Antitumor Complexes of Platinum with Carrier Molecules. I. Sulfadiazine Derivatives of Platinum(II)

ALESSANDRO PASINI* and ERIK BERSANETTI

Dipartimento di Chimica Inorganica e Metallorganica, The University, 20133 Milan, Italy

FRANCO ZUNINO* and GIUSEPPINA SAVI

Divisione di Oncologia Sperimentale B, Istituto Nazionale per lo Studio e la Cura dei Tumori, 20133 Milan, Italy

Received March 8, 1983

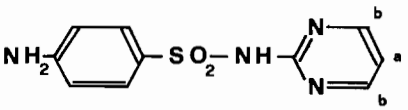
Platinum(II) complexes of sulfanilamidopyrimidine (sulfadiazine, sdH) of formula cis-*[PtCl*$_2$*-(sdH)*$_2$*] and* cis-*K[PtCl*$_2$*sd] (sd = sulfadiazinate anion) were prepared and characterized through IR and* ^{1}*H NMR spectroscopy. The compounds were found cytotoxic* in vitro *to HeLa cells and* cis-*K[PtCl*$_2$*sd] showed antitumor activity against P388 leukemia. To our knowledge this is the first anionic Pt complex with such an activity. Although the potency of these compounds is lower than that of* cis-*dichlorodiammineplatinum(II), they are much less toxic when administered i.p. However the difficulty of parenteral administration of high doses precluded further* in vivo *tests.*

Introduction

cis-dichlorodiammineplatinum(II) (*cis*-DDP) is the prototype of a new class of cytotoxic agents and plays a significant role in the chemotherapy of human cancer [1, 2]. A large number of platinum compounds have been synthesized and evaluated in an effort to identify compounds with increased antitumor effectiveness and/or decreased toxicity [3–5]. As for most antitumor agents, these compounds lack antitumor selectivity; thus damage to sensitive normal host tissues (in particular nephrotoxic effects [2]) is the main limitation in therapeutic use.

In order to overcome this drawback, it has been proposed to bind low molecular weight carriers to antitumor agents, in an attempt to alter drug uptake in a selective way [6]. The relatively high uptake of sulfanilamidopyrimidine (sulfadiazine, (*I*) hereafter sdH) in tumors suggested the use of this compound as a potential carrier molecule [7].

This report describes the synthesis and the biological activity of two platinum complexes of sulfadiazine.



(*I*)

Experimental

Elemental analyses were from the microanalytical laboratory, the University, Milan. Infrared spectra were recorded, in the FT mode, on a Nicolet MX1E instrument as KBr pellets or, in the 600–200 cm^{-1} range, as nujol mulls with CsI windows or polyethylene pellets. ^{1}H NMR spectra were recorded on a Brucker WP80 in deuterated dimethylsulfoxide with tetramethylsilane (Me_4Si) as internal reference. A Philips conductimeter PR9500 was used for conductivity measurements.

Reagent grade chemicals were used throughout.

Preparation of the Complexes

cis-*Dichlorodiammine platinum(II)*

This was synthesized by a standard method [8], and crystallized three times from 0.05 *N* HCl. For biological activity evaluation, the drug was dissolved immediately before use in a small volume of DMSO (typically 20 mg in 0.1 ml) and diluted immediately with distilled water or saline solution, to a final DMSO concentration lower than 5%.

*Authors to whom correspondence should be addressed.

0020-1693/83/$3.00

cis-*Dichlorodisulfadiazineplatinum(II)*

A DMF solution of sulfadiazine (2 mmol in 30 ml) was added to a DMF solution of *cis*-$[PtCl_2(CH_3CN)_2]$ [9] (1 mmol in 5 ml) and heated in the dark at 80 °C overnight. The brown solution was taken to dryness under reduced pressure and the residue was washed with ethanol and water. *Anal.*: found, %: C, 30.0; H, 2.9; N, 14.0; $C_{20}H_{24}N_8O_6S_2Cl_2Pt$ ($PtCl_2(sdH)_2 \cdot H_2O$) requires, %: C, 29.9; H, 2.9; N, 13.9. The compound was dissolved in DMSO prior to use.

Potassium cis-*dichlorosulfadiazinatoplatinate(II)*

This preparation was performed under a nitrogen atmosphere. 1 mmol of sulfadiazine was treated with 10 ml of 0.1 *N* KOH, and the resulting solution was added to a solution of 1 mmol of K_2PtCl_4 in 5 ml of water. Some sulfadiazine precipitates, and the resulting slurry was stirred for 6 h in the dark. The pH was raised to 7 with 0.1 *N* KOH (usually ~2 ml) and the solution was left overnight; it was then concentrated under reduced pressure, treated with methanol, and filtered. *Anal.*: found, %: C, 22.6; H, 2.2; N, 9.4. $C_{11}H_{13}N_4O_3SCl_2PtK$ ($KPtCl_2sd \cdot CH_3OH$) requires, %: C, 22.5; H, 2.2; N, 9.6. This compound is slightly soluble in water, but it dissolves in a pH 7 buffer, from which, however, it precipitates on long standing. Stock solutions for biological tests were prepared by diluting the reaction mixture to the appropriate volume with distilled water. These solutions were stable for 20 days when stored in the dark and under nitrogen.

