Enantiomeric Cisplatin Analogues: an Investigation on their Activity towards Tumors in Mice

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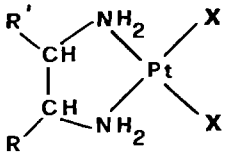
Received June 7, 1983

A series of enantiomeric cisplatin analogues of formula [diamPtCl$_2$] (diam = chiral chelating diamine) and the corresponding sulfato derivatives was prepared and tested for activity against tumors in mice, particularly P 388 leukemia. The configuration of the diamines has practically no influence on the antitumor activity. The effects of the leaving group and of the nature of the diamines are briefly discussed.

Introduction

It is likely, although not widely accepted [1], that the antitumor properties of *cis*-dichlorodiammino-platinum(II) (cisplatin) arise from the electrophilic attack of the platinum atom on the N(7) atom of guanine of DNA [2, 3]. Since DNA is a chiral substrate, the possibility arises, at least theoretically, that chiral drugs, such as some cisplatin analogues in which the two ammonia ligands have been substituted by chiral chelating diamines (see scheme 1) [4], could interact differently depending on the absolute configuration of the diamine. If this assumption is correct, enantiomeric compounds should give rise to different biological activities.



Scheme 1. Cisplatin analogues used in this work. The diamines (diam) and their abbreviations are: R = R' = H, ethylenediamine, en; R = H, R' = CH_3, (*R*)- and (*S*)-propane-1,2-diamine, pn; R = R' = CH_3, (*R*,*R*)-, (*S*,*S*)-, and *meso*-butane-2,3-diamine, bn; R = H, R' = C_6H_5, (*R*)-1-phenylethylenediamine, pen; R = R' = C_6H_5, (*R*,*R*)-, (*S*,*S*)-, and *meso*-1,2-diphenylethylenediamine, dpen; also diam = (*R*,*R*)-, (*S*,*S*)-, and *meso*-cyclohexane-1,2-diamine; chxn. X = Cl for [diamPtCl$_2$]; and X = H_2O for [diamPt$(H_2O)_2$]SO_4.

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We have already studied some model systems and, indeed, in the series of compounds of the type [diamPt(guo)$_2$]$^{2+}$ and [diamPt{guo(–H)}$_2$] (diam = chiral diamine of scheme 1; guo = guanosine; guo(–H) = deprotonated guanosine) certain properties depend on the absolute configuration of the diamine [5, 6]. However, in the case of a more realistic model system, as in the interaction of the diamPt moiety with DNA, electrophoretic and circular dichroism studies on PM2 and calf thymus DNA respectively, have shown that the kinetics of the adduct formation, their distribution ratio, and their chiroptical properties do not depend on the absolute configuration of the diamine [7].

In contrast to these results, a paper has recently appeared which reports a case of enantioselectivity in the interaction of [Zn(phen)$_3$]$^{2+}$ (phen = 1,10-phenanthroline) with DNA [8]. Although in this case intercalation rather than binding to DNA occurs, this paper shows that some chiral recognition is possible in the interaction of DNA with foreign substances. Moreover it has been claimed that the enantiomeric [chxnPtX$_2$] (X = a variety of leaving groups) complexes have different activities against some tumors in mice [9, 10] although the figures reported by these authors are not significantly different. Finally, the two enantiomers of cyclophosphamide [11] have been reported to display different antitumor activity [12], although this finding could not be confirmed by a pharmacokinetic investigation [13].

This body of contrasting evidence prompted us to undertake a thorough study of the antitumor activities of a series of enantiomeric cisplatin analogues of the type depicted in scheme 1. This paper reports the results of such a study.

Experimental

Elemental analyses (C,H,N) were performed at the microanalytical laboratory, the University of Milan; Pt was analyzed by atomic absorption spectroscopy

0020-1693/84/$3.00

on a Varian Techtron instrument with acetylene/air flame.

