Chemical and biological characterization of a series of water soluble 1,2-diaminocyclohexane platinum(II) complexes

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Abstract

A series of water soluble 1,2-diaminocyclohexane platinum(II) complexes have been synthesized and analyzed for their mode of ligand coordination and biological activity. Preliminary *in vitro* and *in vivo* screening tests indicate that these complexes have excellent antitumor activity and are not cross-resistant with DDP. These results suggest that this series of platinum complexes warrant further study for eventual introduction into clinical studies.

Introduction

The antitumor activity of *cis*-diamminedichloro–platinum(II) (DDP) was first reported by Rosenberg *et al.* in 1969 [1]. Since that time DDP has become one of the most widely used antitumor agents in the clinic today. Although selected tumors are remarkably responsive to DDP, the actual spectrum of sensitive human tumors is rather limited [2, 3]. In addition, DDP causes severe, and sometimes life-threatening toxicities such as nausea, vomiting, renal damage, ototoxicity and neurotoxicity [4]. Finally, the clinical utility of this agent is hampered by an apparent potential of DDP to induce resistance in previously response tumors [5, 6].

As a result of these significant shortcomings of DDP as an anticancer drug, a major effort has been put forth by a number of laboratories in an attempt to develop new platinum complexes having a broader spectrum of activity, decreased host toxicity and/or lacking cross-resistance with the parent DDP. A class of platinum analogs using 1,2-diaminocyclohexane (DACH) as the inert amine ligand has received great deal of attention. The initial complex of this class, DACH–Pt–Cl_2, had little cross-resistance with DDP, was relatively non-nephrotoxic and had excellent antitumor activity [7]. Unfortunately, the complex was virtually insoluble in water and was, therefore, not amenable to large scale clinical development. An array of anionic ligands have been introduced into the DACH–Pt complex to enhance the water solubility of the parent DACH–Pt–Cl_2 while maintaining its favorable biological characteristics [8–11]. Of these, only a few have progressed to clinical trials and none have, as of yet, entered large scale clinical testing.

Over the past several years our laboratory has been actively synthesizing new water soluble DACH–Pt analogs [12–14]. The present report describes a series of such complexes that have sugar acids as the anionic leaving group and possess rather encouraging biological activity. Based on these results we believe that one or more of these complexes represent viable candidates for clinical testing.

Materials and methods

Chemicals

Isocitratomonoethylester was purchased from Pfaltz and Bauer, Inc., Waterbury, CT. All other anionic ligands were obtained from Aldrich Chemical Co., Milwaukee, WI. K_2PtCl_4 was supplied by Matthey-Bishop, Inc. All reagents were used as supplied without further purification prior to their use in synthesis.

Synthesis and chemical characterization

A solution of K_2PtCl_4 was mixed with an equal molar concentration of 1,2-diaminocyclohexane in water and allowed to react at room temperature for 6–8 h with constant stirring. The water insoluble *cis*-

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DACH–Pt–Cl_2 was collected by filtration and washed successively with water, ethanol and acetone. After drying *in vacuo* the *cis*-DACH–Pt–Cl_2 was stirred at room temperature with an equal molar concentration of Ag_2SO_4 in water for 24 h in the dark. The water soluble DACH–Pt–SO_4 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 product was then dried over P_2O_5 under vacuum. Finally, the DACH–Pt–SO_4 was dissolved in water and a solution of the appropriate ligand, in equimolar amount in the barium salt form was added to it. The reaction mixture was stirred for 30 min at room 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 solid obtained was subsequently purified from a water and acetone mixture, and the product was dried *in vacuo*.

Prior to entry into biological testing, all complexes were submitted for elemental analysis. If the observed C, H or N values exceeded the calculated values by more than +0.5% the complexes were repurified and reanalyzed. To further characterize these complexes, IR spectra were obtained for complexes reported.

Biological testing

All complexes were evaluated for *in vitro* cytotoxicity against the murine leukemia L1210 cells, both the DDP sensitive wild-type (L1210/0) and the DDP resistant-type (L1210/DDP). Each cell line is maintained in MCoys 5A medium supplemented with glutamine and either 10% horse serum (L1210/0) or 10% fetal bovine serum (L1210/DDP) and were grown in a humidified atmosphere of 90% air:10% CO_2. For testing purpose, 4 ml of cells (10^5 cells/ml) were added to tissue culture tubes to which 40 μm of test compound were added. Each complex was tested for cytotoxicity at final concentration of 10, 1, 0.1 and 0.01 μg/ml of cell suspension. After 72 h of constant exposure to the given complex the cell concentration in each tissue culture tube was determined using a Coulter counter. The ID_{50} value, i.e., the concentration of complex required to inhibit cell growth by 50%, was then calculated for each complex.

