The Cytostatic Activity of a Five-coordinate Pt(II) Complex: Preliminary Results*

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Abstract

The major interest in platinum(II) complexes as anticancer drugs is still centered at present on square complexes and mainly on cisplatin. Since it was observed that the nature and/or the arrangement of the ligands can dramatically affect the relevant steps (such as crossing the cell membrane, hydrolytic processes and intrastrand DNA crosslinking) of the action and the metabolism of the drug, we were prompted to probe the cytostatic activity of the more unusual 18e bpt complexes. We examined the behavior of PtCl(Me)(maleic acid)(*o*-$MeC_5H_4NCH{=}NMe$), which is soluble in water at pH 6.8. For this complex previous experiments showed a toxicity comparable to that of cisplatin towards the lymphoid system in rat embryos. The complex was prepared by a procedure similar to that used for similar dichloro complexes by exchange of maleic acid with the corresponding ethylene complex. An *in vitro* system which has proved very interesting is that of neuroblastoma cells in culture. Neuroblastomas are malignant tumors with reduced sensitivity to common treatments. Then, the study of the control mechanisms of the cellular differentiation gives the possibility of transferring to the therapy the results obtained *in vitro*. The effect of the five-coordinate complex was compared with that of cisplatin by treatment of murine neuroblastoma cells 41A3 for 24 h at a concentration of 10^{-6}–10^{-7} M. A similar cell growth inhibition was observed with both the compounds: these results were also confirmed by the inhibition of ^{3}H-thymidine incorporation into cellular DNA.

The data obtained indicate that a systematic exploration of the cytostatic activity of five-coordinate Pt(II) complexes is of potential interest.

Introduction

Recent literature shows that most of the platinum complexes currently investigated with respect to antitumoral activity are square-planar Pt(II) species and also Pt(IV) complexes. We are not aware of studies on five-coordinated Pt(II) complexes, which can be isolated through a suitable choice of the ligand environment [1]. Several reasons led us to extend the study of antitumoral activity to the latter compounds. Consequently, we note:

(i) The search to find safer and more effective platinum complexes than cisplatin (*cis*-dichlorodiammineplatinum(II)) is still stimulating since so far no significant results have been achieved. At any rate, it has been found that even small variations in the structure of the complexes can induce dramatic changes in their biological activity. Taking into account that the reactivity of the coordinatively saturated five-coordinated complexes is substantially different from that of the square 16e species, it is reasonable to expect substantial differences in pharmacological properties.

(ii) Even though notable advances [2] have been made in the investigation of cisplatin analogs, the mechanistic features of their activity are still obscure. A comparison of the properties of the five-coordinated complexes with those of the square compound can help to clarify some aspects of the interaction with biological substrates. In addition, it should be considered that transient five-coordination is often attained in reactions of square Pt(II) complexes. Thus, it is reasonable to think that five-coordinated species can be involved in the biological pathway of these drugs.

(iii) Preliminary tests of toxicity made on duck [3] and rat [4] embryos showed that the five-coordinated complexes could have a lower or at least comparable toxicity to that of cisplatin.

In this work we report the synthesis of the five-coordinated complex {PtClMe[(Z)–HOOC·CH=CH·COOH)][(6-Mepy–$CH{=}NCH_3$)]} (A), and on its activity towards nervous tumoral cells, *i.e.*, murine neuroblastoma 41A3 [5].

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Experimental

Tissue culture media, fetal calf serum and phosphate buffered saline were obtained from Flow, HEPES was obtained from Sigma, methyl-^3H-thymidine (41 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, U.K.

