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TRYPANOCIDAL AND ANTITUMOUR ACTIVITY OF PLATINUM-METAL AND PLATINUM-METAL-DRUG DUAL-FUNCTION COMPLEXES

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Abstract—A number of antitumour platinum-metal complexes related to *cis*-platin showed trypanocidal activity against *Trypanosoma rhodesiense in vitro* but not *in vivo*. New platinum- and rhodium-metal complexes of diamidine and plenanthridinium trypanocides are described which showed higher therapeutic indices than the parent drugs, due to increased activity in the former drug type and decreased toxicity in the latter. Some evidence of potentiation of antitumour activity was noted in these drug complexes. At the ultrastructural level, complex-treated trypanosomes showed a number of nuclear effects and other lesions specifically attributable to platinum-metal action. Some of the lesions were similar to those induced by *cis*-platin in tumour cells.

The discovery of the antineoplastic activity of *cis*-$[PtCl_2(NH_3)_2]$, (*cis*-platin), and its subsequent clinical development, have renewed interest in the chemotherapeutic properties of transition-metal complexes. In view of analogies between rapidly dividing tumour cells and pathogenic African trypanosomes [1, 2], both in metabolism and in drug reactivity [3], the trypanocidal activity of a number of these metal and metal-drug complexes [4] have been examined more extensively, especially as *cis*-platin has been shown to have trypanocidal activity [5]. The continuing need for new trypanocides justifies such a study, especially when the dual possibility exists of therapeutic activity against both tumours and trypanosomes. The present report includes results of *in vitro* and *in vivo* tests together with an ultrastructural study of the morphological effects of platinum-amine and platinum-drug complexes on trypanosomes.

MATERIALS AND METHODS

All known complexes (Table 1) were prepared by standard procedures. The preparation and characterization of the new complexes, XI–XXI (Table 4) will be reported separately. Where appropriate the structures will be discussed in the text.

Pure Berenil diaceturate was obtained by courtesy of Hoechst AG, Frankfurt, and Ethidium bromide and Samorin by courtesy of Boots Pure Drug Co., Nottingham and May and Baker Ltd., Dagenham, respectively.

Table 1. Activity of metal complexes on *T. rhodesiense in vitro* (values given are maximum titres ($\log_{10} M^{-1}$) producing the effect)

	Complex	Loss of motility	Loss of infectivity: Partial	Loss of infectivity: Total
I	*cis*-$[PtCl_2(NH_3)_2]$	3	5	<3
II	$[PtCl_2(dac)]$	3	5	3
III	$[Pt(DMSO)_2(dac)](BF_4)_2$	3	5	<3
IV	$[RuCl(NH_3)_5]Cl_2$	<3	4	<3
V	$[Ru(NH_3)_6]Cl_3$	<3	3	<3
VI	[Ruthenium red]	<3	4	<3
VII	$[RuCl_2(DMSO)_4]$	<3	<3	<3
VIII	$[RuCl_2(DMSO)_2en^*]$	<3	<3	<3
IX	$[Ru(DMSO)_2(dac)_2]Cl_2$	<3	<3	<4
X	$[Rh_2(acetate)_4 . 2H_2O]$	<3	<3	<3

* en = ethylenediamine.

Trypanocidal activity

Trypanocidal activity was determined with a monomorphic strain of *Trypanosoma rhodesiense* [6], the drug sensitivity of which has been extensively characterized [2, 7]. *In vitro* activity tests were carried out with trypanosome suspensions (10^6/ml) in plastic Microtitre plates [2]; trypanocidal titres ($\log 10^{M-1}$) (M = molarity) using a dilution factor of 10× were determined after 4 hr incubation at 37° for (i) motility and (ii) infectivity by inoculation of well contents into specific-pathogen-free Parkes mice and subsequent check for development of parasitaemia. Compounds with titres < 3 were considered inactive.

Drug activity *in vivo* was determined by intraperitoneal injection of a single drug dose into batches of 10 specific-pathogen-free Parkes mice (20–25 g) infected 24–48 hr previously with *T. rhodesiense*; untreated mice died uniformly within 3–4 days of infection. Drugs were dissolved in water or, if necessary, in dimethylsulfoxide (DMSO) (up to a maximal final concentration of 10% (v/v)). Parasitaemia was checked daily in a coverslip specimen of tail blood examined microscopically (×8 oc, 1/6 obj); curve was taken as absence of parasites for at least 30 days. Median curative and lethal doses (in uninfected mice) were estimated graphically [8].