The chiral diamines were synthesized and resolved according to ref. [4]. 1,2-diaminocyclohexane was purchased from Du Pont (technical grade) as a mixture of the *cis* and *trans* isomers which were separated through their nickel complexes [14] and resolved according to ref. [4].

Cis-dichlorodiamminoplatinum(II)

This was prepared according to a known method [15].

Dichlorodiamineplatinum(II)

The various complexes were prepared following a standard procedure [16].

Diaquodiamineplatinum(II) Sulfate

These complexes were prepared by a slight modification of a literature method [17]. Potassium tetrachloroplatinate(II) was treated, in water, with a large excess of KI at 50 °C for ten minutes. To the resulting cooled solution an aqueous solution of an equimolar amount of the diamine was added, dropwise, and at such a rate that the pH never exceeded 6. The temperature was maintained at about 40 °C. The dark-yellow precipitate of [diamPtI_2] was collected by filtration, washed with water, ethanol, and chloroform and, if necessary, recrystallized from dimethylformamide. The iodides thus obtained were then treated, in water, with silver sulfate (Pt/SO_4 = 1/0.95) and two drops of sulfuric acid 1:3, and heated at 60 °C for 2 hours.

The solution was then centrifuged to remove AgI and evaporated to drops under reduced pressure. The white platinum complexes were crystallized upon addition of a large excess of acetone.

Animal, Tumors, and Tumor Tests

DBA/2 and $CD2F_1$ male mice (20–22 g at the start of the experiment) were obtained from Charles River s.p.a. (Calco Italy). The P388 and L1210 leukemias, originally obtained from Dr. A. Bodgen (Mason Research Institute, Worchester, Mass., USA) were maintained in ascitic form by weekly transfer in DBA/2 mice. For the assessment of antineoplastic activity, 10^6 P388 and 10^5 L1210 viable cells were transplanted intraperitoneally (i.p.), on day 0 into compatible $CD2F_1$ mice, divided into groups of 6 animals. The drugs were dissolved in distilled water (sulfato derivatives) or in klucel (hydroxypropylcellulose) immediately before use. Treatment was performed i.p. 24 hours after tumor implantation (day 1) and activity was expressed as the percent ratio between the median lifespan of treated animals (T) over untreated controls (C). According to standard protocols [18] a T/C% value of 125 was considered the minimal active one.

Results and Discussion

We have prepared a series of complexes of formula [diam$PtCl_2$] and the corresponding sulfato derivatives, where diam is one of the chiral diamines of scheme 1. The dichloro derivatives are well known; the sulfato derivatives were prepared to increase water solubility. These latter compounds have been reported with different formulations (coordinated sulfato group, either monodentate [19] or chelate [20] and different water content [21]). The compounds prepared under our conditions (see Experimental) are better formulated as [diam$Pt(H_2O)_2$]-SO_4 on the basis of elemental analyses (see Table I) and spectroscopic evidence. In fact coordinate sulfato groups should give rise in the infrared spectrum to a number of bands, depending on the mode of binding [22]. The appearance of only one strong band at about 1100 cm^{-1} in the i.r. spectra of our compounds indicates the presence of an ionic sulfato group. In any case this formulation holds in water solutions since the molar conductivities of these compounds are in the range 200–230 $cm^2\ mol^{-1}\ ohm^{-1}$ for about 4×10^{-5} mol dm^{-3} water solutions (See Table I).

The antitumor activities of the compounds are summarized in Tables II and III.

Importance of the Leaving Group

The aquosulfate derivatives have been reported to be more active than the corresponding dichloro compounds [20, 23]. We have found that both the optimal dose and the minimum effective dose are usually lower for the former than for the latter. The toxic dose is also lower for the sulfates. The T/C% values at the optimal and minimum effective doses, however, do not differ much in the case of the analogous chloro and sulfato complexes. Also the therapeutic indexes, an expression of the activity range, are not much different. We therefore believe that these differences in the activities reflect the ease of administration of the water soluble SO_4 salts, rather than a true chemical (leaving group) effect. In fact in a medium of high chloride concentration and at pH 7, as a biological fluid, the bis aquo complex is converted into a dichloro (or chloroaquo, or chlorohydroxo) derivative, which is probably also the species present in the body when the dichloro compound is used [24, 25].