Animals, Tumors, and Antitumor Testing

BDA/2 and BDF1 adult mice of both sexes were obtained from Charles River Laboratories (Calco, Como, Italy). After arrival, the animals were acclimated for about a week and then randomized into experimental groups. Tumor lines were maintained as previously described [10]. The experiments with P388 leukemia were carried out in BDF1 mice inoculated i.p. with 1×10^6 cells per mouse. Drug effects on HeLa cells survivals were determined as previously reported [10].

In the antitumor testing experiments, animals were observed daily. Comparative antitumor effects of various dosages and schedules were determined from the median survival time (MST) in days for treated (T) *versus* control groups (C) and expressed as T/C (%).

Results and Discussion

Preparation and Characterization of the Complexes

Sulfadiazine complexes are known for many metals [11], but to the best of our knowledge no platinum derivative has ever been reported.

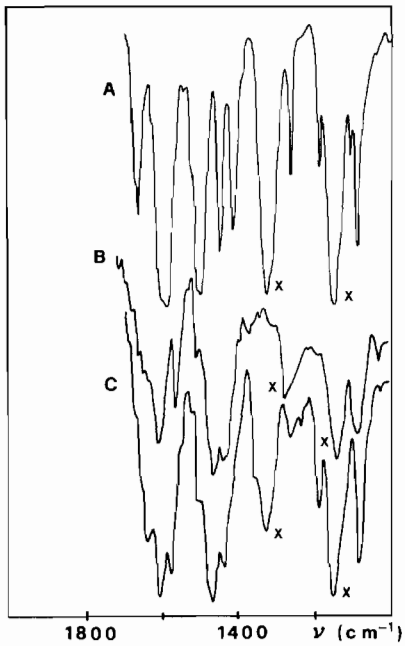


Fig. 1. Infrared spectra in the 1800–1000 cm^{-1} region (KBr pellets) of: A, sdH; B, *cis*-K[$PtCl_2$sd], and C, *cis*-[$PtCl_2(sdH)_2$]. The bands marked with x are due to the SO_2 group [11].

We have prepared two platinum derivatives of sulfadiazine: a neutral compound of formual *cis*-$[PtCl_2(sdH)_2]$ and an anionic derivative of the sulfadiazinate anion, *i.e.* *cis*-K[$PtCl_2$sd].

The compound *cis*-$[PtCl_2(sdH)_2]$ was obtained by reaction of *cis*-$[PtCl_2(CH_3CN)_2]$ and sulfadiazine. During substitution of the acetonitrile moieties the *cis* configuration is retained, as shown by the appearance, in the IR spectrum, of two bands in the Pt–Cl stretching region [12] (322 and 341 cm^{-1}). Comparison of the ^1H NMR spectra (in d_6-DMSO) of the free ligand and the complex shows that the amide protons (δ 9.1 ppm relative to Me_4Si) are almost unaffected, whereas the resonance of the amino group (δ 5.9 in the free ligand) coalesces with the water resonance in the spectrum of the complex. Such a lability of the amino protons could suggest coordination of the amino group to platinum. However, in the IR spectrum, we observed a shift to higher frequencies of the $\nu(NH_2)$ (ν_{as}, 3480 cm^{-1} and ν_{sym}, 3380 cm^{-1}) with respect to the free ligand (3425 and 3355 cm^{-1} respectively) [11], rather than the expected shift to lower frequencies [11]. It is therefore likely that the increased acidity of the NH_2 group derives mainly from some polarization effect, which arises in the complex, rather than from direct coordination to platinum.

The SO_2 stretching vibrations in the complex appear at the same frequency as the ligand (1325 and

TABLE I. Therapeutic Effects of the Pt Derivatives against P388 Leukemia.

Compound	Dose (mg/kg)[a]	T/C (%)[b]	No. of toxic deaths/ No. of treated mice	No. of LTS[c]/ No. of treated mice
cis-$[PtCl_2(NH_3)_2]$	10[d]	242 (200–284)	0/29	5/29
cis-$[PtCl_2(sdH)_2]$	15–120	112 (105–121)	0/48	0/48
cis-K$[PtCl_2sd]$	60	115	0/10	0/10
	120	142	0/10	0/10
	270	155	0/6	0/10
	90 x 4[e]	175	0/10	0/10

[a]Single treatment i.p. on day 1, after i.p. transplantation of 10^6 tumor cells on day 0, except in [e]. [b]Median survival time of treated mice/median survival time of control mice x 100. Range in parenthesis. [c]LTS, long time survivors (>60 days). [d]Optimal dose. [e]I.p. administrations on days 1, 2, 3, and 4.

