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REVIEW ARTICLE

Platinum Complexes: A New Class of Antineoplastic Agents

FRANCIS K. V. LEH and WALTER WOLF ˣ

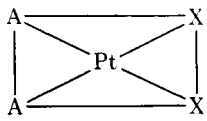
Keyphrases □ Platinum complexes—antineoplastic activity related to chemical structure, biological and toxicological effects, methods of preparation, mechanism of action, pharmacokinetics, review □ Complexes, platinum—antineoplastic activity related to chemical structure, biological and toxicological effects, methods of preparation, mechanism of action, pharmacokinetics, review □ Antineoplastic agents—platinum complexes, activity related to chemical structure, review □ Structure-activity relationships—platinum complexes, antineoplastic activity, review □ Pharmacokinetics—platinum complexes, distribution, retention, and excretion, review □ Toxicity—platinum complexes, effects in various animals, review □ Radioplatinum compounds—labeling, distribution, use, review

The potential of platinum compounds as a new class of antitumor agents was discussed in 1965 (1). Their antineoplastic activity has been demonstrated in many animal tumor-screening systems. Sarcoma 180, L-1210 leukemia, Dunning ascitic leukemia, Walker 256 carcinosarcoma, dimethylbenzanthracine (DMBA)-mammary tumors, and ascitic B-16 melanosarcoma are inhibited by these compounds. *cis*-Dichlorodiammineplatinum(II) (I) is the agent that has been studied most; as NSC-119875, it has been tested clinically in patients (2, 3). The preliminary results of a Phase I clinical trial of the National Cancer Institute (2, 4–8) are encouraging and indicate that tumor growth is inhibited in humans. These preliminary studies intensified the search for related platinum(II) compounds with better antitumor activity.

A large number of platinum complexes have been prepared and tested for their ability to cause regression of tumor cells. Their activities depend on the chemical structures of the complexes. As most such agents at chemotherapeutic dose, they are quite toxic. Therefore, assessments of their biological and toxicological effects are important. This paper reviews our present knowledge of these antineoplastic platinum compounds.

NATURE OF ANTITUMOR PLATINUM COMPOUNDS

The ability of certain platinum coordination compounds to cause regression of tumor cells depends on the chemical structures of the complexes. These active complexes can be represented as:



where A is the carrier ligand(s) [usually amines, either monodentate (NH_3) or bidentate ($NH_2CH_2CH_2NH_2$)], and X is the anionic leaving group(s) [either monodentate (Cl^-) or bidentate ($-OOCCH_2COO^-$)]. Among these complexes, certain common features appear to be required for antitumor activity.

Nature of Metal Ion—The central metal cation must be at a low oxidation state. Such a restriction is apparent in the system of platinum coordination compounds, since most active platinum compounds are square planar platinum(II). In bivalent platinum compounds, the special inertness of the platinum–ligand bond may be the key to the unique activity of platinum(II) complexes as antitumor drugs (9).

Geometry—The platinum(II) complexes have two isomers according to their geometric arrangement.

Table I—Activity of *cis*- and *trans*-$[PtA_2X_2]$

Complex	Solvent[a]	Dose Range[b], mg/kg	Dose Response[c]	Toxic Level[d], mg/kg	T/C[e]	Dose, mg/kg	Reference
cis-$[Pt(NH_3)_2Cl_2]$	S	0.5–20	+	9	1	8	11, 14, 48, 50, 92
trans-$[Pt(NH_3)_2Cl_2]$	S	2.5–40	—	740	83	2.5–40	11, 14, 48, 50, 87
cis-$[Pt(NH_3)_2Br_2]$	B	5–20	+	15	30	14	11
trans-$[Pt(NH_3)_2Br_2]$	B	10–40	—	740	110	10–40	11
cis-$[Pt(CH_3NH_2)_2Cl_2]$	S	10–30	+	10–20	25	15	11, 14
trans-$[Pt(CH_3NH_2)_2Cl_2]$	S	5–100	—	25	101	5–20	11
cis-$[Pt(C_2H_5NH_2)_2Cl_2]$	S	5–50	+	45	14	40	11
trans-$[Pt(C_2H_5NH_2)_2Cl_2]$	SS	5–20	—	720	106	5–20	11
cis-$[Pt(C_6H_5N)_2Cl_2]$	SS	5–200	—	7200	91	50–200	11
trans-$[Pt(C_6H_5N)_2Cl_2]$	SS	5–40	—	740	116	5–40	11
cis-$[Pt(NH_3)_2(SCN)_2]$	S	5–100	—	250	70	20–35	11
trans-$[Pt(NH_3)_2(SCN)_2]$	SS	20–80	—	780	73	20–80	11
trans-$[Pt(CH_2)_2NH]_2Cl_2]$			—				14, 87
trans-$[Pt(NH_2C_2H_4OH)_2Cl_2]$			—				87
trans-$[Pt(C_4H_9N)_2Cl_2]$			—				87
trans-$[Pt[(CH_3)_2SO]_2Cl_2]$			—				87
trans-$[Pt(C_6H_{11}NH_2)_2Cl_2]$			—				87

[a] S = saline, B = 0.04 *M* sodium bromide solution, and SS = slurry. [b] The dose range indicates the maximum and minimum doses administered. [c] The dose response is termed positive (+) for cases where a consistent decrease in tumor size is observed. [d] The toxic level is the highest dose at which survivors are equal to 90%. [e] The ratio of tumor weights of treated and control animals (T/C), expressed as a percentage, is a measure of the potency of the antitumor effect. The T/C values that are less than 50 are generally considered significant.

The *trans*-isomers are always inactive, whereas all active complexes found so far have the *cis*-configuration (Table I).

Nature of Leaving Group—The correlation between the nature of the leaving groups and their antitumor activity can be established from their rates of substitution. Kinetic studies (10) on the reaction $[Pt[H_2NCH_2CH(NH)CH_2NH_2]X]^+ + C_6H_5N \rightarrow [Pt[H_2NCH_2CH(NH)CH_2NH_2]C_6H_5N]^{+2} + X^-$ shows that the order of their rate of leaving for a series of X^- ligands is:

$$NO_3^- > H_2O > Cl^- > Br^- > I^- > N_3^- > SCN^- > NO_2^- > CN^-$$

highly toxic | active | inactive relatively nontoxic

This order reflects the change of activity in the animal test results (10) (Table II).

The complexes consisting of labile ligands such as nitrate ion hydrolyze rapidly, thus preventing a sufficient amount of the complex from reaching the site(s) responsible for antitumor activity (8). The hydrolysis produces the highly toxic diaquo species. On the other hand, ligands (*e.g.*, cyanide ion) that form strong bonds with platinum tend not to interact either rapidly enough or to a sufficient degree to elicit the antitumor response; complexes containing such ligands thus are expected to be inactive (11–13). The most active complexes contain either chloride, bromide, oxalate, or malonate ligands (11) (Tables III–V).

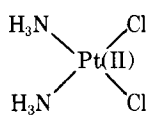
Nature of Carrier Ligand—The carrier ligand A may affect the activity of the complexes due to their difference in basicities and steric and electronic properties. In general, chemotherapeutic activity of *N*-donor atom ligands (Tables I–IV, VI, and VII) decreases along the series: $NH_3 < RNH_2 > R_2NH > R_3N$. The increase in the size of R and the substitution of the hydrogen atom by alkyl or other functional groups similarly decrease activity. As R becomes larger and more hydrophobic, the complex may become less soluble and so less effective (10).

The hydrogen bonding interactions between the amine ligands and biological receptors is believed to be important in stabilizing the receptor–drug complex. A decrease in the strength of hydrogen bonding could be related to the decrease in the activity of alkyl substitution in ammonia and ethylenediamine complexes (11). It has also been noted that toxicity is unrelated to antitumor action; substitution of the hydrogen atom by an alkyl group decreases activity but has virtually no effect in toxicity (11, 14).

The carrier ligands A found so far that are capable of producing active complexes are ammonia (I–III), ethyleneimine (IV), pyrrolidine (V), alicyclic amines (VI), cyclohexane-*trans*-1,2-diamine (VII), and *o*-phenylenediamine (VIII).

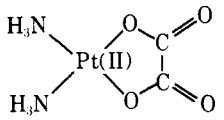
A group of pyrimidines and substituted pyrimidines constitute another series of active complexes (Table VIII). They exhibit a deep-blue color and are called generically "platinum blues," but their structures are unknown (15, 16).