Antitumour activity

A mastocytoma cell line (P815) [9] was maintained by intraperitoneal inoculation (10^7 cells) in inbred CBA/DBA$_2$F1 mice (16–20 g); the infection was uniformly fatal in 2–3 weeks. Percent increase in life span of batches of drug-treated mice over controls was computed by estimation of median survival time [10].

Electron microscopy

Buffy coat layers from trypanosome-infected mouse blood or from *in vitro* suspensions (10^7/ml in 50/50 rat serum/Krebs saline–glucose) were fixed and processed for electron microscopy as described by McLaren [11]. All samples were subjected to primary fixation in ice-cold 2.5% glutaraldehyde in 50 mM sodium cacodylate buffer, pH 7.2, containing 0.22 M sucrose for 18 hr at 4°. Specimens were then washed 3 times in sodium cacodylate buffer and either dehydrated in graded alcohols and embedded in Araldite directly, or subjected to post-fixation procedures before dehydration. In the first case, secondary fixation was carried out in 1% osmium tetroxide in *s*-collidine buffer for 1 hr at 0°. The samples were then washed in distilled water and in some cases, subjected to tertiary fixation for 30 min at 0° in 0.5% aqueous uranyl acetate, pH 5.0, containing 45 mg/ml sucrose. After being washed in distilled water the specimens were dehydrated in graded ethanols and embedded in Araldite. Sections prepared with a Reichert Ultracut and a diamond knife were collected on naked 300 mesh copper grids; some were left unstained while others were stained with lead citrate or uranyl acetate or both. Sections were examined with a Jeol 100 CX electron microscope. Representative areas of thick sections were subjected to X-ray emission probe analysis by courtesy of Jeol (UK) Ltd.

Table 2. Comparison of acute toxicity for mice of [PtCl$_2$(dac)] and [Pt(DMSO)$_2$(dac)]$^{2+}$

Complex	LD_{50}* (i.p.) (mg/kg)
II [PtCl$_2$(dac)]	28 (17.5–44.8)
III [Pt(DMSO)$_2$(dac)]$^{2+}$	140† (112.9–173.6)
	236‡ (190.3–292.6)

* Confidence limits (p 0.95) in parentheses. Complex III is significantly (2.9–8.6 times) less toxic than complex II (p 0.95).

† Dose expressed as [PtCl$_2$(dac)] equivalent (MW III/MWII = 1.686).

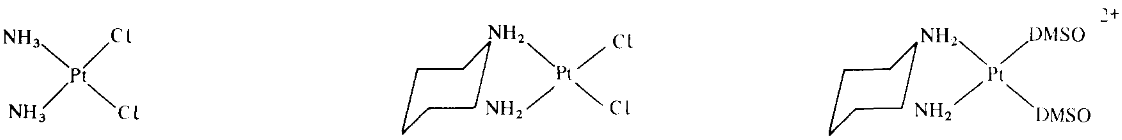
‡ Actual dose.

RESULTS AND DISCUSSION

(i) *Metal complexes*

The *in vitro* results obtained with the metal complexes are shown in Table 1 and the *in vivo* results using two selected compounds are given in Table 2. The data show that platinum complexes I, II and III, Fig. 1, are the most active, while the ruthenium complexes IV and V are slightly active. The remaining complexes with titres <3 may be considered to be inactive.

Since the initial discovery of *cis*-platin activity, a large number of complexes based on the *cis*-PtX$_2$(amine)$_2$ structure have been synthesized in attempts to improve the toxicity and water-solubility of the parent drug and a number of 'second generation' complexes with good pharmaceutical properties have been selected for detailed study [12]. Of these, the chelating diamine, 1,2-diaminocyclohexane (dac), has been widely used [13] and it is of interest to note that, in our case, substitution of dac for NH_3 does not significantly alter the activity (Table 1), although in mice the toxicity appears to be decreased. However, solubility still remains a handicap but the use of DMSO, as leaving group, in place of chloride greatly enhances the water-solubility and markedly decreases toxicity, again without great alteration of activity (Tables 1 and 2) [14]. In this respect the [Pt(dac)(DMSO)$_2$]$^{2+}$ cation presents considerable advantages over other analogues [15]. Independent



I II III

Fig. 1. Structures of trypanocidal platinum complexes.