The Effect of the Diamine

We have confirmed that the derivatives of the type [chxnPtX_2] are rather effective antitumor agents, as found even in the case of solid slowly growing tumors [26] and that the complexes with diamines which carry phenyl groups generally show low activity [27]. Of interest is the moderately high activity displayed by the compounds with butanediamine,

TABLE I. Analytical and Conductivity Data.

Compound	Found (calcd.), % C	H	N	Pt	Λ_M[a]
cis-[$(NH_3)_2PtCl_2$]		2.02 (2.00)	9.25 (9.33)	64.9 (65.0)	
[$enPtCl_2$]	7.79 (7.36)	2.55 (2.45)	8.42 (8.59)	59.2 (59.8)	
[$enPt(H_2O)_2$]SO_4	6.10 (6.19)	2.79 (3.10)	7.18 (7.23)	49.2 (50.4)	235
[(*R*)-$pnPtCl_2$]	10.21 (10.59)	3.09 (2.94)	8.05 (8.23)	56.3 (57.4)	
[(*R*)-$pnPt(H_2O)_2$]SO_4	8.82 (8.97)	2.82 (3.49)	6.85 (6.98)	46.9 (48.6)	235
[(*S*)-$pnPtCl_2$]	10.83 (10.59)	2.71 (2.94)	8.07 (8.23)	56.5 (57.4)	
[(*S*)-$pnPt(H_2O)_2$]SO_4	8.88 (8.97)	3.24 (3.49)	6.49 (6.98)	48.2 (48.6)	200
[(*R*,*R*)-$bnPtCl_2$]	13.41 (13.56)	3.48 (3.39)	8.05 (7.91)	54.5 (55.1)	
[(*R*,*R*)-$bnPt(H_2O)_2$]SO_4	11.00 (11.56)	3.52 (3.85)	6.48 (6.74)	44.3 (45.0)	220
[(*S*,*S*)-$bnPtCl_2$]	13.75 (13.56)	3.33 (3.39)	8.00 (7.91)	54.8 (55.1)	
[(*S*,*S*)-$bnPt(H_2O)_2$]SO_4	10.91 (11.56)	3.57 (3.85)	6.22 (6.74)	44.1 (45.0)	210
[*meso*-$bnPtCl_2$]	13.81 (13.56)	3.32 (3.39)	8.02 (7.91)	54.2 (55.1)	
[*meso*-$bnPt(H_2O)_2$]SO_4	11.00 (11.56)	3.69 (3.85)	6.53 (6.74)	44.5 (45.0)	
[(*R*,*R*)-$chxnPtCl_2$]	18.71 (18.94)	3.42 (3.68)	7.50 (7.37)	50.5 (51.3)	
[(*R*,*R*)-$chxnPt(H_2O)_2$]SO_4	16.86 (16.33)	3.92 (4.08)	6.12 (6.35)	43.7 (44.2)	220
[(*S*,S)-$chxnPtCl_2$]	19.10 (18.94)	3.75 (3.68)	7.30 (7.37)	50.9 (51.3)	
[(*S*,*S*)-$chxnPt(H_2O)_2$]SO_4	16.20 (16.33)	4.12 (4.08)	6.47 (6.35)	43.8 (44.2)	220
[*meso*-$chxnPtCl_2$]	19.15 (18.94)	3.51 (3.68)	7.42 (7.37)	50.4 (51.3)	
[*meso*-$chxnPt(H_2O)_2$]SO_4	16.15 (16.33)	4.00 (4.08)	6.52 (6.35)	43.7 (44.2)	200
[(*R*)-$penPtCl_2$]	23.78 (23.88)	2.82 (2.98)	6.94 (6.96)	48.6 (48.5)	
[(*R*)-$penPt(H_2O)_2$]SO_4	20.85 (20.73)	3.26 (3.46)	5.76 (6.05)	40.9 (42.1)	210
[(*R*,*R*)-$dpenPtCl_2$]	35.06 (35.14)	3.37 (3.35)	5.58 (5.86)	40.1 (40.8)	
[(*R*,*R*)-$dpenPt(H_2O)_2$]SO_4	30.95 (31.16)	3.38 (3.71)	5.03 (5.19)	35.6 (36.2)	200
[(*S*,*S*)-$dpenPtCl_2$]	35.29 (35.14)	3.41 (3.35)	5.70 (5.86)	40.1 (40.8)	