Little work has been reported on carrier ligands with phosphorus, oxygen, and sulfur donor atoms



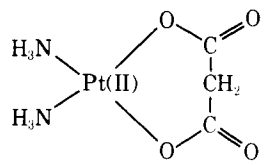
$[Pt(NH_3)_2Cl_2]$

cis-dichlorodiammine-platinum(II)
I



$[Pt(NH_3)_2(C_2O_4)]$

cis-oxalatodiammine-platinum(II)
II



$[Pt(NH_3)_2(H_2C_3O_4)]$

cis-malonatodiammine-platinum(II)
III

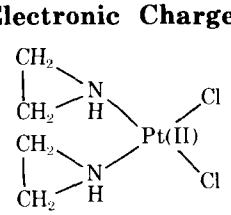
Table II—Changes in Activity on Varying X in *cis*-[PtA_2X_2]

A	X	Solvent[a]	Dose Range[b], mg/kg	Dose Response[c]	Toxic Level[d], mg/kg	T/C[e]	Dose, mg/kg	Reference
NH_3	NO_3^-	W	6–12	—	7[f]	54	6	11
NH_3	NO_3^-	S	2.5–12	+	11	8	10	11
NH_3	H_2O	W	2–20	—	5[f]			11
NH_3	Cl^-	S	0.5–20	+	9	1	8	11, 87
NH_3	Br^-	B	5–20	+	15	30	14	11
NH_3	Br^-	S	10–25	+	15	30	15	11
NH_3	Br^-	S	2–6	+	5–6	13	5	11
NH_3	I^-	WS	10–25	—	725	110	10–25	11
NH_3	SCN^-	S	5–100	—	~50	70	20–85	11
NH_3	NO	SS	5–100	—	>100	99	5–100	11
CH_3NH_2	Br^-	B	5–40	±	20–40	60	~20	11
	I^-	W	10–80	—	780	~87	10–40	11
$(CH_3)_2NH$	Br^-	BS	10–120	+	>120	~65	10–80	11
	I^-	WS	5–30	±	30	~77	5–30	11
$(CH_3)_2NH$	Br^-	BS	10–120	+	>120	~65	10–80	11
$(CH_3)_2NH$	I^-	WS	5–30	±	>30	~77	5–30	11
$C_2H_5NH_2$	Br^-	BS	20–80	—	>80	~75	20–80	11
$HOC_2H_4NH_2$	I^-	W	5–150	+	~125	~40	60–100	11
$(C_2H_5)_2NH$	Cl^-	SS	15–60	—	>60	75	60	88
					ID_{90}	LD_{50}	TI	
Ethyleneimine	Cl	Oil	2.5–10	+	2.6	56.6[g]	21.7	14, 87
Cyclopropylamine	Cl	Oil	1.25–80	+	2.8	56.6	24.6	
Cyclohexylamine	Cl	Oil	10–1250	—	12.0		>267	14, 87
Cyclohexylmethylamine	Cl			+			2.6	88
Cyclopentylamine	Cl	Oil	12.5–400	+	2.4	565.5	235.7	14, 87
2-Aminomethyl-1-cyclopentylamine	Cl			+			40.7	88
Cyclobutylamine	Cl	Oil	0.25–128	+	2.9	90[g]	31.0	14, 87
Cycloheptylamine	Cl	Oil	5–625	—	18	625[g]	34.7	87
Isopropylamine	Cl	Oil	40	+	0.9	33.5	37.2	87
Isoamylamine	Cl	Oil	12–1500	—	>12	7125	>1500	87
2-Aminohexane	Cl	Oil	12–1500	—	>12	670	55	87
Acetonitrile	Cl			—	>27.0	>1.0	27.0	87
Benzonitrile	Cl			—	>90.0	>90.0	1.0	87
1-Aziridinoethanol	Cl			—				87
Di(ethylaminoethylchloro)	Cl			—				87
Methylamine	Cl			—				87
Hexamethyleneimine	Cl			—				87
2,2′-Dichloro-*N*-methyldiethylamine	Cl			—				87
Piperidine	Cl			—				87
Cyclooctylamine	Cl			—				87
Pyridine	Cl			+	10.8	131	14.1	14, 87
N-Ethylaminopyrrolidine	Cl			+	10.8	131	14.1	14, 87
N-Ethylaminopyrrolidine	Cl			+			11.1	88
N-(2-Hydroxyethyl)-ethyleneimine	Cl			—	>90	90	1.0	14

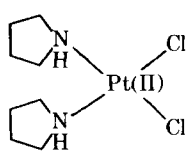
[a] W = water, S = saline, B = 0.4 *M* sodium bromide solution, WS = water slurry, SS = slurry, and BS = sodium bromide slurry. [b–e] See footnotes in Table I. [f] Highly toxic—convulsions. [g] Only 50% survivors.

(9–11, 17). The data for these complexes are listed in Table IX, with only the triphenylphosphine complex showing a marginal response. Other complexes have not shown any significant activity as antitumor agents.

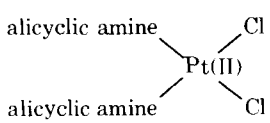
Electronic Charge—Only neutral platinum(II) complexes are known to possess antitumor activity. The charged complexes have shown no antitumor activity (18, 19) (Table X). This phenomenon may be connected with the problem of drug transport through cell membranes. The neutral species pass through the cell membranes more easily than do



$[Pt((CH_2)_2NH)_2Cl_2]$
cis-dichlorobis(ethyleneimine)-platinum(II)
IV



$[Pt((CH_2)_4NH)_2Cl_2]$
cis-dichlorobis(pyrrolidine)-platinum(II)
V



alicyclic amines=
cyclopropylamine, cyclobutylamine, cyclopentylamine, and cyclohexylamine

[Pt(alicyclic amine)$_2$Cl$_2$]
cis-dichlorobis(alicyclic amine)platinum(II)
VI

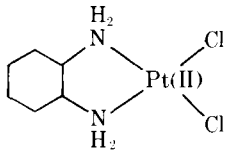
Table III—Changes in Activity on Varying X in *cis*-[$PtAX_2$]

A	X	Solvent[a]	Dose Range[b], mg/kg	Dose Response[c]	ID	TI	LD	Reference
DL-Cyclohexane-*trans*-1,2-diamine	Cl	Oil	0.3–40	+	2.1	6.9	14.1	14, 81, 89
4,5-Dimethyl-*o*-phenylenediamine	Cl	Oil		±				
2-Aminomethyl-1-cyclopentylamine	Cl	Oil	20	+	0.59	40.7	24	87
Ethionine	Cl			—	>41.0	>1.0	41.0	14, 87
Methionine	Cl			—	>300	>1.0	>300	14, 87
Cysteamine	Cl			—				87
2-Thia-4-aminobutane	I			—				87
2-Thia-4-aminobutane	Cl			—				87
Proline	Cl			—				87
2-Chloroethylamine	Cl			±	17.5	2.6	45.0	87
Edetic acid	Cl			—				87
2-Pipecoline	Cl			—				87
4-Pipecoline	Cl			—				87
Morpholine	Cl			—	>18.0	>1.0	18.0[f]	14, 87
Ethylenediamine-*N,N'*-diacetic acid	Cl			—				87
Benzimidazole	Cl			—	>9.0	>1.0	9.0[f]	14, 87
N-Ethylaminomorpholine	Cl			—				87
2-Aminoethylpyridine	Cl			—				87
1,4-Diazocyclo[2.2.2]octane	Cl			—				87
L-*N*-β-Diethylaminoethylaziridine	Cl			—				87
o-Phenylenediamine	Cl			+	2.35	20.4	48	87
					Toxic Level[d], mg/kg	T/C[e]	Dose, mg/kg	
N,N'-Dimethylethylenediamine	Br^-	BS	25–100	—	>100	83	25–100	11
	I^-	WS	25–100	—	>100	128	25–100	11
N,N-Dimethylethylenediamine	Br^-	B	20–120	±	100	49	80–90	11
N,N'-Diethylethylenediamine	Br^-	B	5–100	—	>100	75	10–100	11
N,N,N',N'-Tetramethylethylenediamine	Br^-	BS	10–80	—	>80	65	10–80	11
	I^-	WS	10–80	—	>80	100	10–80	11
	H_2O	W	2–16	±	5	25	4	11
	Cl^-	S	2.5–32	+	14	27	12	11
	Cl^-	S	1–10[g]	+	6	4	5	11
	Br^-	S	8–16	+	>16	71	16	11
	I^-	SS	5–40	—	>40	82	5–40	11
	SCN^-	W	10–40	—	>40	83	10–40	11
	NO_2	SS	14–100	—	>100	71	75–100	11

[a] BS = sodium bromide slurry, WS = water slurry, B = 0.04 *M* sodium bromide solution, W = water, S = saline, and SS = slurry. [b–e] See footnotes in Table I. [f] Only 50% survivors. [g] Daily injection for 9 days.