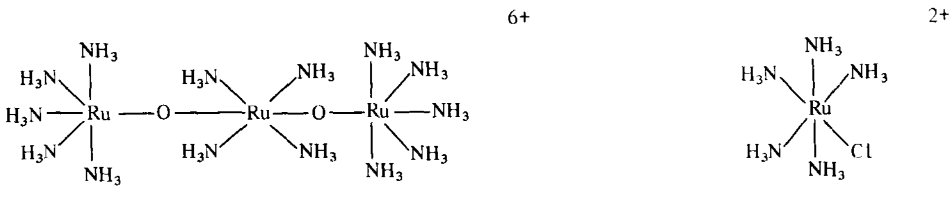


Fig. 2. Structure of ruthenium complexes with trypanocidal activity.

results show much the same pattern of activity in *T. venezuelense* (D. G. Crianeoscu, personal communication).

Although complexes I, II and III show trypanocidal activity *in vitro* (Table 1), they have no curative or therapeutic activity *in vivo*. Their trypanocidal activity extends only to a prolongation of the survival time of infected mice, and production of abnormal trypanosomes, without any clearing effect on the parasitaemia, and is demonstrable only at maximum tolerated doses (see Morphological Effects). Thus, modification of the basic skeleton of *cis*-$PtCl_2(NH_3)_2$ appears not to affect activity, and the platinum complexes, as a family, may be considered potentially active trypanocides. The use of disulfiram in the amelioration of toxicity of *cis*-platin in trypanosomiasis has also been reported [16].

A further set of complexes with activity, but less than that of the platinum series, are the ruthenium species IV and VI whose structures are shown in Fig. 2. Ruthenium red was first shown to have some activity against *T. brucei* in 1932, in a study of metal complexes undertaken by Fischl and Schlossberger [17]. Ruthenium red is also a well-known inhibitor of calcium transport [18] and this fact prompted a study of the complex as part of a series with this property, where the trypanocidal activity was again confirmed [2]. The structural similarity to known mononuclear complexes suggested a study of these also, but only $[RuCl(NH_3)_5]Cl_2$, (VI) had some slight activity. The chemically inert $[Ru(NH_3)_6]Cl_3$ was less active and substitution of the dac for NH_3 in this case (complex IX), resulted in loss of activity. Extensive studies have been made on the antitumour activity of ruthenium complexes; in general, compared with similar complexes of platinum, in approximate order of magnitude decrease in activity but reduced toxicity is observed [19]. Both ruthenium red [20] and $[RuCl(NH_3)_5]Cl_2$ [21] are active against tumours and this correlation appears also to hold in trypanosomiasis. Other complexes with reported antitumour but not trypanocidal activity are rhodium acetate [21], (X) *cis*-dichlorotetrakis (dimethylsulfoxide)-ruthenium [11] $[RuCl_2(DMSO)_4]$ (VII).

(ii) *Metal-drug complexes*

Where the structure of known drugs presents possible metal-binding sites, metal-drug complexes can

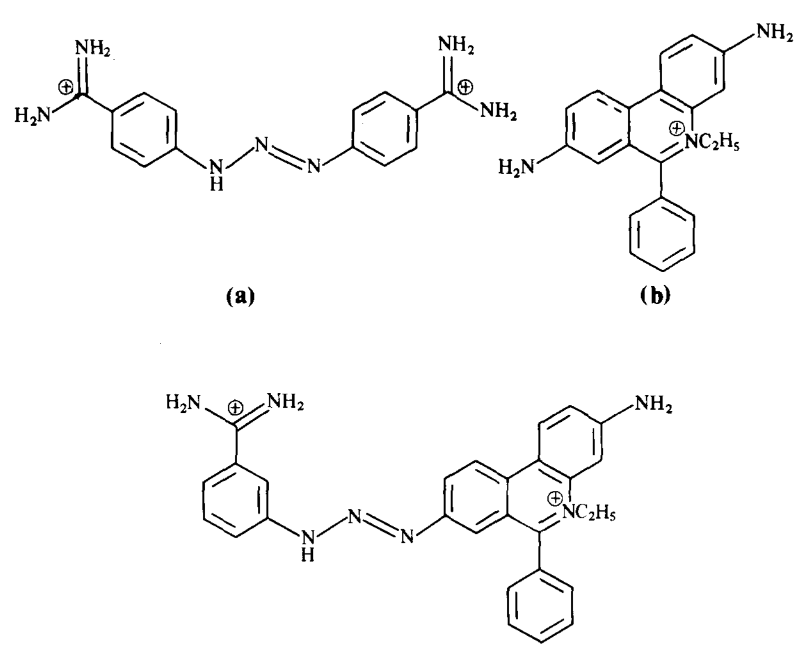


Fig. 3. Structural relationships of Berenil (a), Ethidium (b) and Samorin (isometamidium) (c) salts. Counter anions are omitted for clarity.

