Chemical Properties of Rare Platinum Metal Complexes Having Antitumor Activity*

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Abstract

After the discovery of the antineoplastic activity of *cis*-$[PtCl_2(NH_3)_2]$ · (cisplatin) several transition metal complexes have been synthesized and tested with the aim of developing new antitumor agents with comparable activity and reduced host toxicity. Some rare platinum metal complexes seem to possess such properties; their chemical behavior has been compared and related to their biological activity. In particular, the latest results on the antitumor activity of Ru(III) and Ru(II) compounds is reported and their future perspectives emphasized.

Introduction

After the discovery of the antineoplastic activity of *cis*-$[Pt(II)Cl_2(NH_3)_2]$ (cisplatin) [1], several Pt(II) derivatives have been synthesized and tested with the aim of developing new drugs having more pronounced antitumor activity and reduced host toxicity [2].

Meanwhile, the mechanism of action of the complex has been studied in detail and in particular its interactions with DNA, which seems to be the main cellular target. The platinum complex was found to bind to DNA mainly through two *cis*-coordination sites and, since the corresponding *trans*-isomer is practically inactive, this interaction seems to be responsible for its activity [3–5].

Although up to now only 3 out of more than 1500 platinum derivatives tested have reached the stage of clinical trials [6], recent work shows that this number is still growing [7, 8]. As a consequence, it is worthwhile to extend the screening to transition metals other than platinum. In this field, particularly in the early stage, the study of the antitumor activity of row II and III transition metal complexes has been carried out keeping in mind the main chemical features of cisplatin, that is: square-planar geometry; two *cis* leaving groups (Cl^-); good stability of the metal–nitrogen bond; ligands such as Cl^- and NH_3; neutrality of the complex; good stability of the oxidation state; a given substitution rate. Of course these features are responsible for the activity of the complex; some of them concern in particular its interactions with the cellular target (DNA), while others are important in the stage of reaching the target itself.

This paper will deal with the rare platinum metal complexes (Ru, Os, Rh, Ir); initially, those compounds will be examined which have as many chemical features as possible in common with cisplatin.

Results

In Fig. 1 some examples of chloro-ammine complexes of Ru, Os, Rh and Ir, in both their *cis*- and *trans*-configurations, are reported; they can be anionic, neutral or cationic in nature, according to the Cl^-/NH_3 ratio and to the metal oxidation state. Although these complexes have octahedral geometry, most of them have in common with cisplatin two Cl^- anions in the *cis* position so that they could interact with DNA in a way similar to the platinum(II) derivative [4]. Moreover, such chloro-ammine derivatives generally form very stable metal–nitrogen

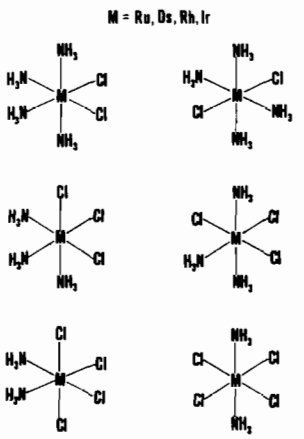

Fig. 1. Examples of octahedral chloro-ammine complexes in the *cis*- and *trans*-configurations.

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bonds and the species $[M(II)Cl_2(NH_3)_4]$ and $[M(III)Cl_3(NH_3)_3]$ are neutral. On the other hand, these compounds can exist in at least two oxidation states of comparable stability (e.g., Ru(II), Ru(III)) and, unlike the Pt(II) derivatives (being d^5 and d^6 low-spin complexes), they are generally very inert toward substitution and therefore should be biologically inactive; an inert complex in fact will react very slowly, or not at all, with the various cellular components.

The stereochemical differences between *cis*- and *trans*-isomers, which play a very important role in the biological interactions of square-planar complexes (in this case the substitution reactions are stereospecific), may be found to be not essential in the case of octahedral derivatives; in fact, assuming a dissociative path, with formation of the same trigonal bipyramidal intermediate from the two isomers, no difference will result at the biological level between the *cis*- and *trans*-starting species.