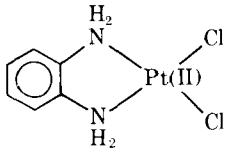
charged species. However, no definitive data on rate of membrane transfer are available.

In summary, to synthesize platinum complexes possessing antitumor activity, the following requirements are important: (*a*) the complexes must be neutral, (*b*) the central metal atom should be at the platinum(II) oxidation state, (*c*) the complex should contain a pair of *cis*-leaving groups of intermediate lability, (*d*) the complex should contain relatively inert carrier ligands, and (*e*) the complex should have good solubility and excretion rates to meet pharmacological and pharmacokinetic requirements.



[$Pt(C_6H_{10}(NH_2)_2)Cl_2$]
cis-dichloro(cyclohexane-*trans*-1,2-diamine)-platinum(II)

VII



[$Pt(C_6H_4(NH_2)_2)Cl_2$]
cis-dichloro(*o*-phenylenediamine)platinum(II)

VIII

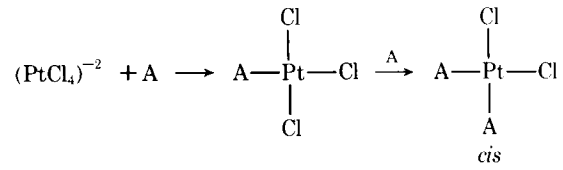
METHODS OF PREPARATION

The general methods for preparation of platinum compounds of the type *cis*-[PtA_2X_2] can be summarized in four classes:

1. Direct reaction of dipotassiumtetrachloroplatinum(II) with carrier ligand A. Because platinum belongs to a class of metals that are soft acceptors, whereas chloride ion is a fairly hard donor, this constitutes a weak metal–ligand bond (10). The chloride ion can be easily replaced by soft donors A to form new complexes (11, 14). This reaction may lead to the formation of either *cis*- or *trans*-products, depending on the *trans*-effect of the ligands. The approximate order of the decreasing *trans*-effect has been estimated as:

$$CO, CN^-, C_2H_4 > PR_3, H > CH_3, SC(NH_2)_2 > C_6H_5^-$$
$$NO_2^-, I^-, SCN^- > Br^-, Cl^- > Py, NH_3, OH^-, H_2O$$

If the *trans*-directing influence of the chloride ion is greater than that of A, *e.g.*, amines, then ligand A will enter the *trans*-position to chloride to give a *cis*-isomer (Scheme I).



Scheme I

More often, the tetraiodoplatinumate ion is used in the synthesis of the desired *cis*-platinum(II) complexes because the iodide ion has a greater *trans*-effect than the chloride ion.

2. Indirect reaction, *via* the intermediate dipotassiumtetraiodoplatinum(II), which can be prepared quantitatively by the reaction of dipotassiumtetrachloroplatinum(II) with potassium iodide (Scheme II). The iodo derivative is then converted to *cis*-(PtA_2I_2) when treated with ligand A (Scheme III). If a chloro derivative is desired, the iodo derivative can be converted to the diaquo complex by silver nitrate treatment (Scheme IV), from which the chloro product can be obtained readily with potassium chloride (Scheme V).

$$K_2(PtCl_4) + 4KI \longrightarrow K_2(PtI_4) + 4KCl$$

Scheme II

$$K_2(PtI_4) + 2A \longrightarrow cis\text{-}(PtA_2I_2) + 2KCl$$

Scheme III

$$cis\text{-}(PtA_2I_2) + 2AgNO_3 + 2H_2O \longrightarrow cis\text{-}[PtA_2(H_2O)_2]^{+2} + 2AgI + 2NO_3^-$$

Scheme IV

$$[PtA_2(H_2O)_2]^{+2} + 2KCl \longrightarrow cis\text{-}(PtA_2Cl_2) + 2K^+ + 2H_2O$$

Scheme V

This method has more general application due to its high yield and low ratio in the formation of by-products.

3. Reactions of *cis*-(PtA_2X_2) (X = Cl, I) with monodentate or bidentate anionic ligands Y to give the complexes of the type *cis*-(PtA_2Y_2) (Scheme VI).

$$cis\text{-}(PtA_2X_2) + 2Y^- \longrightarrow cis\text{-}(PtA_2Y_2) + 2X^-$$

Scheme VI

4. Exchange of ligand X in *cis*-(PtA_2X_2) *via* the diaquo species, which is formed when *cis*-(PtA_2Cl_2) is treated with silver nitrate (Scheme IV). The new complexes *cis*-(PtA_2X_2) are produced by addition of the appropriate anion X to the diaquo complex (Scheme VII).

$$cis\text{-}[PtA_2(H_2O)_2]^{+2} + 2X^- \longrightarrow cis\text{-}(PtA_2X_2) + 2H_2O$$

Scheme VII

Table V—Activity of Neutral Complexes of the Type [PtA_2]

Complex	Dose Response[a]	Reference
cis-[Pt(glycinate)$_2$]	—	87
trans-[Pt(glycinate)$_2$]	±	87
cis-[Pt(NH_3)$_2$(malonate)]	+	14
Pt(*N*-oxyethylethylene-*N,N,N*-triacetic acid)Cl	—	87

[a] The dose response is positive (+) for cases where a consistent decrease in tumor size is observed.

MECHANISM OF ACTION

The discovery of the action of platinum compounds on biological systems was first noted by observing their disruptive effect on growth processes in bacterial systems (1); cells did not divide but produced filamentous masses where individual cells of

Table IV—Changes in Activity on Varying X in *cis*-[PtA_2X]

A	X	Solvent[a]	Dose Range[b], mg/kg	Dose Response[c]	Toxic Level[d], mg/kg	T/C[e]	Dose, mg/kg	Reference
NH_3	Oxalate^{-2}	Dimethyl sulfoxide (slurry)	5–20	+	16–20	9	15	11
		WS	12–18	+	17	24	14–16	11
		W	10–20	+	~10	0	20	11
		W	0.5–6	+	3–4	25	25	11
NH_3	Malonate^{-2}	WS	10–60	+	~35	7	30	11, 87
		W	5–24	+	20–24	28	20–24	11
NH_3	Methylmalonate^{-2}	W	10–80	+	65	7	60	11
	Ethylmalonate^{-2}	W	30–80	+	>80	17	70–80	11
	1,1-Cyclobutanedicarboxylic acid^{-2}	W	20–160	+	~150	18	120	11
	Hydroxymalonate^{-2}	WS	10–40	±	>40	52	30–40	11
NH_3	Pyrophosphate^{-4}	WS	5–160	±	>160	70	5–160	11
CH_3NH_2	Oxalate^{-2}	W	5–80	+	20–30	21	30	11
	Malonate^{-2}	W	80–180	+	>180	~21	120–180	11
	Pyrophosphate^{-4}	W	20–80	—	40	68	40	11
C_2H_5NH	Oxalate^{-2}	W	10–80	±	~20–40	21	10	11
$CH_2(NH_2)CH(NH_2)CH_3$	Malonate^{-2}	W	45–90	+	65	9	60	11
$CH_2(NH_2)CH_2CH_2(NH)$	Malonate^{-2}	W	20–80	+	~90	28	60–80	11
1,2-$C_6H_{10}(NH_2)_2$	Malonate^{-2}	WS	50–200	±	~100	41	100	11
	Oxalate^{-2}	W	0.25–16	—	35	73	0.23–2	11
	Malonate^{-2}	W	5–80	+	45–60	18	40	11, 87
	Malonate^{-2}	S	45–60	±	>60	41	50–60	11
	Malonate^{-2}	S	5–20[f]	+	6–10	24	5	11
	Methylmalonate^{-2}	W	30–90	+	>90	4	90	11
	Ethylmalonate^{-2}	WS	40–120	±	>120	51	90–120	11

[a] WS = water slurry, W = water, and S = saline. [b–e] See footnotes in Table I. [f] Daily injection for 9 days.