(continued overleaf)

TABLE I *(continued)*

Compound	Found (calcd.), % C	H	N	Pt	Λ_M[a]
[(*S,S*)-dpenPt(H_2O)$_2$]SO_4·H_2O	30.21 (30.16)	4.11 (3.95)	4.94 (5.03)	34.5 (35.0)	195
[*meso*-dpenPtCl_2]	35.31 (35.14)	3.34 (3.35)	5.70 (5.86)	40.9 (40.8)	
[*meso*-dpenPt(H_2O)$_2$]SO_4	31.02 (31.16)	3.60 (3.71)	5.05 (5.19)	35.5 (36.2)	

[a] Λ_M values in $cm^2\ mol^{-1}\ ohm^{-1}$, for $\sim 4 \times 10^{-5}$ *M* water solutions.

TABLE II. Antitumor Activities of Various Platinum Complexes towards P388.[a]

Compound	OD (T/C%)		MED (T/C%)		Toxic dose	TI
cis-[(NH_3)$_2$PtCl_2]	5	(170)	<1.25	(140)	15	4
[enPtCl_2]	3.12	(185)	0.78	(140)	12.5	4
[enPt(H_2O)$_2$]SO_4	3.12	(172)	0.78	(140)	12.5	4
[(*R*)-pnPtCl_2]	12.5	(159)	<3.12	(136)	25	4
[(*S*)-pnPtCl_2]	12.5	(163)	<3.12	(154)	25	4
[(*R*)-pnPt(H_2O)$_2$]SO_4	3.12	(176)	<0.78	(140)	12.5	4
[(*S*)-pnPt(H_2O)$_2$]SO_4	6.25	(184)	<0.78	(145)	12.5	8
[(*R,R*)-bnPtCl_2]	25	(181)	6.25	(127)	>50	4
[(*S,S*)-bnPtCl_2]	25	(181)	3.12	(127)	50	8
[*meso*-bnPtCl_2]	25	(170)	<1.56	(127)	>25	16
[(*R,R*)-bnPt(H_2O)$_2$]SO_4	3.12	(170)	<0.78	(160)	12.5	4
[(*S,S*)-bnPt(H_2O)$_2$]SO_4	6.25	(180)	<0.78	(135)	12.5	8
[*meso*-bnPt(H_2O)$_2$]SO_4	12.5	(155)	<0.78	(150)	25	16
[(*R,R*)-chxnPtCl_2]	6.25	(180)	1.56	(136)	12.5	4
[(*S,S*)-chxnPtCl_2]	12.5	(168)	6.25	(148)	>12.5	2
[*meso*-chxnPtCl_2]	12.5	(163)	1.56	(127)	>12.5	16
[(*R,R*)-chxnPt(H_2O)$_2$]SO_4	6.25	(195)	0.39	(125)	12.5	16
[(*S,S*)-chxnPt(H_2O)$_2$]SO_4	6.25	(172)	0.39	(127)	12.5	16
[*meso*-chxnPt(H_2O)$_2$]SO_4	6.25	(165)	0.39	(127)	12.5	16
[(*R*)-penPtCl_2]	25	(172)	3.12	(136)	>50	8
[(*R*)-penPt(H_2O)$_2$]SO_4	3.12	(160)	0.78	(135)	12.5	4
[(*R,R*-dpenPtCl_2]	50	(136)	25	(127)	[b]	2
[(*S,S*)-dpenPtCl_2]	50	(131)	50	(131)	[b]	1
[*meso*-dpenPtCl_2]	50	(136)	25	(127)	[b]	2
[(*R,R*)-dpenPt(H_2O)$_2$]SO_4	1.56	(135)	1.56	(135)	25	1
[(*S,S*)-dpenPt(H_2O)$_2$]SO_4	25	(152)	6.25	(145)	50	4
[*meso*-dpenPt(H_2O)$_2$]SO_4	3.12	(160)	0.78	(160)	25	4