On the other hand, if the incoming ligand attacks stereospecifically the trigonal bipyramid or if the intermediate has square-pyramidal geometry, the substitution reactions will be stereospecific as in the case of square-planar species; in this case different biological behavior between the two starting stereoisomers is to be expected. Moreover, when the metal atom becomes a chiral center, as in the case of the *cis*-$[M(chel)_2Cl_2]$ complexes (chel = bidentate chelating ligand), the reaction would proceed also with retention of configuration and, since DNA is a chiral target, different biological behavior between the Λ and Δ optical isomers will be observed.

On this subject it has been reported recently [9] that the Λ isomer of the *cis*-$[Ru(phen)_2Cl_2]$ complex (phen = 1,10-phenanthroline) selectively interacts with B-DNA forming covalent bonds, while the other one (Δ) practically does not react. The high selectivity observed may be due to the close interaction between the chiral metal center and the chiral target (DNA); such a favorable situation cannot be achieved in the case of square-planar complexes.

Since the inert complexes should be biologically inactive, first of all it should be experimentally verified whether or not the d^5 and d^6 low-spin octahedral complexes show some biological activity and then, in a further stage, their possible antineoplastic activity should be evaluated.

In the case of cisplatin the discovery of its antitumor properties was based on the observation that the complex induced filamentous growth in strains of *Escherichia coli* [1]; likewise this microbiological property has been chosen here to compare the biological activity of a series of rare platinum metal octahedral complexes. Table I reports the values of filamentous growth induced by complexes bearing the following ligands: Cl^-, H_2O and Cl^-, NH_3 and Cl^- [10], dimethylsulfoxide (DMSO) and Cl^- [11], pyridine (py) and Cl^- [10].

The row III metal anionic chloro derivatives are bacteriocidal, including the platinum one, while the complexes of Rh(III) and Ru(III) induce filamentous growth, the Rh(III) derivative being the most active; a similar trend is also found with the chloro-aqua complexes, where the ruthenium derivative is inactive; however going from the chloro-aqua to the chloro-ammine derivative (*fac*-isomer) a filamentous growth close to 100% is observed, very similar to that induced by cisplatin. Therefore, in the case of ruthenium, a sharp increase in activity is obtained by the substitution of water with ammonia.

While no data can be found on the corresponding Rh(III) and Os(III) derivatives, the $[IrCl_3(NH_3)_3]$ complex is inactive. Finally the derivative of Ru(II) with DMSO and that of Rh(III) with pyridine both induce filamentous growth similar to cisplatin.

These data clearly point out that most of the systems examined are biologically active and that their activity (the induction of filamentation) depends on the nature of both the metal and the ligands; besides cisplatin, the ruthenium and rhodium derivatives show some promising features and therefore deserve to be tested as antitumor agents.

Of all the chloro-ammine derivatives of Ru and Rh tested, the neutral species *fac*-$[RuCl_3(NH_3)_3]$ [12] and *mer*-$[RhCl_3(NH_3)_3]$ [10] give the best antineoplastic activity. The former shows an activity

TABLE I. Effect of Transition-Metal Complexes in Producing Elongation in *Escherichia coli*[a]

Complex	Elongation (%)	Complex	Elongation (%)
$(NH_4)_3[RuCl_6]$	10	*fac*-$[RuCl_3(NH_3)_3]$	100
$(NH_4)_3[RhCl_6]$	75	*mer*-$[RhCl_3(NH_3)_3]$	?
$(NH_4)_2[OsCl_6]$	bacteriocidal	$[OsCl_3(NH_3)_3]$	?
$(NH_4)_2[IrCl_6]$	bacteriocidal	*mer*-$[IrCl_3(NH_3)_3]$	inactive
$(NH_4)_2[PtCl_6]$	bacteriocidal	*cis*-$[PtCl_2(NH_3)_2]$	100
$RuCl_3$(aq)	inactive	*cis*-$[RuCl_2(DMSO)_4]$[b]	100
$RhCl_3$(aq)	75	*trans*-$[RhCl_2(Py)_4]^+$	85

[a]See ref. 10. [b]See ref. 11.