Table VI—Changes in Activity on Varying A in *cis*-[PtA_2Cl_2] (A = Monodentate and N = Donor Atom)

A	Solvent[a]	Dose Range[b], mg/kg	Dose Response[c]	Toxic Level[d], mg/kg	T/C[e]	Dose, mg/kg	Reference
NH_3	S	4–10	+	9	3	8	11
ND_3	S	4–10	+	~9	17	~9	11
CH_3NH_2	S	10–30	+	12–20	14	14[f]	11, 14
$(CH_3)_2NH$	S	30–150	+	~100	25	80	11
$C_2H_5NH_2$	S	5–50	+	~40	14	40	11
	S	3–9	+	5–7	13	5	11
$(C_2H_5)_2NH$	SS	15–60	—	>60	75	60	11
$HOC_2H_4NH_2$	S	20–225	+	~125	22	125	11
$(CH_3)_2CHNH_2$	SS	20–50	±	>50	33	30	11
C_5H_5N	WS	5–40	—	>40	94	5	11, 18
3-HO_2C—C_5H_5N	W	4–80	±	>80	51	40–60	11
$C_6H_5NH_2$	SS	10–50	+	>50	33	10–20	11

[a] S = saline, SS = slurry, WS = water slurry, and W = water. [b–e] See footnotes in Table I. [f] Only 66% survivors.

Escherichia coli attained lengths up to 300 times those of normal cells. Subsequently, studies conducted using ^{191}Pt (20) to determine the distribution of some platinum complexes in *E. coli* after the induction of filaments suggested that there were two different modes of action. In the filamentous cells induced by action of *cis*-dichlorodiammineplatinum(II), the platinum(II) is associated with nucleic acid and cytoplasmic proteins (20). In cells inhibited by the tetrachloroplatinumate ion, the platinum(II) is associated only with the cytoplasmic protein. Furthermore, Gram-positive cells of *Bacillus cereus* and *Staphylococcus aureus* show no filamentous growth in the presence of *cis*-dichlorodiammineplatinum(II). This finding led Renshaw and Thomson (20) to suggest that after the metal complex penetrates the cell wall of these bacteria, it is metabolized (hydrolyzed?) to intermediates such as B, $[Pt(NH_3)_2Cl]^+$, and C, $[Pt(NH_3)_2]^{+2}$, where the starting material A, $[Pt(NH_3)_2Cl_2]$, is suggested as being substantially devoid of activity *per se* against the test system. Intermediate B inhibits synthesis of DNA, RNA, and protein; and C is highly selective toward inhibition of DNA synthesis and is devoid of activity against RNA and protein synthesis. This hypothesis attempts to correlate the antitumor action of *cis*-dichlorodiammineplatinum(II) with the rate of synthesis of DNA, RNA, and protein (2, 21). At 12–24 hr postinjection, both RNA and protein syntheses are gradually restored; they return to normal rates after 72–96 hr. The inhibition of DNA synthesis is persistent for several days after injection of *cis*-dichlorodiammineplatinum(II) (12).

Other hypotheses that could account for the delayed onset of inhibitory action are: (*a*) a slow rate of diffusion into the cell of these lipid-insoluble complexes through membrane pores, and (*b*) a platinum–membrane interaction, which is time dependent and which gradually increases the rate of diffusion of the compound into the cell (12).

Gale *et al.* (22) studied the inhibition of the syntheses of DNA, RNA, and protein by *cis*-dichloro(4,5-dimethyl-*o*-phenylenediamine-*N,N'*)platinum(II). Cellular DNA, RNA, and protein became more acid soluble upon incubation *in vitro* with this agent, leading Morris and Gale (23) to suggest that the most probable point of platinum–DNA association would be at the derepressed area of DNA undergoing RNA synthesis.

The rate of loss of viability of all suspensions incubated with *cis*-dichloro(4,5-dimethyl-*o*-phenylenediamine-*N,N'*)platinum(II) was considerably greater than the rate obtained upon incubation of cells with an equivalent concentration of *cis*-dichlorodiammineplatinum(II). More direct correlations are needed to verify if this proposed mechanism is really op-

Table VII—Changes in Activity on Varying A in *cis*-[$PtACl_2$] (A = Bidentate and N = Donor Atom)

A	Solvent[a]	Dose Range[b], mg/kg	Dose Response[c]	Toxic Level[d] mg/kg	T/C[e]	Dose, mg/kg	Reference
Ethylenediamine	S	2–32	+	16	27	12	11
N-Methylethylenediamine	S	7.5–20	±	10–15	51	15[f]	11
N,N-Dimethylethylenediamine	S	20–80	±	25–35	26	30	11
N,N-Dimethylethylenediamine	SS	25–100	—	75–100	60	25–75	11
N,N,N',N'-Tetramethylethylenediamine	SS	10–40	—	>40	64	20–40	11
N,N-Dimethyl-*N'*-ethylethylenediamine	SS	50–125	±	~120	62	100	11
N,N'-Diethylethylenediamine	SS	75–225	—	>225	96	75–225	11
N,N-Diethylethylenediamine	S	10–100	±	>100	54	75–100	11
1,2-Propylenediamine	SS	5–20	±	8–12	62	12[f]	11
1,3-Propylenediamine	SS	8–30	±	10–15	58	12[f]	11
1,2-Cyclohexanediamine	SS	10–30	±	20–35	62	15–30	11
1,2-Phenylenediamine	SS	20–80	—	>80	119	20–80	11, 14
1,2-Phenylenediamine	Oil	5–40	+				87
1,10-Phenanthroline	SS	10–30	—	~15	60	10	11, 87
1,10-Phenanthroline	SS	10–30	+	2.4	69	48.0	11, 87
Adenosine	SS	20–220	+	>220	32	160–220	11

[a] S = saline, and SS = slurry. [b–e] See footnotes in Table I. [f] Only 66% survivors.

Table VIII—Activity of "Platinum Blue" Complexes against Ascites Sarcoma 180 in Swiss Mice[a]