Synthesis of Complex A

The ethylene complex corresponding to **A** was prepared as previously described from $[PtClMe(C_2H_4)]_2$ and 6-Mepy-2-CH=NMe. The synthesis of **A** was performed by adding an excess of maleic acid to a solution of the ethylene complex in acetone/methanol (1/2). After standing 24 h at room temperature a crop of yellow crystals precipitated. After filtering, washing with methanol and drying, the crude product was obtained in 86% yield. Analytical data proved this material to be nearly pure. *Anal.* Calc. for $C_{13}H_{17}ClN_2O_4Pt$: C, 31.49; H, 3.46; N, 5.65. Found: C, 31.87; H, 3.56; N, 5.42%. Recrystallization can be made from acetone. The ^1H NMR spectrum (270 MHz, acetone-d^3) displays two different sets of signals having unequal intensity for each group of chemically equivalent protons. This is consistent with the presence in solution of two conformers in about a 3:2 ratio, which are not rapidly interconverted by free rotation around the Pt–olefin bond.

Conformer **1** (40% abundance), δ: 9.40 (q, N=CH, $^4J_{HH}$ = 1.5 Hz, $^3J_{HPt}$ = 56 Hz); 8.19 (d, C(4)H); 8.05 (t, C(5)H); 7.87 (d, C(3)H, $^4J_{HPt}$ = 9 Hz); 4.33 (d, CH=C, $^2J_{HPt}$ = 72 Hz); 4.23 (d, C=CH, $^2J_{HPt}$ = 80 Hz); 4.02 (d, N–CH_3, $^4J_{HH}$ = 1.5 Hz, $^3J_{HPt}$ = 24 Hz); 3.07 (s, C–CH_3, $^4J_{HPt}$ = 5 Hz); 0.24 (s, Pt–CH_3, $^2J_{HPt}$ = 68 Hz).

Conformer **2** (60% abundance), δ: 9.36 (q, N=CH, $^4J_{HH}$ = 1.5 Hz, $^3J_{HPt}$ = 56 Hz); 8.15 (d, C(4)H); 8.05 (t, C(5)H); 7.87 (d, C(3)H, $^4J_{HPt}$ = 9 Hz); 4.05 (d, N–CH_3, $^4J_{HH}$ = 1.5 Hz, $^3J_{HPt}$ = 24 Hz); 3.45 (d, CH=C, $^2J_{HPt}$ = 80 Hz); 3.22 (d, C=CH, $^2J_{HPt}$ = 70 Hz); 3.02 (s, C–CH_3, $^4J_{HPt}$ = 5 Hz); 0.36 (s, Pt–CH_3, $^2J_{HPt}$ = 68 Hz).

Cells and Media

Murine 41A3 neuroblastoma cells were grown in a monolayer in Dulbecco's modified Eagle medium supplemented with $NaHCO_3$ (1.2 g/l), HEPES buffer 5 g/l, penicillin (50 units/ml), streptomycin (50 μg/ml) and 5% fetal calf serum. The average doubling time under these conditions was approximately 20 h. The plating efficiency was about 50%.

Growth Inhibition Studies

All growth studies were performed on 41A3 cells in logarithmic growth. The cells (1×10^4) were plated in 60 mm Corning plastic dishes and incubated at 37 °C in an atmosphere of 5% CO_2–95% air, and treated 24 h later with the compounds indicated. The cells were counted with a Burker hemocytometer 48 h after treatment. The percentage of growth inhibition was determined and the ID_{50} values, the drug concentration that decreases the growth rate to 50% of the control, were deduced graphically.

Cytotoxicity Assay

Cell survival after drug exposure was determined by cloning cells after treatment for 24 h. The colonies formed 7 days after treatment were fixed and stained with 50% methanol and methylene blue (5 g/l) for 10 min. The colonies were counted with an illuminating lamp.

DNA Synthesis

41A3 Cells were plated in Costar 24 well tissue culture plates (1×10^4 cells per well) on a tissue culture cover slip and incubated as previously described. After a preincubation of 24 h the cells were treated for 24 h with the compounds, followed by a 4 h pulse with methyl-^3H-thymidine (41 Ci/mmol). The incubation was carried out with 250 μl of medium, containing 5% dyalized fetal calf serum, and 0.25 μCi methyl-^3H-thymidine for each well. After the pulse the cells on the cover slip were washed twice with PBS and DNA was precipitated with cold 10% TCA and washed with cold 5% TCA. Incorporated radioactivity was determined by counting in a Model LS 8100 Beckman liquid scintillation system. The experiments were performed in triplicate.