be prepared in an attempt to impart synergistic effects to the combination. The known drug may transport the metal ion or metal complex species to the target molecule within the cell, thus increasing efficiency of action. Applying these principles to trypanocidal drugs, our attention first focused on the diamidine trypanocide Berenil (4,4'-diazoaminodibenzamidine-bis(*N*-acetylglycinate)) (Fig. 3), and a number of metal complexes of the drug have been prepared and studied [23].

Berenil was chosen for a number of reasons:

(1) It has widespread use in the treatment of animal trypanosamiasis because of its high therapeutic index and the low incidence of drug-resistant strains induced by it.

(2) The mechanism of action has been well-studied and is known to involve DNA combination. The drug binds to nuclear and kinetoplast DNA by polar interaction of the amidine groups with phosphate anions leading to inhibition of synthesis [24, 25].

(3) The triazene linkage is a well-known metal-binding site and is distinct from the active site of the molecule (the amidine groups). The platinum-triazene structure may also be considered as an analogue of *cis*-$[PtCl_2(NH_3)_2]$.

(4) Berenil does not react with simple nucleosides such as adenosine, and since heavy metal complexes are known to react with the nitrogen atoms of purine and pyrimidine rings [26], the possibility existed of synthesis of a dual function agent.

Interaction of Berenil with K_2PtCl_4 in aqueous solution gives an immediate yellow precipitate which chemical studies confirm to be $(Berenil)^{+}$ $[PtCl_4]^{-}$, by exchange of counter anion; on stirring, this species transforms to the orange $PtCl_2$ complex. The hydrolysis behaviour has been studied and is shown in Fig. 4. This behaviour is relatively complicated and as some of the species produced will exist under the conditions of biological experiments [23], our initial tests were carried out on the salt, $[(Berenil)PtCl_2].2HCl$.

Following encouraging results with the Berenil complexes, similar complexes of two phenanthridine trypanocides, Ethidium and Samorin, (a hybrid of '*meta*Berenil' and Ethidium) (Fig. 3) were studied. The former was widely used for treatment of animal trypanosomiasis until it gave rise to widespread drug resistance; the latter, like Berenil, is still in field use. These complexes were also isolated as the $PtCl_4$ salts by reaction in solution with K_2PtCl_4.

In addition to the study of the drugs as their tetrachloroplatinate salts, we set out to prepare complexes containing covalent bonds between the drug and the metal, besides those shown in Fig. 4, as analogues of the platinum-amine complexes and to study structure–activity relationships. A number of complexes were prepared with Berenil and the methods of preparation and the structures are given in Table 3. In some of these cases palladium analogues were synthesized, and in others, ruthenium and