Compound	Solvent[b]	Dose Range[c], mg/kg	Toxic Level[d], mg/kg	Best Percent ILS[e]	Dose of Best Percent ILS[e], mg/kg	Physical State[f]	Number of Cures	Reference
cis-$[Pt(NH_3)_2(H_2O)_2]$ + "Amide" → Class IA and Class IB Complexes								
Class IAπ								
Uracil	W	50–400	400	91	200	S	5	90
Uracil	S	50–400	400	80	100	S	1	90
5,6-Dihydrouracil	W	20–800	400	92	200	S	4	90
Thymine	W	150–600	45	72	300	S	2	90
Thymine	S	50–200	>200	67	150	S	1	90
5,6-Dihydro-6-methyluracil	W	50–400	200	89	50	S	2	90
6-Methyluracil	S	200–800	>800	87	600	S	3	90
5,6-Dimethyluracil	W	50–400	>400	100	400	S	5	90
5,6-Dihydrothymine	S	50–400	400	87	200	S	2	90
1-Methyluracil	S	50–400	>400	85	400	S	3	90
1-Methylthymine	S	50–400	400	94	100	S	3	90
1-Ethyluracil	S	50–400	200	38	50	S	0	90
5-Fluorouracil	W	50–400	200	90	100	S	4	90
5-Chlorouracil	W	50–400	200	67	50	S	3	90
6-Chlorouracil	S	25–200	>200	88	200	S	3	90
5-Bromo-1-methyluracil	S	50–400	>400	88	200	S	1	90
5-Iodouracil	PO	25–600	500	8	250	S1	0	90
5-Hydroxymethyluracil	S	50–400	200	98	100	S	2	90
5-Carbethoxyuracil	S	25–200	200	56	200	S	0	90
6-Carbomethoxyuracil	S	25–200	>200	38	25	S	0	90
Uridine deoxyribose	S	25–200	>200	46	200	S	0	90
Thymidine	S	50–400	200	46	50	S	1	90
5-Iodouridine deoxyribose	PO	50–200	>200	23	200	S1	0	90
2′,3′,5′-Triacetyluridine	S	50–1000	800	79	600	S	2	90
2′,3′,5′-Tribenzoyluridine	S	50–400	>400	19	100, 200	S	0	90
2′,3′-Isopropylidine-uridine	S	50–400	400	61	50	S	2	90
Acetamide	S	25–200	25	−94	25	S	0	90
Trimethylacetamide	S	3.1–25	12.5	10	6	S	0	90
3-Chloropropionamide	S	3.1–25	>25	61	25	S	2	90
Benzamide	S	50–400	23	23	400	S1	0	90
Class IB								
Uracil	W	25–675	500	95	340	S	4	90
Thymine	S	50–400	>400	60	200	S	0	90
1-Methylthymine (yellow)	S	50–400	400	−14	100	S	0	90
1-Ethyluracil	S	50–400	100	15	50	S1	0	90
5-Fluorouracil	S	25–200	>200	37	200	S	0	90
Hydantoin	S	25–200	>200	60	50	S1	1	90
Benzamide	S	3.1–25	25	14	3.1, 6.2	S	0	90
Trimethylacetamide	S	3.1–25	3.1	−55	3.1	S	0	90
$[Pt(R-CN)_2Cl_2 + 2AgNO_3]$ or [Class III Complex + $2AgNO_3$] → Class IIA and Class IIB Complexes								
Class IIA								
Acetamide	W	100–400	>400	93	200	S	1	15
Fluoroacetamide	S	50–400	>400	94	400	S	0	15
Chloroacetamide	S	50–400	>400	96	400	S	1	15
Propionamide	2% C_2H_5OH	50–400	>400	89	200	S	2	15
n-Butyramide	S	50–400	>400	23	100	S1	0	15
Benzamide	S	50–400	>400	14	100	S1	0	15
Class IIB								
Acetamide	S	50–400	400	14	100	S1	0	15
$Pt(CH_3CN)_2Cl_2 + R-CO-NH_2$ → Class III Complexes								
Class III								
Acetamide (purple)	S	50–400	400	31	400	S	1	15
Acetamide (blue)[g]	S	50–200	200	76	150	S1	0	15
Acetamide (blue)	S	25–200	200	39	100	S	0	15
Fluoroacetamide	S	50–400	400	66	400	S1	0	15
Chloroacetamide	S	50–400	400	53	200	S	0	15
Trimethylacetamide	S	50–400	400	67	50	S	0	15
Acetamide (red)	S	6.3–400	100	91	25	S	2	15
Miscellaneous								
Dichlorobis(acetonitrile)-platinum(II)	S	6.3–50	50	83	25	S	1	15

[a] Average day of death, untreated controls = 17.5 (*SD* ± 2.16). Six animals per test; cures are considered as having *no* distention of abdominal cavity but do include formation of solid tumors at site of injection in some cases. Single injections given intraperitoneally on Day 1 only. [b] W = water, S = saline, and PO = peanut oil. [c] The dose range indicates the maximum and minimum doses administered. [d] The toxic level is the highest dose at which survivors are equal to 90%. [e] Percent ILS = percent increase in lifespan, with the maximum being 100%. [f] S = solution, and S1 = slurry. [g] Prepared by hydrolysis of dichlorobis(acetonitrile)platinum(II).

Table IX—Activity of Complexes of Type *cis*-$[PtL_2Cl_2]$[a]

L	Solvent	Dose Range[b], mg/kg	Dose Response[c]	Toxic Level[d], mg/kg	T/C[e]	Dose, mg/kg
Triphenylphosphine	Saline	25–100	±	100	53	25–100
	Saline–dimethyl sulfoxide	40–160	±	160	78	40–160
Dimethyl sulfoxide	Saline	6–12	—	12	86	6–12
Diethyl sulfide	Dimethyl sulfoxide	5–40	—	40	67	5–20
Glycinate	Saline	10–200	—	125–150	79	40–100

[a] Reference 11. [b–c] See footnotes in Table I.

erative.

The suppression of DNA synthesis in the rat kidney has also been suggested to proceed *via* direct interaction of platinum compounds with renal cell nucleic acid. By using ^{14}C-labeled dichloroethylenediamineplatinum(II), the effect of this drug upon DNA appeared to be mediated *via* the guanine, cytosine, and adenine bases (24–26). The change in UV absorption was measured, and a hyperchromic change was observed. This change was proposed to be due to a direct interaction of *cis*-dichlorodiammineplatinum(II) with some of the DNA bases (27). The binding of *cis*-dichlorodiammineplatinum(II) to DNA is markedly lowered when the concentration of chloride ion is increased.

Further studies on nucleosides showed that guanosine, adenosine, and cytidine interact with *cis*-dichlorodiammineplatinum(II), whereas uridine and thymidine show very little interaction (28, 29). Since the formation constants of platinum–guanosine complexes are the largest in magnitude (Table XI), guanosine in DNA may be the primary target for complexation. It was speculated that such a complex might result in partial denaturation of the DNA helix, which would open other sites on cytidine and adenosine for further interactions with *cis*-dichlorodiammineplatinum(II).

In the case of DNA-containing bacteriophage T, the inactiviation by *cis*-dichlorodiammineplatinum(II) is correlated to the interstrand cross-linking of neighboring bases on the same nucleic acid chain as well as to intrastrand cross-linking of nucleic acid (30) with platinum. The binding of *cis*-dichlorodiammineplatinum(II) to DNA can be estimated from the changes of buoyant density and sedimentation rates, which parallel the change in the conformation of the DNA molecule. An increase in sedimentation efficiency follows an increase in molecular weight and a decrease of partial specific volume (31).

The ready formation of platinum–nucleic acid bonds is probably the determinant in the inhibitory actions of platinum compounds on tumor growth and DNA synthesis. *cis*-Dichlorodipyridineplatinum(II) forms an acid-resistant bond when incubated with intact cells and DNA but does not bind appreciably with proteins, polysaccharides, or erythrocyte membranes. The last interaction with DNA is inhibited by sodium chloride and nitrogen mustard complexation (32), and this finding may indicate that the antitumor and antimitotic action of the square-planar platinum complex depends on the dissociation of one or both chlorine atoms from the central platinum atom.

cis-Dichlorodiammineplatinum(II) has two neighboring leaving chlorine groups and may act as a bifunctional agent, whereas *trans*-dichlorodiammineplatinum(II) probably behaves as a monofunctional agent. The study on the interaction of *cis*- and *trans*-dichlorodiammineplatinum(II) with purines, substituted purines, pyrimidines, and substituted pyrimidines shows that the *cis*-isomer forms a bidentate chelate with either 6-NH_2 and N-7 or 6-NH_2 and N-1 of adenosine and 4-NH and N-3 of cytidine. The

Table X—Activity of Miscellaneous Charged Complexes

Complex	Solvent[a]	Dose Range[b], mg/kg	Dose Response[c]	Toxic Level[d], mg/kg	T/C[e]	Dose, mg/kg	Reference
$K_2[PtCl_4]$	S	10–100	—	40–50	127	25	11
$K_2[PtBr_4]$	S	3.5–60	±	~45	73	15–30	11
$K_2[Pt(C_2O_4)_2]\cdot 2H_2O$	W	5–160	—	~40	91	10–40	11
$K_2[Pt(C_3H_2O_4)_2]\cdot 2H_2O$	W	20–250	±	>250	60	20–250	11
$K[Pt(C_2H_4)Cl_3]$	S	5–40	—	>40	122	5–40	11
$K[Pt(H_2NCH_2CO_2)Cl_2]$	S	10–200	—	125–150	79	40–100	11
$[Pt(NH_3)_4][Pt(NH_3)Cl_2]_2$	SS	20–80	+	~50	35	40	11
$K_2[Pt(NO_2)Cl_3]$	S	5–200	—	~75	81	50	11
$[Pt(NH_3)_4]Cl_2$	S	5–200	—	>200	100	5–200	11
$[Pt(H_2NCH_2CH_2NH_2)_2]Cl_2$	S	5–40	—	>40	90	5–40	11
$[Pt(H_2NCH_2CH(NH)CH_2NH_2)Cl]Cl$	S	10–100	—	>100	71	10–100	11
$[Pt(H_2NCH_2CH(NH)CH_2NH_2)Br]Br$	S	10–80	—	>80	93	10–80	11
$K[Pt(C_{12}H_8N_2\cdot H_2O\text{-}5\text{-}SO_3)Cl_2]$	S	5–40	—	>40	58	5–40	9
$[Pt[CH_3S(CH_2)_2CH(NH_2)CO_2H]_2]Cl_2$			—				87
$[Pt(CH_3SCH_2CH_2NH_2)_2][PtCl_4]$			—				87
$[Pt[H_2NCH_2CH_2NH(C_2H_4OH)]Ce]Cl$			—				87
$[Pt((CH_2)_2NH)_4][PtCl_4]$			—				87

[a] S = saline, W = water, and SS = slurry. [b–e] See footnotes in Table I.