[a] Single injection treatment (on day 1), for details see Experimental. OD, optimal dose; MED, minimum effective dose; all doses are given in mg/kg of body weight. TI, therapeutic index (OD/MED). [b] These materials were highly insoluble and presumably only a small amount of the administered dose was adsorbed. As a matter of fact the T/C% values for these compounds were not dose dependent over a wide range. The toxic dose could not be evaluated.

TABLE III. Antitumor Activities of Some Platinum Complexes towards L1210.[a]

Compound	OD (T/C%)		MED (T/C%)		Toxic dose	TI
[(*R,R*)-chxnPtCl_2]	12.5	(182)	1.56	(130)	>12.5	8
[(*S,S*)-chxnPtCl_2]	12.5	(158)	0.78	(129)	>12.5	16
[(*R,R*)-chxnPt(H_2O)$_2$]SO_4	6.25	(164)	0.78	(125)	12.5	8
[(*S,S*)-chxnPt(H_2O)$_2$]SO_4	6.25	(158)	0.78	(127)	12.5	8
[*meso*-chxnPt(H_2O)$_2$]SO_4	6.25	(141)	0.78	(129)	12.5	8

[a] See footnote of Table II.

especially in the case of the meso form of this diamine. [Meso-bnPtCl_2] was also tested for a multiple treatment scheme. The results show an increased efficiency after a three day (days 1, 5, and 9) treatment.

With regard to the effect of the absolute configuration of the diamines, the compounds with diamines of *R*-, or *R,R*-absolute configuration are usually slightly more active at lower doses, but also slightly more toxic than the corresponding derivatives of the *S*- or *S,S*-diamines. In fact the T/C% values of the former isomers diminish more rapidly than those of the latter, as the doses increase. These differences however, are only marginal and not easily detected. Moreover we have observed rather small differences even for the enantiomeric chxn complexes, in contrast with an early report on their different activities toward both P388 and L1210 [9, 10]. We have also repeated the test on this latter tumor system without finding any significant difference between the various isomers of the chxnPt type compounds (Table III).

Conclusions

The differences, if any, in the activity of the enantiomeric couples of cisplatin analogues reported in this paper are in agreement with some published observations on the antitumor activities of another enantiomeric couple of anticancer compounds, *i.e.* the two optical isomers of cyclophosphamide [11–13].

It appears therefore that no or very little chiral discrimination is operative in the tumor cell–drug interaction. In the case of the chiral cisplatin analogues here described we have recently shown that the absence of such a discrimination exists also at the DNA level [7]. To our knowledge no other extended study of this type on other chiral antitumor drugs and their interaction with DNA has appeared in the literature. In the absence of other data, therefore, it may be that factors such as drug transport, drug uptake, or metabolism, are relevant to the observed absence of chiral recognition.

In the case of cisplatin, however, our reported study [7] suggests that cisplatin induces such an alteration of the secondary structure of DNA that it destroys its helical feature (at least at a local level) making any chiral recognition impossible. The fact that for intercalating substances a certain degree of stereoselectivity seems to be operative [8, 28] is a reflection of the different mechanism of action of molecules that bind to DNA in a covalent fashion.

Acknowledgements

The support of the Italian C.N.R. is gratefully acknowledged (Piano Finalizzato Chimica Fine e Secondaria, no. 80.02079.95). Potassium chloroplatinite was a generous loan from the C.N.R. Centre for the Synthesis and Structure of Compounds of Transition Metals in Low Oxidation States.

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