To determine the *in vivo* antitumor activity of a given complex, C57B1/6xDBA mice (hereafter referred to as BDF mice) were inoculated intraperitoneally (i.p.) with 10^6 L1210 cells. Tumor bearing mice were then administered the test complex i.p. either as a single treatment on day 1 or as 3 i.p. injections on days 1, 5 and 9 following tumor implantation. Mice were then observed daily for survival for 60 days following inoculation of tumor. The day of death for each animal was carefully recorded and the mean survival time (MST) of treatment group calculated. Mice alive after the 60 day observation period were considered to be long term survivors (LTS) and were not included in the MST calculations but are noted as LTS in the appropriate Tables. To compare the relative efficacy of each complex was then compared by calculating the % *T/C* value, i.e. MST (treated)/MST (control).

The relative toxicity of selected complexes was compared by injecting test compound i.p. to female CD mice, weighing 20–25 g and observing animals daily for survival and toxic signs. After 14 days, all surviving animals were sacrificed and gross necropsy was performed on each animal. Similarly all animals that died during the study were necropsied and gross abnormalities noted. Using the probit analysis, as described by Finney [15], LD_{10}, LD_{50} and LD_{90} were calculated for each complex tested.

Results

All platinum complexes were submitted for elemental analysis prior to being evaluated for biological activity. As can be seen in Table 1, all seven complexes had observed C, H and N values in close approximation to the calculated values. These results suggest a stoichiometric relationship of 1 DACH to 2 leaving groups for complexes **1** and **2** and 1 DACH to 1 leaving group for complexes **3** to **7**. The IR data reported in Table 1 support the idea that the leaving groups are coordinated to the platinum through the carboxylate functionalities. Taken together this information suggests the structural formula depicted in Table 2 for the complexes under study.

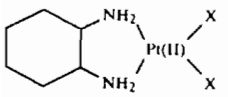
Initial biological evaluation of these platinum complexes revealed that all seven drugs had approximately the same potency against the L1210 cells *in vitro* with ID_{50} values ranging from 1 to 3 μg/ml (Table 3). Further, none of the complexes exhibited cross-resistance with DDP against the L1210/DDP cells *in vitro*.

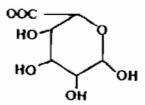
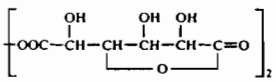
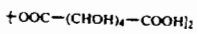
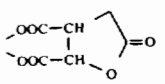
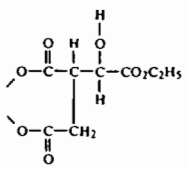
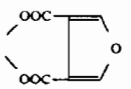
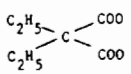
To assess the *in vivo* efficacy of these seven complexes, BDF tumor bearing mice were administered the test compound as either a single i.p. injection 24 h after tumor inoculation (day 1) or 3 i.p. injections on days 1, 5 and 9. As can be seen from the results reported in Table 4, all but complex **1** had criterion level of antitumor activity, defined as % $T/C > 140$, when given as single injection. The *in vivo* efficacy of this class of compounds appeared to be strikingly schedule dependent. All five of the complexes had significantly increased activity when administered on days 1, 5, 9, with a marked increase

TABLE 1. Elemental analysis and IR data for the 1,2-diaminocyclohexane platinum(II) complexes

Complex no.	Analysis: found(calc.) (%)			IR (cm^{-1})	
	C	H	N	C=O	C–O
1	29.19(29.53)	4.57(5.00)	3.41(3.83)	1627	1401
2	29.66(29.70)	4.15(3.85)	3.53(3.85)	1630	1363
3	29.00(28.99)	4.42(4.56)	3.63(3.76)	1630	1392
4	28.65(28.85)	4.13(4.00)	5.42(5.61)	1631	1397
5	30.10(29.84)	4.46(4.97)	5.32(4.97)	1618	1372
6	28.84(28.80)	3.53(4.00)	5.33(5.60)	1571, 1541	1400
7	31.02(31.00)	4.95(5.56)	5.43(5.56)	1592, 1554	1451