Table XI—Formation Constant of the Stoichiometry of $[Pt(NH_3)_2Cl_2]$–Nucleoside Complexes[a]

Ligand L	$K_f(cis)$	$K_f(trans)$	Type of Complexes
Adenosine	0.24×10^5	0.64×10^5	PtL
Cytidine	0.66×10^5	—	$Pt^{II}L$
Guanosine	1.06×10^9	1.06×10^9	$Pt^{II}L_2$

[a] Reference 27.

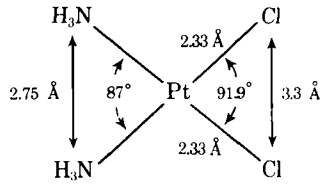
trans-isomer interacts monofunctionally at either N-7 or N-1 of adenosine and at N-3 of cytidine; it does not bind to the free amino groups (33, 34). Both isomers bind monofunctionally to N-7 of guanosine and inosine but do not bind to uridine or thymidine.

In DNA, both adenosine and cytidine bases are the probable sites available for binding with *cis*-dichlorodiammineplatinum(II) (35). In the latter, the spacing between the two chlorine atoms is 3.3 Å (36), which is very close to the 3.4 Å of the stacking spacing of the bases in the Watson–Aich model (IX). It appears, therefore, that such platinum complexes could act like bifunctional alkylating agents to form interstrand and intrastrand cross-links (37). This assumption is supported by recent evidence that the platinum complexes inhibit a single-stranded bacteriophage as well as the double-stranded bacteriophage (38). Other evidence supporting interstrand cross-links are obtained from circular dichroism measurements (39) and from isolation of hybrid cross-link species of DNA and *cis*-dichlorodiammineplatinum(II) (40).

A different hypothesis relates the antitumor effect of platinum compounds with the stimulation of immune processes (41), since immunity plays an important role in the arrest of malignant diseases (42). In mice, studies on antibody formation (43), graft *versus* host reaction (44), and phytohemoagglutinin-induced lymphocyte blastogenesis (45) show that *cis*-dichlorodiammineplatinum(II) has strong immunosuppressive effect. This effect is more prominent when the drug is administered at the time of sensitization and is also effective within 2 days either before or after the sensitization and will last for 3 days after a single injection.

cis-Dichlorodiammineplatinum(II) suppresses antibody formation in cells after they have been exposed to the antigen. The diminished weight of spleen and suppression of antibody plaque-forming cells indicate that this drug has definite effects on the normal lymphoid tissue (43). *cis*-Dichlorodiammineplatinum(II) suppresses the graft *versus* host reaction but prolongs the survival of skin graft against the host's histocompatibility barrier during the initial phase of graft injection. A delay in the administration of the drug leads to abolition of the effect (46).

cis-Dichlorodiammineplatinum(II) not only affects the tumor cell but also affects the normal lymphocytes during their blastic transformation (45). In patients being treated with *cis*-dichlorodiammineplatinum(II), an immediate effect on the lymphocytes was observed, as evidenced by inhibition of blastogenesis 15 min following the administration of the drug in dose ranges from 1 to 4 mg/kg (47). The immunosuppressive effect lasted for 18–72 hr, a relatively short period compared to other immunosuppressive drugs. The short-lived immunosuppressive effect of *cis*-dichlorodiammineplatinum(II) may prove to be advantageous, because a rapid recovery of the immune system may help in the control of the malignant disease.



spacial structure of *cis*-dichlorodiammineplatinum(II)
IX

ANTITUMOR ACTIVITY

The platinum complexes studied have been shown to have activity against a wide range of animal tumors. Rosenberg *et al.* (48) first reported the inhibition of sarcoma 180 and L-1210 leukemia in mice by *cis*-dichlorodiammineplatinum(II). Some treated mice appeared normal and healthy and remained in remission for 10 months (37). The initial tumor transplant disappeared. *cis*-Dichlorodiammineplatinum(II) was also active in inhibiting and causing regression of large solid sarcoma 180 tumors in 63–100% of the animals with no apparent irreversible damage to the host (41). This compound is the first chemotherapeutic agent able to accomplish such complete regression and is superior to other agents, such as mercaptopurine, that have been used with sarcoma 180 tumors with approximately 50% recovery (49).

In the rat, *cis*-dichlorodiammineplatinum(II) had strong antileukemic effects on myeloid and lymphatic leukemias but no detectable effect on the normal cells of the marrow, gonads, and intestine (50). The effectiveness of *cis*-dichlorodiammineplatinum(II) against Dunning ascitic leukemia and intramuscular Walker 256 carcinosarcoma was reported (51). A single treatment with *cis*-dichlorodiammineplatinum-(II) on Day 1 inhibited the development of Dunning ascitic leukemia in Fisher 344 rats. Delayed treatment even on Day 7 can cause significant regression of tumor development and extend the survival time. *cis*-Dichloro(4,5 -dimethyl-*o*- phenylenediamine -*N*,-*N'*)platinum(II) exhibited the same effect as *cis*-dichlorodiammineplatinum(II). It extended up to 175% the survival time of mice bearing the Ehrlich ascitic tumor and up to 74% the survival time of mice bearing the L-1210 leukemia (22).

Two other types of tumors that are more relevant to the cancers occurring in humans are those caused by chemical agents (carcinogens) and by viruses. The platinum compounds are also capable of exhibiting antitumor activity against carcinogenically or virally induced tumor systems. The effectiveness of *cis*-dichlorodiammineplatinum(II) was reported in treating a virus-induced reticulum cell sarcoma and in pro-

Table XII—Effectiveness of Chemotherapeutic Agents in Mice with L-1210 Leukemia (93)

Tumor Site[a]	Drug	Treatment Schedule[b], days	Percent ILS[c]
ip	Doxorubicin (Adriamycin)	q3h/1	60
ip	Daunorubicin	1, 5, 9	20
ip	Camptothecin	1, 5, 9	112
sc	*cis*-Dichlorodiammine-platinum(II)	q3h/5, 9, 13	67
sc	Cytosine arabinoside	5, 7, 9, 11, 13	100
ip	Harringtonine	1–9	40
sc	5-Azacytidine	1, 5, 9	117
sc	Methotrexate	5, 9, 13	83
sc	Cyclophosphamide	5, 9, 13	279
sc	Isophosphamide	5, 13, 21	144

[a] ip = 10^5 L-1210 ascites cells inoculated intraperitoneally; sc = 10^6 L-1210 ascites cells inoculated subcutaneously. [b] Days of treatment after tumor inoculation; q3h = injections every 3 hr for 24 hr on 1 day only or on every 4th day. [c] Percent ILS = percent increase in lifespan.

moting regression of carcinogen-induced rat mammary tumors (52) and *cis*-dichlorodiammineplatinum(II) was studied against the dimethylbenzanthracene-induced mammary tumor (53). In the latter study, complete regression of a large number of extensively developed mammary tumors was observed in the rats, with three out of 14 animals completely "cured" of all tumors. The same compound was tested against the Rous sarcoma virus-induced tumor in the chicken (54). Again, regression of this tumor in 95% of the animals was seen after the treatment with *cis*-dichlorodiammineplatinum(II). The platinum compounds have one of the broadest spectra of action of any class of antitumor agents yet discovered. They cause regression of large tumors and rescue animals even injected a few days prior to their death.

The data in Table XII summarize the effectiveness of *cis*-dichlorodiammineplatinum(II) in mice with L-1210 leukemia in comparison with other drugs tested in this model system. This comparison only provides limited indication, since the percent increase of lifespan varies greatly with the test model chosen and the treatment schedule. To date, there is no precise way to compare the effectiveness of drugs by a single animal test system. It would be desirable to compare *cis*-dichlorodiammineplatinum(II) with chemotherapeutic agents in various tumor models.