Results and Discussion

Complex A

Complex **A** was synthesized by exchange of the unsaturated ligand from the corresponding ethylene compound.

The choice of this compound for the preliminary study was based on the following grounds. Typical environments able to stabilize a five-coordinated Pt(II) complex include an olefin, a bidentate $\widehat{N\ N}$ ligand having suitable steric requirements and two chloride (or one chloride and one methyl) ligands [1]. We have chosen maleic acid as the unsaturated ligand, since its presence allows a moderate solubility of the complex at physiological pH values. In fact, lack of solubility can be a prohibitive handicap in the use of the drug. The presence of the methyl group in **A** promotes substitution reactions at the opposite apical position, with retention of the coordination number. Finally, the $\widehat{N\ N}$ ligand* is apt to stabilize five-coordination [1].

*Similar ligands were used in the synthesis of complexes demonstrating antineoplastic activity; see for instance ref. 6.

TABLE I. Effect of PtCl(Me)(Ins)(N^N) and Cisplatin on Cell Growth[a]

Treatment	Concentration (μM)	Inhibition (%)
PtCl(Me)(Ins)(N^N)	0.1	10
	0.5	13
	1.0	20
	5.0	22
	20.0	30
	40.0	39
	60.0	55
	80.0	73
Cisplatin	0.1	13
	0.5	27
	1.0	34
	2.0	42
	4.0	55
	6.0	63

[a]41A3 cells were treated 24 h after plating and counted 48 h after treatment.

TABLE II. Effect of PtCl(Me)(Ins)(N^N) and Cisplatin on Cytotoxicity[a]

Treatment	Concentration (μM)	Cell death (%)
PtCl(Me)(Ins)(N^N)	0.1	8
	0.5	23
	1.0	22
	5.0	32
Cisplatin	0.1	15
	0.5	40
	1.0	73

[a]Cells were incubated for 24 h with drugs and clones formed were counted after 168 h.

Growth Inhibition Studies

The percentage of growth inhibition is shown in Table I. At lower concentrations, ranging between 0.1–1 μM, the effect of PtCl(Me)(Ins)(N^N) is comparable with that of cisplatin. At higher concentrations cisplatin gives a higher inhibition than the other compound. The ID_{50} value is 3.5 μM for cisplatin and 50 μM for PtCl(Me)(Ins)(N^N). However this compound still produces a substantial inhibition at higher concentrations.

Cytotoxicity Assay

The ability of the cells treated with the two compounds to form clones was tested and results are shown in Table II. The efficiency of the two drugs is similar at lower concentrations, while an increase in concentration does not cause a corresponding increase of the cellular death with the compound PtCl(Me)(Ins)(N^N). If we compare the cytotoxicity *versus* the growth inhibition we observe that the cytotoxicity is lower with the five-coordinate complex (at 1 μM).

TABLE III. Effect of PtCl(Me)(Ins)(N^N) and Cisplatin on DNA Synthesis[a]

Treatment	Concentration (μM)	Radioactivity (%)	
		24 h	48 h
PtCl(Me)(Ins)(N^N)	0.1	100	93
	0.5	96	85
	1.0	85	83
Cisplatin	0.1	100	91
	0.5	96	69
	1.0	85	69

[a]Cells treated 24 h after plating were incubated, at indicated times, for 4 h with 0.25 μCi of methyl-^3H-thymidine (41 Ci/mmol).

DNA Synthesis

The results, summarized in Table III, show that the activity of the two compounds is similar.

Finally we can say that the effect of complex **A** is comparable to that of cisplatin; however the cytotoxicity is lower with the five-coordinate complex.

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