TABLE 2. Proposed structures for 1,2-diaminocyclohexane platinum(II) complexes



Complex no.	Anionic ligand (X) Chemical name	Chemical structure
1	Galacturonate	
2	Saccharatolactone	
3	Saccharate	
4	Isocitratolactone	
5	Isocitratomono-ethylester	
6	Furanedicarboxylate	
7	Diethylmalonate	

in % *T/C* values for each complex and long term survivors occurring for all complexes except complex **4** (Table 5). It is interesting to note that the maximally effective dose for both treatment schedules was approximately the same suggesting a lack of increased

TABLE 3. *In vitro* cytotoxicity of the 1,2-diaminocyclohexane platinum(II) complexes

Complex no.	ID_{50} (μg/ml)[a]		Resistance factor
	L1210/0	L1210/DDP	
1	1.00	1.50	1.5
2	3.00	4.00	1.33
3	2.80	0.90	0.32
4	1.00	3.00	3
5	2.00	2.90	1.45
6	1.00	2.50	2.5
7	2.00	3.50	1.75
DDP	0.15	4.00	27

[a]L1210 cells sensitive (L1210/0) or resistant (L1210/DDP) to DDP were grown in 1 ml of McCoy's 5A medium (10^4 cells/ml) to which test complex (10, 1, 0.1, 0.01 μg/ml, final concentration) was added. After 48 h of drug exposure the cell concentrations of treated and control cultures were determined and the ID_{50} values for each complex calculated.

TABLE 4. *In vivo* antitumor activity of 1,2-diaminocyclohexane platinum(II) complexes when administered as a single intraperitoneal injection

Complex no.	Maximum effective dose[a] (mg/kg)	% *T/C*
1	25	129
2	50	160
3	50	156
4	50	145
5	25	150
6	25	143
7	25	156
DDP	7.5	185

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

TABLE 5. *In vivo* efficacy of 1,2-diaminocyclohexane platinum(II) complexes administered as multiple intraperitoneal injections

Complex no.	Maximum effective dose[a] (mg/kg)	% *T/C*	Long term survivors
1	25	228	4/6
2	25	207	2/6
3	50	225	1/6
4	25	219	0/6
5	25	230	4/6
DDP	5	220	1/6

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

TABLE 6. Acute toxicity of 1,2-diaminocyclohexane platinum(II) complexes administered as a single intraperitoneal injection to mice

Complex	Calculated values (mg/kg)[a]		
	LD_{10}	LD_{50}	LD_{90}
3	115	155	220
5	80	100	130
DDP	13	17	19

[a]CD_1 albino mice were administered a single i.p. injection of the test complex (6 mice/dose) and mice were observed daily. Lethal dose (*LD*) values were calculated using probit analysis [15].

toxicity when multiple doses are administered. Finally, the acute toxicity of complexes **2** and **3** were evaluated by administering a single i.p. injection of test compound to CD mice (Table 6). Both complexes were significantly less toxic than DDP and while renal function tests were not performed in this study the kidneys of the DACH–Pt treated mice had no grossly apparent lesions whereas the kidneys taken from mice administered DDP were pale in color.

Discussion

Although DDP has remarkable antitumor activity against selected human tumors, there are attributes of this agent that severely limit the usefulness of this compound. Several investigators have delineated guidelines for properties desirable in second and third generation platinum based antitumor complexes and include decreased toxicity, broadened spectrum of action, enhanced water solubility and lack of cross-resistance with DDP [16, 17]. Work in our laboratory, as well as others, has clearly demonstrated that it is possible through modulation of either the inert amine ligand or the labile anionic ligand to attain one or more of the above stated guidelines.

The data summarized in the present communication indicate that the water solubility of the DACH–Pt complexes can be significantly enhanced by the substitution of the chlorides with acidic sugars such as those used herein. These complexes were active *in vitro* and were apparently non-cross-resistant with DDP. Further, as has been reported previously with other highly water soluble platinum complexes, the *in vivo* efficacy of the platinum complexes reported in the present communication is highly schedule dependent. Thus, the platinum complexes appear to have many of the attributes needed for successful introduction of a second or third generation platinum complex into large scale clinical trials.

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