Clinical trials on *cis*-dichlorodiammineplatinum(II) have been conducted in hospitals. Phase I studies on clinical toxicology (7, 13, 55) showed that renal toxicity was the most serious side effect, and damage was noted in the bone marrow and GI tract. Ototoxicity (initially high tone hearing loss and eventually deafness) also was described.

Phase II trials for antitumor activity of *cis*-dichlorodiammineplatinum(II) were performed by many investigators to assess its effectiveness toward different types of malignancies (Table XIII). Very good results were reported with *cis*-dichlorodiammineplatinum(II) in nine of 11 (56) and seven of 16 (57) patients with testicular tumors. Good responses in carcinoma of the bladder, breast, and thyroid also were noted. Talley *et al.* (58) observed objective remission in neuroblastoma, malignant thymoma, and three out of five lymphomas. *cis*-Dichlorodiammineplatinum(II) was inferior to fluorouracil for colon carcinoma (59). The toxicity of *cis*-dichlorodiammineplatinum(II) does limit its use in cancer chemotherapy as a single agent, but very promising developments were observed when this agent was used in combination therapy with fluorouracil (60), doxorubicin (adriamycin) (61), and other agents (62).

Thus, by using combination therapy of flurouracil and *cis*-dichlorodiammineplatinum(II), over 50% tumor regression was obtained in nine of 25 patients (63, 64). Four of nine patients with colon carcinomas showed 50% tumor regression, and one of these patients had previously failed on fluorouracil treatment as a single agent. Of the 50% or better responders, mammary, jejunal, and tonsillary adenocarcinoma were included. Encouraging response was found in patients treated with combined *cis*-dichlorodiammineplatinum(II) and doxorubicin (61). In two patients with embryonal carcinoma of the testis, one had complete regression of a large retroperitoneal mass and another had partial regression of an abdominal mass. Other responses were seen in anaplastic carcinoma of the lung, squamous cell carcinoma of the lung, and adenocarcinoma of the sigmoid colon. The promising antitumor activity of *cis*-dichlorodiammineplatinum(II) in the treatment of squamous cell carcinoma, lymphosarcoma, adenocarcinoma of the pancreas, testicular carcinoma, and endometrial carcinoma was also found in 201 patients (62).

BIOLOGICAL DISTRIBUTION

Both biological activity and side effects of drugs have some relation to their distribution, retention, and excretion in the various organs of animals and humans as well as to the rate of distribution (pharmacokinetics). The use of radioplatinum has proven most useful for such studies.

Table XIII—Responses to *cis*-Dichlorodiammineplatinum(II) in Various Malignant Diseases

Malignant Disease	Number of Patients	Number of Complete Responses	Number of Partial Responses[a]	Number of Improvements[b]	Total Response Rate, %	Reference
Testicular carcinoma	16	7	3	3	81	57, 91
	11	9			81	56
Lymphoma	16	2	7	1	63	4, 8, 57, 97
	5	3			60	58
Squamous cell carcinoma of the head and neck	17	0	1	6	41	13, 91
Ovarian carcinoma	20	0	5	3	40	91

[a] Greater than or equal to 50% tumor reduction. [b] Less than 50% tumor reduction and significant subjective improvement.

Platinum possesses 28 radioactive isotopes and six stable nuclides. Of the radionuclides, a few possess physical characteristics that are desirable for biological and/or clinical studies. Table XIV summarizes the main radionuclides of platinum. Most of these are γ-emitters and are amenable to external detection. Their half-lives are also suitable for *in vivo* studies.

The distribution of *cis*-dichlorodiammineplatinum(II) labeled with ^{193m}Pt and ^{195m}Pt was evaluated in mice, rabbits, and humans (65, 66). The tumor to blood ratio of radioactivity varied from 1:3 to 2:0 (4 hr to 5 days after injection) in mice bearing sarcoma 180. In rats bearing the Walker 256 carcinosarcoma, the tumor did not exhibit selective uptake of the drug inasmuch as the tumor to blood ratio was near unity (67, 68). However, the rate of clearance in tumor animals was significantly higher than in control animals, an effect observed within the first few minutes postinjection. This result suggests that, in the tumor animals, the renal clearance mechanism has been altered, a possible paraneoplastic effect, or that the blood contains (or lacks) a protein with unique and specific binding characteristics to *cis*-dichlorodiammineplatinum(II). This effect was dependent on tumor size and may be of potential interest in objective assessment of remission status of a tumor. Otherwise, the organ distribution in mice, rats, and rabbits with tumors was similar to the control animals. The kidneys and the liver are the principal targets of localization of platinum compounds (15, 37). Renal retention appears to involve irreversible binding of the platinum complex to the renal cell and is cleared slowly.

The distribution of the labeled *cis*-dichlorodiammineplatinum(II) has been studied in patients (66, 69). Early concentration is in the kidneys and in the head regions, but the brain area is relatively devoid of activity. Plasma disappearance reveals an initial rapid fall in radioactivity following intravenous injection and then a slow phase. About 60–70% of the radioactivity injected is retained for 2 days and excreted in the urine. After 40 hr, organs containing most of the radioactivity are the kidney, liver, and the intestine. The pattern of clearance of *cis*-dichlorodiammineplatinum(II) from the plasma, kidney, liver, and whole body is similar to that observed in animals. The half-lives of retention in humans are in the order of 8–10 days.

The subcellular distribution of ^{195m}Pt- and ^{14}C-ethylenediamine-labeled *cis*-dichloroethylenediammineplatinum(II) was studied in the liver, kidneys, and two transplanted tumors (70). This drug shows a distribution pattern similar to that for *cis*-dichlorodiammineplatinum(II) in normal and tumor tissues. Among nuclear cell debris, mitochondria, lysosomes, microsomes, and cytosol, *cis*-dichloroethylenediammineplatinum(II) is localized mainly in the cytosol in the form of low molecular weight complex. Similar results (71) were noted in the subcellular distribution studies of ^{195m}Pt-*cis*-dichlorodiammineplatinum(II) and other platinum complexes.

The study of the distribution of *cis*-dichloro-bis-(cyclopentylamine)platinum(II) in Walker 256 and in control rats illustrated that such a highly insoluble complex not only diffused very slowly out of the site of injection but was also retained at the kidney to an inordinate amount (15). Thus, animals injected at 100 mg/kg retained over 50% of the injected dose at the kidney even after 8 days.

It has been recommended that kidney function be closely monitored and correlated to platinum compounds to prevent severe renal toxicity, the main undesirable side effect of these complexes.

Table XIV—Potentially Useful Radionuclides of Platinum-99[a]

Nuclide	$t_{1/2}$	Nature of Radiation[b]	Main Energies	Production
188	10.2 days	EC	140 kev, 190 kev	^{191}Ir (p, 4n)
189	10.9 hr	EC	Complex	^{191}Ir (p, 3n)
191	3 days	EC	129 kev, 360 kev	^{191}Ir (d, 2n)
^{m}193	4.3 days	IT	Pt X-rays: 66 kev, 77 kev	^{193}I (d, 2n) ^{192}Pt (n, γ)
^{m}195	4.0 days	IT	99 kev, 129 kev, Pt X-rays	^{194}Pt (n, γ)
197	17 hr	β^-	0.670 Mev	^{196}Pt (n, γ)

[a] Reference 93. [b] EC = electron capture, and IT = internal transition.

TOXICITY AND DOSAGE

In animals, treatment with high doses of platinum compound elicits severe acute toxicity such that death or severe morbidity occurs within 3–4 days after termination of treatment. The nontoxic dose range of *cis*-dichlorodiammineplatinum(II) established for dogs is 0.625 mg/kg as a single dose or 0.185 mg/kg/day for 5 days. Monkeys can tolerate 0.156 mg/kg/day for 5 days without ill effects (72). The minimum lethal dose for the dog is a single injection of 2.5 mg/kg or five daily consecutive injections of 0.75 mg/kg. For the monkey, the minimum lethal dose is five daily doses of 2.5 mg/kg. The LD_{50} for Swiss mice is 13.4 mg/kg for males and 12.32 mg/kg for females (73). Animals receiving maximally tolerated doses recover from the toxic effects within 14–124 days. Toxic signs include damage to the GI tract, bone marrow, lymph nodes, liver, and kidneys. The renal lesions are the most severe toxic changes and are characterized by increasing excretion of urinary lactic dehydrogenase. Thus, the urinary lactic dehydrogenase excretion can be used as a monitor of *cis*-dichlorodiammineplatinum(II)-induced renal toxicity (74).

