepared *in situ* by the addition of 0.3 g of $Ba(OH)_2 \cdot 8H_2O$ to an aqueous solution of 0.172 g of cyclopropanecarboxylic acid) was added thereto. The reaction mixture was stirred for 30 min at room

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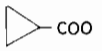
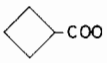
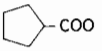
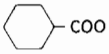
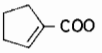
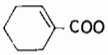
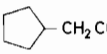
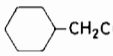
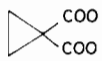
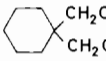
TABLE I. Elemental Analysis and Infrared Data for the Carboxylato-(1,2-diaminocyclohexane)–Platinum(II) Complexes.

Compound	X	% Observed (% Calculated)			IR (cm^{-1})
		C	H	N	
1		34.15(33.80)	5.13(5.23)	5.65(5.63)	C=O 1602; C–O 1410
2		36.61(36.55)	5.72(5.71)	5.21(5.33)	C=O 1667; C–O 1383
3		38.90(39.05)	6.01(6.14)	5.49(5.40)	C=O 1600; C–O 1387
4		41.23(41.30)	6.93(6.54)	5.46(4.82)	C=O 1603; C–O 1381
5		40.26(40.66)	5.65(5.65)	5.05(5.27)	C=O 1632; C–O 1398
6		41.20(41.56)	5.47(5.89)	4.64(4.85)	C=O 1654; C–O 1391
7		37.73(37.73)	5.77(5.34)	4.74(4.40)	ND ND
8		41.49(42.00)	6.94(7.00)	4.98(4.46)	C=O 1561; C–O 1392
9		30.11(30.13)	4.47(4.38)	6.09(6.39)	C=O 1612; C–O 1388
10		36.58(36.55)	5.75(5.71)	5.03(5.33)	C=O 1600; C–O 1382

temperature and the $BaSO_4$ precipitate was removed by filtration. The filtrate was evaporated to dryness at 45 °C under reduced pressure using a rotary evaporator. The yellow solid was obtained, which was subsequently purified from methanol, and the product was dried *in vacuo* with a yield of 70%. The other complexes were prepared by a similar method.

Prior to entry into biological studies all complexes were submitted for elemental analysis. If the observed values for C, H or N exceeded the calculated values by more than ±0.5% the complexes were again purified with methanol and reanalyzed. The general synthetic pathway is shown below:

$$K_2PtCl_4 + DACH \xrightarrow{R.T.} Pt(Cl)_2(DACH)\downarrow + 2KCl$$

$$Pt(Cl)_2DACH + Ag_2SO_4 \xrightarrow{R.T.} Pt(SO_4)(DACH) + 2AgCl\downarrow$$

$$Pt(SO_4)(DACH) + Ba(ligand) \longrightarrow Pt(ligand)(DACH) + BaSO_4\downarrow$$

To further characterize these complexes, IR spectra were obtained for **9** of the **10** complexes reported.

Antitumor Testing

Each complex was assayed for *in vitro* cytotoxicity against an L1210 leukemia cell line. The cell line was routinely maintained in McCoy's 5A supplemented with glutamine, penicillin, streptomycin and 10% horse serum (L1210/0). For testing purposes, 4 ml of cells (10^5 cells/ml) were added to culture tubes and the appropriate concentration of test compound added to the culture (0.01, 0.1, 1.0 or 10 μg/ml final concentration). After 72 h incubation in a humidified atmosphere of 90% air:10% CO_2, the cell concentration of control and test cultures were determined using a Coulter counter (Coulter Electronics, Hialeah, Fla.). When grown under these conditions, L1210 cells have a doubling time of approximately 15 h and control cultures are in exponential growth kinetics after 72 h of incubation.

To assess the *in vivo* antitumor activity of the carboxylate (DACH) platinum complexes, male BDF_1 mice (weighing 18–20 gm), purchased from Jackson Laboratories, Bar Harbor, Maine, were inoculated intraperitoneally (i.p.) with 10^6 viable L1210/0 leukemic ascites cells (day 0). Drug administration was begun the next day (day 1) as a single i.p. injection or 3 i.p. injections on days 1, 5 and 9. Animals were observed daily for signs of toxicity and sur-

vival. Deaths occurring on or before day 7 were considered to relate to drug toxicity (mean survival time of non-treated tumor bearing controls was 8.8 ± 0.6 days). After 60 days, the study was ended and all surviving mice were autopsied. The efficacy of the platinum complexes administered according to these treatment schedules was calculated using the following formula:

$$\%T/C = \frac{\text{Mean Survival Time Treated}}{\text{Mean Survival Time Control}} \times 100$$

The other means of evaluating the antitumor effect of the test complexes was the number of long-term surviving mice, defined as animals alive 60 days after tumor inoculation and no gross evidence of tumor at autopsy.

Results and Discussion

The platinum complexes were submitted for elemental analysis prior to biological evaluation. As depicted in Table I, the observed C, H and N values were within close approximation of the calculated values. Although no X-ray crystallographic analyses were obtained on any of the complexes, the elemental analyses were consistent with the stoichiometric relationship of 1-1,2-diaminocyclohexane:1-platinum and 2 carboxylate ligands for complexes **1** through **8** and 1-1,2-diaminocyclohexane:1-platinum and 1 carboxylate ligand for complexes **9** and **10**. That the anionic ligands are coordinated to platinum through the carboxylate functionalities is supported by the IR data summarized in Table I. These pieces of evidence suggest the structural formula shown in Fig. 1.