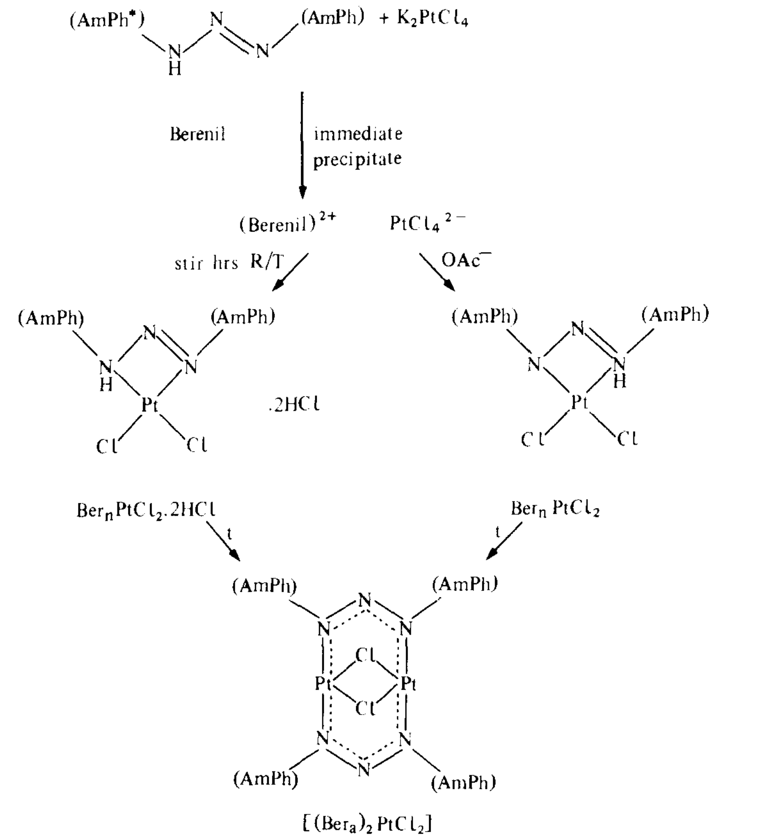


Fig. 4. Hydrolysis behaviour of platinum–Berenil complexes. Ber_n = neutral triazene, Ber_a = anionic triazene. See ref. 23 for a full discussion. * *p*-amidinophenyl residue; t = time; RT = room temperature.

Table 3. Preparation and structures of metal-Berenil complexes

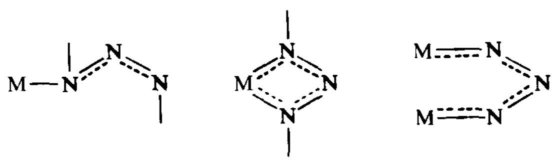
Starting complex	Conditions	Product*	Berenil bonding mode†
cis-$[PtCl_2(DMSO)_2]$	$AgNO_3$ MeOH/Et_3N	XII	Bidentate
$[PtCl_3(DMSO)]^-$	H_2O/Et_3N	XIII	Bidentate
$[PdCl_2(DMSO)_2]$	MeOH/Et_3N	XV	Bidentate
$[Rh_2(acetate)_4.2H_2O]$	MeOH	XVI	Monodentate
$[Rh_2(acetate)_4.2H_2O]$	MeOH/Et_3N	XVII	Bridging
$[Rh_2(butyrate)_4]$	MeOH/Et_3N	XVIII	Bridging
cis-$[RuCl_2(DMSO)_4]$	MeOH	XIX	Bidentate

* Numbers refer to complexes listed in Table 4.

† All Berenil ligands act as uninegative anions except for XVI and XIX where a neutral ligand is indicated [23].

rhodium complexes were prepared as derivatives of the antitumour complexes, VII and X, tested in Table 1.

The rationale behind the synthesis is as follows. Triazenes may bind to metals in one of these ways, similarly to carboxylates:



Examples of all three types have been structurally characterized [27–29]. Neutral triazenes are rare [30], generally due to employment of forcing conditions in the synthesis (e.g. base to deprotonate the nitrogen). In our study, physical methods showed the structures to be as indicated in Table 3. The DMSO complexes were prepared as analogue to those in Table 1. In the case of complex XVI, its identity was confirmed by comparison with $[Rh_2(OAc)_4(diphenyltriazene)_2]$, for which an X-ray crystal structure determination has been carried out (N. Farrell, unpublished results). Indeed, comparison with simple diphenyltriazene complexes is a necessary adjunct for structural characterization of all these complexes.

In vitro tests of a number of the complexes, (Table 4), showed that those with greater activity than the parent drug were $Pt(DMSO)_2(Berenil)^+$, $[Rh_2(acetate)_4(Berenil)_2]$ and $[Rh_2(butyrate)_3(Berenil)]$; the palladium and ruthenium species being less active. Butyrate substitution in rhodium carboxylates has been shown to give higher antitumour activity than other analogues and may be responsible for the difference in trypanocidal activity also, although there is a corresponding increase in toxicity which renders the rhodium carboxylate series impractical for *in vivo* use [21].