TABLE II. Antitumor Activity (P388 leukemia) of Ru(III) Derivatives with Different *N*-donor Ligands

Complex	Therapeutic dose (mg/kg)	I.L.S. (%)	Toxic dose (mg/kg)
fac-$[RuCl_3(NH_3)_3]$	50	189	100
$ImH^+[RuCl_4(Im)_2]^-$	150	250[a]	500
cis-$[PtCl_2(NH_3)_2]$	8	150[a]	

[a]Direct comparison. Im = imidazole. For more details see ref. 13.

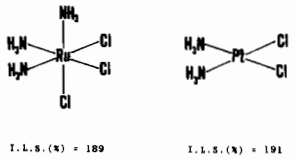

Fig. 2. Structure and antitumor activity (P388 leukemia) of *fac*-$[RuCl_3(NH_3)_3]$ and *cis*-$[PtCl_2(NH_3)_2]$.

as good as cisplatin towards P388 leukemia (I.L.S. (%) = 189 *versus* 191) (Fig. 2) while the latter is remarkably active towards sarcoma 180, reducing the tumor growth to 17% with respect to controls [10].

Using imidazole instead of ammonia, a ligand more able than NH_3 to stabilize Ru(II) with respect to the Ru(III) oxidation state, a ruthenium complex is obtained which is more active than *fac*-$[RuCl_3(NH_3)_3]$ (and therefore also than cisplatin) towards P388 leukemia [13]. This anionic complex, $[RuCl_4(Im)_2]^-ImH^+$ (Im = imidazole), has octahedral geometry with the two imidazole molecules *trans* to each other and the four Cl^- anions on the equatorial plane [13].

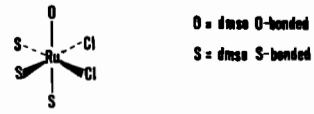

Fig. 3. Schematic structure of *cis*-$[RuCl_2(DMSO)_4]$.

As can be seen from Table II this complex, in the case of mice bearing P388 leukemia, gives an increase in life span of up to 250%, to be matched with 189% given by *fac*-$[RuCl_3(NH_3)_3]$ and 150% given by cisplatin (direct comparison); moreover this Ru(III)–imidazole derivative is fairly active (more active than cyclophosphamide) in reducing the primary tumor growth of mice bearing B16 melanoma (13% with respect to the controls) [13].

The use of dimethylsufoxide as a ligand remarkably stabilizes the +2 oxidation state of ruthenium [14]. The activity of the *cis*-$[Ru(II)Cl_2(DMSO)_4]$ complex will now be examined; its structure is schematically reported in Fig. 3. This ruthenium derivative [15, 16] has octahedral geometry with the two chlorine atoms in the *cis*-position; three out of the four DMSO molecules are bonded through the sulfur atom in a facial configuration, while the

TABLE III. Effects of *cis*-$[PtCl_2(NH_3)_2]$ and *cis*-$[RuCl_2(DMSO)_4]$ on Primary Tumor Growth and on the Formation of Spontaneous Metastases in Mice Bearing Lewis Lung Carcinoma

Complex	Daily dose (mg/kg)	Primary tumor weight	Lung metastases	
			number	weight
Controls		100	100	100
cis-$[PtCl_2(NH_3)_2]$	0.52[a]	43	30	25
cis-$[RuCl_2(DMSO)_4]$	610[a]	32	36	21

[a]Equitoxic dosages ($LD_{0.05}$). For more details see ref. 18.

TABLE IV. Effects of *cis*-$[PtCl_2(NH_3)_2]$ and *cis*-$[RuCl_2(DMSO)_4]$ on Primary Tumor Growth and on Survival Times of Mice Bearing B16 Melanoma and MCa Mammary Carcinoma[a]

Tumor line	Complex	Daily dose (mg/kg)	Primary tumor weight	I.L.S. (%)
B16 melanoma	Controls		100	100
	cis-$[PtCl_2(NH_3)_2]$	0.52	76	124
	cis-$[RuCl_2(DMSO)_4]$	610	37	109
MCa mammary carcinoma	Controls		100	100
	cis-$[PtCl_2(NH_3)_2]$	0.52	104	94
	cis-$[RuCl_2(DMSO)_4]$	610	55	125

[a]For more details see ref. 18.