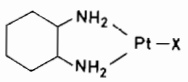


Fig. 1. Suggested structural formula, where X = two monodentate carboxylato ligands or one bidentate carboxylato ligand.

The initial antitumor testing performed with these complexes was the *in vitro* cytotoxicity against L1210/0 cells (Table II). The ID_{50} values for the carboxylato-(1,2-diaminocyclohexane)–platinum(II) complexes ranged from 0.32 to 1.30 μg/ml indicating that all of the complexes had acceptable cytotoxicity. To evaluate the *in vivo* efficacy, the complexes were administered as a single i.p. injection to BDF_1 mice inoculated the previous day with 10^6 viable L1210/0 cells. All 10 platinum complexes had at least criterion levels of activity (%T/C > 140%) and were approximately equivalent to DDP (Table III). Selected complexes (CDP 1, 3, 5 and 9) were further evaluated for *in vivo* efficacy when administered on a multiple treatment. As can be seen in Table IV, the antitumor activity of all **4** complexes was significantly enhanced when administered i.p. on days 1, 5 and 9. Indeed, complexes **1**, **5** and **9** produced several long-term survivors (alive 60 days after tumor inoculation) and were at least equivalent to DDP in activity.

In spite of the marked effectiveness of DDP in selected human malignancies, certain deficiencies in DDP have prompted an extensive search for an improved second or third generation platinum analog. Burchenal *et al.* [16] and more recently Harrap [17] have established guidelines for such synthesis programs and include increased efficacy, decreased

TABLE II. *In Vitro* Cytotoxicity of the Carboxylato-(1,2-diaminocyclohexane)–Platinum(II) Complexes.

Complex	ID_{50} (μg/ml)
1	0.32
2	0.44
3	0.32
4	0.55
5	1.30
6	1.00
7	0.66
8	0.61
9	0.41
10	0.59
DDP	0.10

TABLE III. *In Vivo* Efficacy of Carboxylato-(1,2-Diaminocyclohexane)–Platinum(II) Complexes Administered as a Single Intraperitoneal Injection.

Compound	Max. Effective Dose[a] (mg/kg)	% T/C
1	12.5	196
2	25	159
3	25	178
4	100	170
5	12.5	185
6	50	165
7	12.5	145
8	12.5	182
9	12.5	170
10	25	180
DDP	5	165

[a]Mice were inoculated intraperitoneally with 10^6 L1210 cells (day 0) and administered a single intraperitoneal injection of the test compound the following day. The results reported here represent the dose of compound that produced the greatest % T/C of all the doses tested.

TABLE IV. *In Vivo* Efficacy of Carboxylato-(1,2-diaminocyclohexane)–Platinum(II) Complexes Administered as Multiple Intraperitoneal Injections.

Compound	Dose[a] (mg/kg)	% T/C	LTS
1	25	144	–
	12.5	337	2/6
	6.25	265	2/6
3	25	253	–
	12.5	246	–
	6.25	206	–
5	25	300	1/6
	12.5	356	1/6
	6.25	190	–
9	15	357	2/6
	10	452	4/6
	5	140	–
DDP	10	240	1/6
	5	220	1/6

[a]Mice were inoculated intraperitoneally with 10^6 L1210 cells (day 0) and were administered test compound intraperitoneally on days 1, 5 and 9. Long-term survivors (LTS) are defined as animals alive 60 days after tumor inoculation.

toxicity, enhanced water solubility and broader spectrum of activity. Our laboratory has been actively pursuing the possibility of enhancing the usefulness of platinum as an anticancer drug in two ways. One method has been to modify the stable amine ligand which has resulted in a series of highly active complexes that are unfortunately relatively water insoluble [18]. Our primary emphasis, however, has been placed on systematic substitution of the anionic ligand of the 1,2-diaminocyclohexane–platinum(II) class of heavy metal complexes. Using this technique we [11–13] and others [14, 15], have found that it is indeed possible to enhance the water solubility of the parent dichloro-(1,2-diaminocyclohexane)–platinum while maintaining many of the desirable biological properties of the parent complex.

The data summarized in the present communication indicate that the water solubility can be significantly increased by substitution of the two chloride anions with mono or bis carboxylates. The use of these anionic ligands produced highly water soluble 1,2-diaminocyclohexane–platinum(II) complexes having significant antitumor activity both *in vivo* and *in vitro*. Further, as we have observed with other water soluble 1,2-diaminocyclohexane–platinum(II) complexes, the efficacy of the carboxylato-(1,2-diaminocyclohexane)–platinum(II) complexes is highly schedule dependent. In fact, when administeron days 1, 5 and 9, selected complexes (CDP 1, 3, 5 and 9) exceeded the antitumor activity of the prototype DDP.

In summary, the carboxylato-(1,2-diaminocyclohexane)–platinum(II) complexes possess sufficient water solubility and biological activity to warrant further biological and chemical investigation.

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