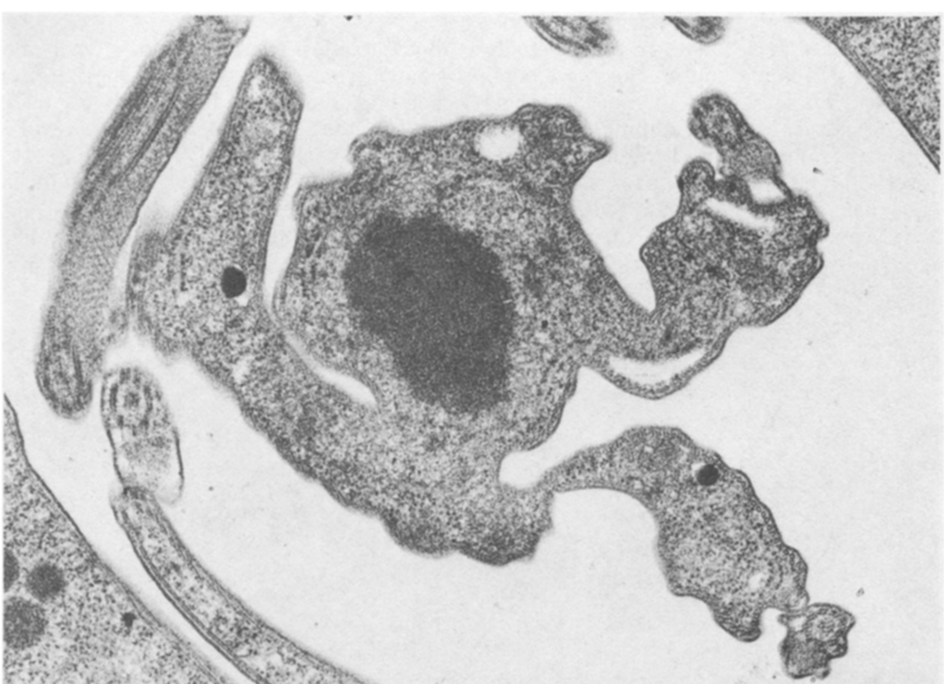


Fig. 5. Section showing an abnormally-shaped trypanosome with an enlarged nucleolus 23 hr after treatment of infected mice with 50–100 mg/kg $[PtCl_2(dac)]$ (×27,060).

Table 4. Activity of metal-drug complexes on *T. rhodesiense in vitro* (values given are maximum titres ($\log_{10} M^{-1}$) producing the effect)

Complex	Loss of motility	Loss of infectivity Partial	Total
Berenil	<3–4	6	4–5
XI [(Berenil)$PtCl_2$.2HCl]	3	5	5
XII [Pt(DMSO)$_2$Berenil]NO_3	<3	8	5
XIII [PtCl(DMSO)Berenil]	3	5	4
XIV [(Berenil)$PdCl_4$]	3	6	3
XV [PdCl(DMSO)Berenil]	3	4	3
XVI [Rh_2(acetate)$_4$(Berenil)$_2$]	3	7	5
XVII [Rh_2(acetate)$_3$(Berenil)]	<3	4	4
XVIII [Rh_2(butyrate)$_3$(Berenil)]	<4	8	5
XIX [$RuCl_2$(DMSO)$_2$(Berenil)]	3	3	3
Ethidium	4	5	4
XXI [(Ethidium)$_2PtCl_4$]	4	6	3
Samorin	3–4	6–7	5–6
XXI [(Samorin)$PtCl_4$]	4	6	5

Table 5. Comparison of activity of free drugs and metal-drug complexes on *T. rhodesiense* in mice (doses of metal-drug complexes are expressed as weight content of the parent drug; confidence limits of median doses in parentheses)

Compound	Mol. wt.	LD_{50}	CD_{50}	LD_{50}/CD_{50}
Berenil	515	140 (82–238)	1.90 (1.40–2.58)	74
XI [(Berenil)$PtCl_2$.2HCl]	618	221* (190–256)	1.91* (1.68–2.14)	116
XVI [Rh_2(acetate)$_4$(Berenil)$_2$]	1000	118* (87–160)	0.97† (0.72–1.30)	122
Ethidium bromide	394	38 (31– 46)	3.8 (2.26–6.38)	10
XX [(Ethidium)$_2PtCl_4$]	963	189† (136–262)	3.5* (2.19–5.60)	54
Samorin	496	44 (28– 69)	0.42	105
XXI [(Samorin)$PtCl_4$]	797	156† (123–198)	0.54	288

* Not significantly.
† Significantly different from parent drug (p 0.95).

The acute toxicity (LD_{50}) and curative activity (CD_{50}) of Berenil, Ethidium and Samorin are compared in Table 5 with those of the corresponding metal-drug complexes. Metal-substitution improves the chemotherapeutic index of all three drugs, but for [Rh_2(acetate)$_4$(Berenil)$_2$] the increase is due to greater curative activity without reduction of mammalian toxicity. In the case of the phenanthridine compounds, metal-substitution does not appear to affect the trypanocidal activity, but it significantly reduces the toxicity. The improvement in chemotherapeutic index is about two-fold for [Rh_2(acetate)$_4$(Berenil)$_2$], three-fold for Samorin and about five-fold for Ethidium.