TABLE V. Summary of the Toxicity of *cis*-$[PtCl_2(NH_3)_2]$ in Comparison with *cis*-$[RuCl_2(DMSO)_4]$ in Animal Models[a]

Complex	Spleen	Bone marrow	Intestinal mucose	Blood
cis-$[PtCl_2(NH_3)_2]$	+ + + +	+ + +	+ + +	+ +
cis-$[RuCl_2(DMSO)_4]$	+ +	–	+	–

[a] + = toxicity; – = lack of toxicity. For more details see ref. 18.

last one is bonded through the oxygen atom [17]. Table III reports the activity of the complex towards Lewis lung carcinoma, a metastasizing tumor of the mouse. These results clearly show that, using equitoxic dosages (the ruthenium derivative is considerably less toxic than the platinum one), the *cis*-$[RuCl_2(DMSO)_4]$ complex has an activity similar to that of cisplatin towards both primary tumor growth (32% *versus* 43%) and metastases formation [18].

In the case of two other murine metastasizing tumors, B16 melanoma and MCa mammary carcinoma (Table IV), the ruthenium derivative shows an activity greater than cisplatin, especially towards primary tumor growth [18]; furthermore, in mice bearing the B16 melanoma, when the treatment is followed by surgical amputation of the primary tumor, the number cured rises from 8% for the controls to 20% for animals treated with cisplatin and up to 36% for those treated with *cis*-$[RuCl_2(DMSO)_4]$.

Finally, as shown in Table V, the ruthenium complex clearly has a less pronounced toxicity than cisplatin [18].

Discussion

From the results reported it can be seen that, in spite of the few complexes tested at present, the antitumor activity shown by ruthenium derivatives is very similar to that of cisplatin; moreover, on modifying the nature of the ligands, there is a strong possibility of obtaining more active systems, in particular by tuning the redox potential of the Ru(III)/Ru(II) couple and using pure enantiomers when the metal is a chiral center (the importance of this latter aspect has already been mentioned).

The use of pure optical isomers is however useless if the species undergo a fast racemization reaction; in this case it is possible to stabilize the chiral information on the metal by introducing a second chiral center, configurationally stable, on the non-labile ligands. By varying the nature of the substituents at this second chiral center it should be possible to shift the diastereoisomeric equilibrium towards one or other of the two isomers (Fig. 4) [19], which should be tested in turn. Finally, the hypothesis has been put forward, in particular by Clarke [12],

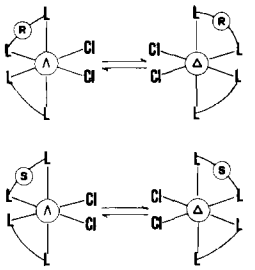

Fig. 4. Diastereoisomeric equilibria of octahedral complexes having a chiral center on the metal and a second one on a ligand.

that the Ru(III) amino derivatives, before interacting with the cellular components, would be reduced to the corresponding Ru(II) derivatives, so that the Ru(III) complexes should be considered as prodrugs. Such a hypothesis is based on the fact that, as already reported in this paper, the Ru(III) derivatives are usually inert and consequently inactive, while the corresponding Ru(II) derivatives are labile and therefore active. For example, substitution of an NH_3 molecule in the hexa-amine complexes of Ru(III) and Ru(II) takes place with half-lives ($t_{1/2}$) of 1.6 years and 1 day, respectively [12]. Therefore, when an inert complex is introduced into an organism, it could stay essentially unchanged, and therefore fairly innocuous, until it is reduced to the corresponding labile Ru(II) species, which interact with the target and so induce toxicity [12].

The use of Ru(III) derivatives as prodrugs, a concept applicable also to other metal redox couples, could have further advantages: (a) it would allow the drug to reach the target in higher concentrations, with consequent greater efficacy and lower toxicity; (b) the complex would selectively attack the solid hypoxic tumors, since Ru(III) would be more easily reduced inside the tumor than in normal tissues.

Conclusions

On this basis it is reasonable to assess that the use of rare platinum metal complexes, in particular of octahedral ruthenium derivatives, could give rise to drugs remarkably more active and less toxic than cisplatin.

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