In rats, a single intraperitoneal injection of a toxic dose of *cis*-dichlorodiammineplatinum(II) (12.2 mg/kg) causes leukopenia, the decrease of both neutrophiles and lymphocytes, and the depression of the number of circulating platelets, but it does not produce significant alteration for circulating erythrocytes, packed cell volumes, and hemoglobin. At such dosage, blood urea nitrogen values, serum glucose levels, and serum albumin levels are elevated, whereas total protein levels are depressed. Serum levels of uric acid, calcium, and inorganic phosphorus are not altered (75).

The histological alterations in rats are most pronounced in tissues having cellular constituents. Thymic atrophy, splenic depletion of lymphoid elements, intestinal epithelial denudation, and bone marrow problems are most severe. Renal tubular sloughing also occurs. Rats surviving the intoxication regenerate the cellular constituents in those tissues affected (75).

The histological, hematological, and serum alterations observed in animals after a toxic dose may indicate that platinum compounds possess antitumor properties as a result of their general cytoinhibitory effects.

In humans, single doses of 1.95 mg/kg of *cis*-dichlorodiammineplatinum(II) are likely to produce renal impairment and may result in severe morbidity (76). Thus, human tolerance for the platinum compound is quite similar to that of dogs and monkeys. Patients receiving doses of more than 0.75 mg/kg experience nausea and vomiting within several hours; these effects are more intense at the higher dose levels (2). At doses above 2.0 mg/kg, toxic effects are hematological, renal, and neural (eighth nerve) (13, 76). Reversible leukopenia and thrombopenia also occur. Nephrotoxicity manifested by azotemia, rise in serum creatinine and uric acid, fall in creatinine and uric acid clearances, and concentration defects happen in some patients (13, 77). Partially reversible, high frequency nerve deafness is usually the earliest sign of toxicity. A minimal decrease in lymphodenopathy is observed in patients with undifferentiated carcinoma or with advanced lymphosarcoma (4). For patients with advanced unsusceptible cancer who have failed to respond to conventional therapy, dose levels have been used in increments up to a total cumulative dose of 8.5 mg/kg. In such high doses, renal toxicity increases (7, 13).

CONCLUSION

This review has discussed the design of platinum compounds that would show antitumor activity. The compounds should be neutral and contain a pair of *cis*-leaving groups. The carrier groups also play an important role in the determination of activity. They should be inert and neutral. The new derivatives should be synthesized with high tumor affinity and low renal toxicity—problems yet to be overcome.

Although the platinum compounds have shown a broad spectrum of action on various tumors, their immunosuppressive effect, prolonged retention, slow urinary elimination, and severe renal toxicity pose a possible clinical danger. Drug treatments scheduled at too close intervals may lead to accumulation of a toxic body load of platinum. Therefore, the choices of dose level and time schedule are important factors in the treatment. Based on the preclinical studies of tumor inhibition (41, 51) and pharmacological data (2, 76), a widely spaced treatment schedule is desired. Fiserova *et al.* (78) reported that no apparent and serious manifestation of toxicity was observed during the treatment of patients with *cis*-dichlorodiammineplatinum(II) at weekly intervals and a low dose.

In view of the toxicity and suppression of the immune response of platinum compounds, the combination of platinum compounds with certain other antitumor agents proves to be more practical than using them as single agents (79). Enhanced therapeutic effects of *cis*-dichlorodiammineplatinum(II) were observed against L-1210 leukemia in mice when it was combined with cyclophosphamide, isophosphamide, 1,2-bis(3,5-dioxopiperazine-1-yl)propane, thioguanine, 5-thiouracil, methotrexate, daunomycin, or other antitumor agents (79–83). The treatment of neoplastic disease with this combination gave greater survival than the maximum survival obtainable after treatment with either drug alone.

Conran and Rosenberg (84) studied the combination of immune therapy with *cis*-dichlorodiammineplatinum(II) chemotherapy against sarcoma 180 tumors in mice. They found that an immunodepressant, *e.g.*, hydrocortisone, reduced the antitumor activity of the platinum compound, whereas a nonspecific immune stimulant produced less effect on the drug activity. Alternatively, the usage of cysteine and other nucleophiles with the platinum compound affords protection against the toxicity of these drugs (85). In a search of platinum compounds with the promising antitumor activity and a low toxic side effect, Leh and Wolf (86) recently reported a new type of platinum–bleomycin compound. These compounds show higher tumor affinity and reduced kidney retention than *cis*-dichlorodiammineplatinum(II). It is hoped that some new platinum compounds will be synthesized and regarded as a new class of drugs to be added to the medical armamentarium.

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RESEARCH ARTICLES

Effects of Lipids on Bioavailability of Sulfisoxazole Acetyl, Dicumarol, and Griseofulvin in Rats

DUANE C. BLOEDOW * and WILLIAM L. HAYTON *

Abstract □ The effects of hexadecane, oleyl alcohol, polysorbate 80, trioctanoin, and triolein on the bioavailability of sulfisoxazole N^1-acetyl, dicumarol, and griseofulvin were investigated. Compared to administration of the drugs in water, the rate of absorption of the drugs was either decreased or not changed by the lipids. The extent of absorption of sulfisoxazole acetyl and dicumarol was significantly increased by polysorbate 80 and triolein and not affected by hexadecane or oleyl alcohol. Trioctanoin increased the extent of absorption of sulfisoxazole acetyl but had no effect on the absorption of dicumarol. Compared to the aqueous vehicle, the extent of absorption of griseofulvin was decreased by hexadecane, oleyl alcohol, and triolein, increased by polysorbate 80, and not affected by trioctanoin. The extent of absorption of sulfisoxazole acetyl was not affected by the amount of triolein in which it was administered nor by emulsification of triolein prior to administration.

Keyphrases □ Bioavailability—sulfisoxazole acetyl, dicumarol, griseofulvin, effect of lipids, rats □ Sulfisoxazole acetyl—effect of lipids on bioavailability, rats □ Dicumarol—effect of lipids on bioavailability, rats □ Griseofulvin—effect of lipids on bioavailability, rats □ Lipids—effect on bioavailability of sulfisoxazole acetyl, dicumarol, and griseofulvin, rats

The bioavailability of orally administered drugs having low aqueous solubility may be incomplete due primarily to slow dissolution in the lumen of the GI tract. Several reports indicate that the bioavailability of poorly water-soluble drugs, particularly drugs that are also lipophilic, can be improved by coadministration of a lipid, which apparently increases the rate of dissolution of such drugs. For example, the bioavailability of indoxole in humans is increased following oral administration of the drug in a lipid emulsion compared to administration as an aqueous suspension (1). Similar results in rats were reported following the oral administration of indoxole dissolved or suspended in cottonseed oil or dissolved in polysorbate 80 (2).

The bioavailability of griseofulvin in humans increases with the amount of lipid in the diet (3) and is greater following oral administration with corn oil (4) or corn oil emulsion (5) than with water. The therapeutic effect of N^1-acyl derivatives of sulfanilamide in mice is increased following oral administration of suspensions of the drugs in olive oil compared to administration in water (6). In rats and humans, the bioavailability of sulfisoxazole N^1-acetyl is greater following oral administration of the drug in a vegetable oil-in-water emulsion than it is when administered in water (7).

While bioavailability may be improved by lipids, there are also examples of lipids decreasing the bioavailability of lipophilic substances. The absorption of chlorophenothane (DDT), as reflected by LD_{50} values, is increased when it is administered in corn oil or olive oil but decreased when given in mineral oil compared to its administration in an aqueous vehicle (8). And ingestion of potato chips containing small amounts of methyl polysiloxane, a lipid-like agent that enhances crispness, apparently significantly reduced the absorption of warfarin and phenindione in patients taking these drugs (9).

The purpose of this study was to examine physicochemical and physiological properties of lipids that may alter the bioavailability of lipophilic, poorly water-soluble drugs. The drugs used and their aqueous solubilities in milligrams per liter were: sulfisoxazole acetyl, 70 (10); dicumarol, 0.5 at low pH (11); and griseofulvin, 8.8 (12). The bioavailabilities of the drugs were determined following their oral administration in selected lipid vehicles and water.

The lipid vehicles (Table I) were selected on the basis of their diverse physicochemical and physiological properties. Because the environment of the GI lumen is aqueous, the primary physicochemical prop-