Platinum did not affect the inactivity of Berenil against a Berenil-resistant strain of *T. rhodesiense,* in accord with the general supposition that such resistance arises, not from differences in uptake at

Table 6. Antitumour activity of metal drug complexes on mastocytoma P815 in CBA/DBA$_2$F1 mice

Test compound	Dose i.p. (mg/kg)	T/C*
Cyclophosphamide	100 × 1	138
Daunorubicin	1.88 × 1	128
[$PtCl_2$(dac)]	10 × 1	128
Berenil	50 × 1	87
[Rh_2(acetate)$_4$(Berenil)$_2$]	50† × 1	103
Ethidium	25† × 2	102
[(Ethidium)$_2PtCl_4$]	25† × 2	104, 105, 156
Samorin	100 × 1	110
[(Samorin) $PtCl_4$]	100 × 1	114

* T/C = (mean survival time of treated mice/mean survival time of control mice) × 100; acceptable activity ≥ 125% [43].
† Dose expressed as weight content of parent drug.

the final lethal target (DNA) but at intermediate uptake stages.

The possibilities of platinum-complex synergy with Ethidium have yet to be explored, but preliminary experiments with $[PtCl_2(dac)]$ and Berenil showed that no significant synergy of trypanocidal activity was discernible.

The antitumour activity of some of these complexes is shown in Table 6. Berenil itself can be considered to be inactive but a slight potentiation is observed for $[Rh_2(acetate)_4(Berenil)_2]$. In the case of Ethidium the improved LD_{50} values in its platinum complex allow for use of larger doses with some potentiation. Various treatment schedules failed to give T/C values greater than 125%, but in one exceptional case, two separate doses of 25 mg/kg gave a value of 156%, which would warrant further investigation.

As Berenil represents a phosphate-binding ligand whereas Ethidium is an intercalator, it was of interest to see if these results could be extended to other drugs and ligands which bind to DNA in the same manner. Complexes of platinum and rhodium were therefore prepared with the antimalarial chloroquine and spermidine. The complexes, $[Rh_2(acetate)_4spermidine]$, $[(Rh_2(acetate)_4$ chloroquine], chloroquine $PtCl_4$ and $[(PtCl_2)_2$ spermidine] had only weak trypanocidal action.

MORPHOLOGICAL EFFECTS

Metal complexes

Examination by light microscopy of Giemsa-stained thin blood films taken from the tail blood of infected mice at varying short-term intervals after treatment with maximum tolerated doses of complexes I and II, showed that abnormal trypanosomes appeared in proportion to the dose and length of time after treatment.

Abnormal division forms (anucleate, multinucleate, giant, and multiple, incompletely divided forms) indicated effects on nuclear DNA; the giant forms were approximately twice as long and five times as wide as the normal parasite.

At the ultrastructural level, complexes I and II at 3½ hr after treatment with doses of 25 mg/kg and 50–100 mg/kg respectively, were seen to have produced central clumping of the nuclear chromatin of deformed trypanosomes (loss of peripheral chromatin with apparent enlargement of the nucleolus) (Fig. 5), but there were no effects on the kinetoplast. Complex I (*cis*-platin) induced the formation of microvesicles (approx. 50 nm dia.) on the red cell membrane. Similar 'nanovesicle' membrane blebbing has been observed in erythrocytes and other cells after exposure to calcium ionophores [31], or as a result of ageing [32]. A possible cause is microfilament interaction [34] which has been noted in the present study in trypanosomes treated with complex II and with $[(Berenil)PtCl_2]$ (Fig. 8) (see below).

The effects of complex II on trypanosome ultrastructure *in vivo* were progressive. In samples taken from infected mice 3½ hr after a dose of 50–100 mg/kg, large numbers of empty membrane-bound vesicles were produced in the cytoplasm. At 23 hr after treatment, apart from nucleolar enlargement and general loss of peripheral nuclear chromatin, increased numbers of microbodies and dense lysosomal vesicles were seen. In samples fixed with glutaraldehyde alone, short (5 s) lead staining of sections showed dense particulate deposits in the lysosomal vesicles *cf* [34] which were still visible after longer (5 min) lead staining. This result differed from that obtained with the normal untreated trypanosomes where prolonged lead staining of sections caused total loss of the vesicle from the section, giving a 'punched-out' appearance. Particulate lysosomal deposits occurred also in trypanosomes treated with complex I, indicating some lysosomal interaction with the complexes, possibly related to extensive autophagic vacuole formation (Fig. 6).