1155 cm^{-1}, see Fig. 1) and the only significant difference between the IR spectra of the complex and the free ligand appears in the 1580–1200 cm^{-1} region in the bands attributable to some vibrations of the pyrimidine ring [13] (Fig. 1). This difference is accompanied by a different pattern in the ^1H NMR spectrum of the resonances of the pyrimidine protons: both the triplet attributable to proton *a* and the doublet of protons *b* of (*I*) (δ 8.5 and 7.0 ppm respectively) become complex multiplets. This fact can be explained by coordination of Pt to one nitrogen atom of the pyrimidine moiety, which would make protons *b* no longer equivalent. Such a mode of coordination of sdH to Pt is reasonable, since Pt usually chooses the softer basic centre of a potentially multidentate ligand [14, 15]. Although chromatography on a Sephadex LH 20 column gave only one band, the untidiness of the NMR spectrum of the complex suggests the presence of some linkage isomerism. Finally the compound is non electrolyte in DMSO solution, only at high dilution is a weak conductivity observed, which is suggestive of some dissociation which was not investigated in detail.

The monosulfadiazinato derivative was obtained by reaction of Ksd with K_2PtCl_4. A solid compound of formula K$[PtCl_2sd]$ could be obtained by addition of methanol. For this compound also the *cis*-configuration was established by the presence, in the infrared spectrum, of two Pt–Cl stretching bands at 330 and 325 cm^{-1} [12]. The lowering of the SO_2 stretching frequencies (1270 and 1135 cm^{-1}), the difference in the IR spectrum of the aromatic region with respect to the free ligand (Fig. 1) and again, the complexity of the resonances of the pyrimidine protons, are in agreement with the chelation to Pt through one oxygen atom of the SO_2 group and one nitrogen atom of the pyrimidine ring [11]. This mode of coordination has also been found, through X-ray crystallography, in a silver sulfadiazine derivative [16]. Finally the ionic structure has been assigned on the basis of conductivity measurements in DMSO (Λ_M = 35 ohm^{-1} cm^2 mol^{-1} for 10^{-3} *M* solutions; reported data for 1:1 electrolytes in DMSO range from 23 to 42 ohm^{-1} cm^2 mol^{-1} at the same concentrations [17]).

TABLE II. Effect of the Pt Derivatives on Survival of HeLa Cells.[a]

Compound	ID_{50}[b]
cis-$[PtCl_2(NH_3)_2]$	0.12
cis-$[PtCl_2(sdH)_2]$	90
cis-K$[PtCl_2sd]$	150

[a]Cells were exposed to the drugs for 3 h. After treatment, the medium was removed and the cells were washed and suspended in Eagle's minimal essential medium containing 10% fetal calf serum and plated. Colonies were counted after 8 days of incubation. [b]Concentration (μg/ml) required for 50% inhibition of colony forming ability.

This compound is not stable either in the solid state or in water solution at pH 7, from which a dark brown precipitate separates upon long standing. Both this precipitate and the aged, insoluble, solid have the same elemental analysis as the starting *cis*-K$[PtCl_2sd]$ and are probably polymeric.

Biological Studies

The platinum complexes were evaluated for antitumor activity in mice bearing P388 leukemia (Table I) and compared with the activity of *cis*-DDP as a standard compound of this class of agents. Only *cis*-K$[PtCl_2sd]$ was found to be active (% T/C > 125), following single injection therapy, in the i.p. transplanted leukemia model. The percent T/C value for this derivative was somewhat increased (up to

175) when multiple treatments were used (90 mg/Kg on days 1, 2; 3; and 4). This treatment schedule was employed only for this derivative, which is the more active. The biological activity of these complexes was not shared by the ligand alone, since sulfadiazine was found to be inactive up to 300 mg/kg.

Although following multiple injection treatments the monosulfadiazinato derivative produced therapeutic effects only slightly lower than those of *cis*-DDP, both compounds were much less potent than *cis*-DDP, since the former produced maximal effects at much higher doses. The reduction in potency is accompanied by a reduction in cytotoxic activity assayed on HeLa cells (Table II). However, the lower potency *in vivo* does not parallel the much lower cytotoxicity, since the sulfadiazine complexes are more potent than expected from their low cytotoxicity (*cf.* Tables I and II).

A general property of these platinum complexes is their low toxicity, compared with *cis*-DDP. This is also accompanied, as noted above, by a reduced potency. The poor potency of these complexes precluded further *in vivo* evaluations, especially as a consequence of difficult i.v. administration of high doses of drug; thus they were not subjected to further testing.

A possible *in vivo* selective effect cannot be excluded by these experiments, however it remains to be documented by detailed pharmacokinetic studies.

Conclusions

Different approaches have been proposed to design more selective antitumor agents. The most relevant include the use of drugs that possess latent activity (prodrugs) [18] and the use of suitable carriers to afford a differential delivery of drug at the tumor cell site [6].

The limited success obtained with the sulfadiazine–platinum complexes reported here in the attempt to design more selective antitumor agents further stresses the generally recognized difficulty of the rational development of drugs with high therapeutic indices [19]. For instance, the results of a similar approach, using a sulfadiazine-mustard derivative [7], have been disappointing, since the chemical modification alters the *in vivo* distribution. It is likely that changes in the chemical and physicochemical properties of the specific carrier molecule influence the transport characteristics. Relevant to this point is the observation that the therapeutic and pharmacologic properties of the platinum complexes used in this study are appreciably different, suggesting a critical role of the chemical structure in the biological properties.

Finally the biological activity of *cis*-K[$PtCl_2$sd] is interesting since until recently the neutrality of *cis*-DDP and its analogues has often been assumed as a prerequisite for antitumor activity [3]. However some cationic antitumor complexes have been reported (see, for instance refs. 20–22); our findings show that anionic complexes also can be active.

Acknowledgements

We wish to thank the CNR Centro per la Sintesi e Struttura dei Composti dei Metalli di Transizione nei Bassi Stati di Ossidazione for a generous loan of K_2PtCl_4.

References

1 B. Rosenberg, in 'Metal Ions in Biological Systems', Vol. 11, E. Sigel, ed., Marcel Dekker, New York, 1981, p. 127.
2 M. Rozencweig, D. D. Von Hoff, R. Abele and F. M. Muggia, in 'Cancer Chemotherapy 1981', H. M. Pinedo, Ed., *Excerpta Medica*, Amsterdam, 1981, p. 120.
3 M. J. Cleare and P. C.Hydes, in 'Metal Ions in Biological Systems', Vol. 11, E. Sigel, Ed., Marcel Dekker, New York, 1981, p. 1.
4 A. W. Prestayko, J. C. D'Aoust, B. F. Issell and S. T. Crooke, *Cancer Treat. Rev., 6,* 17 (1979).
5 W. C. Rose, J. E. Schurig, J. B. Huftalen and W. T. Bradner, *Cancer Treat. Rep., 66,* 135 (1982).
6 D. S. Zahrko, M. Przybylski and V. T. Oliverio, *Meth. Cancer Res., 16,* 347 (1979).
7 C. Abel, T. A. Connors, W. C. Ross, N. H. Nam, H. Hoellinger and L. Pichot, *Eur. J. Cancer, 9,* 49 (1973).
8 G. B. Kauffman and D. O. Cowan, *Inorg. Synth., 7,* 239 (1963).
9 K. A. Hofmann and G. Brugge, *Berichte, 40,* 1776 (1907).
10 F. Zunino, F. Giuliani, G. Savi, T. Dasdia and R. Gambetta, *Int. J. Cancer, 30,* 465 (1982).
11 A. Bult, *Pharm. Week. Sci. Ed., 3,* 213 (1981).
12 J. R. Ferraro, 'Low Frequency Vibrations of Inorganic and Coordination Compounds', Plenum Press, New York, 1971.
13 G. Socrates, 'Infrared Characteristic Group Frequencies', Wiley–Interscience, New York, 1980.
14 A. Adeyemo, Y. Teklu and T. Williams, *Inorg. Chim. Acta, 51,* 19 (1981).
15 L. G. Marzilli, B. DeCastro and C. Solozzano, *J. Am. Chem. Soc., 104,* 461 (1982).
16 D. S. Cook and M. F. Turner, *J. Chem. Soc. Perkin II,* 1021 (1975).
17 W. J. Geary, *Coord. Chem. Rev., 7,* 81 (1971).
18 P. Workman and J. A. Double, *Biomedicine, 28,* 255 (1978).
19 P. J. Cox and P. B. Farmer, *Cancer Treat. Rev., 4,* 47 (1977).
20 A. B. Robins, *Chem.-Biol. Interact., 13,* 205 (1976).
21 R. E. Cramer, P. L. Dahlstrom, M. J. T. Seu, T. Norton and M. Kashiwagi, *Inorg. Chem., 19,* 148 (1980).
22 N. Farrel, *J. Chem. Soc. Chem. Commun.*, 331 (1982).