Cis-platin (complex I) is known to produce giant multinucleate tumour cells (transplantable fibrosarcoma) 4–6 days after treatment of infected mice with a single intraperitoneal dose of 8–9 mg/kg [34, 35]. Large numbers of dense lysosome-like bodies appeared at this time. Giant multinucleate fibrosarcoma cells were also produced within 2 hr *in vitro* in the presence of 2 μg/ml *cis*-platin [34]; in both cases, cell fusion and cytokinesis inhibition appear to be responsible.

In vitro, after 1¾–2½ hr at 37°, complex II $[PtCl_2(dac)]$, at a maximum concentration (1 mM ≡ 373 μg/ml) chosen to produce pre-lethal effects detectable in the light microscope, had little apparent effect on the ultrastructure of trypanosomes, as compared to control untreated suspensions. Some treated parasites showed dense deposits in the matrix of the kinetoplast-mitochondrial tubule labyrinth and regions of the mitochondrion showed myelin-like figures (Fig. 7). Similar mitochondrial matrix deposits occur in renal tubes after *cis*-platin treatment [33]. Despite the high concentration of drug used here, platinum was not detectable by X-ray

Table 7

Complex	Dose (mg/kg)	% Abnormal forms after 5 hr	24 hr
I *cis*-$PtCl_2(NH_3)_2$	25		5
	50	1–3	36–44 (16)
II $PtCl_2$ (dac)	100	3	54 (32)
	50	2	40

Values in parentheses are % anucleate forms.

Fig. 6. Autophagic vacuoles seen in trypanosomes 23 hr after treatment of infected mice with 50–100 mg/kg [$PtCl_2$(dac)] (×18,000).

emission probe analysis of representative areas in thick sections of the trypanosomes. This technique has demonstrated platinum localization in the nucleolus and inner nuclear membrane of HeLa cells after *in vitro* exposure for 4 hr to a lethal dose of 60 μg/ml *cis*-platin [37].

Metal-drug complexes

A primary reason for attaching heavy transition metals to known drugs was to investigate the possibility that such electron-dense markers might be used to localize drugs in electron micrographs of treated trypanosomes. As with the metal complexes, parasite

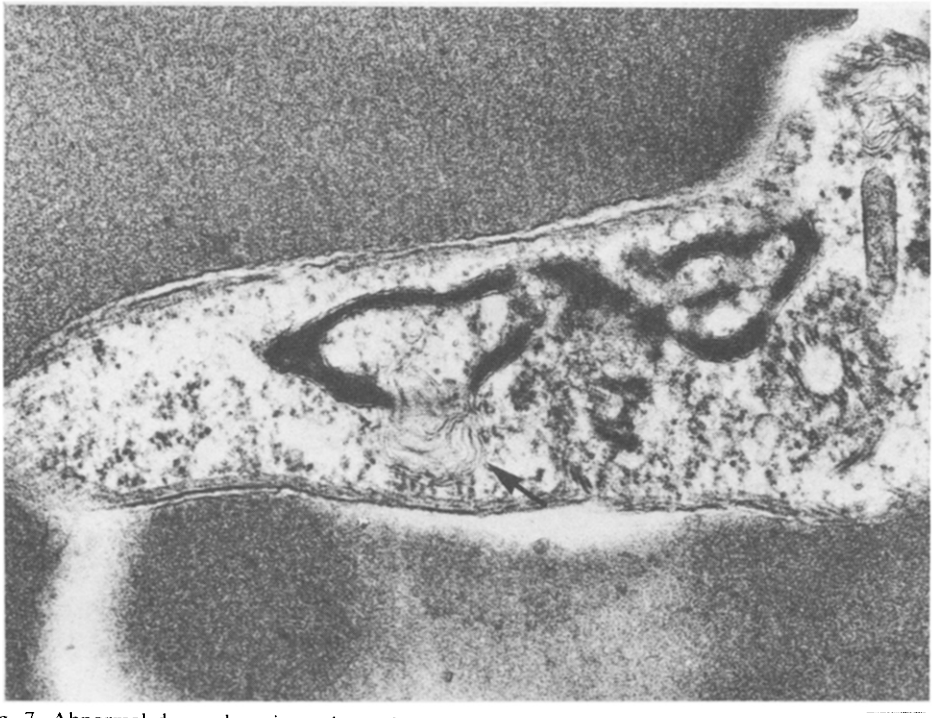


Fig. 7. Abnormal dense deposits and membraneous configurations (arrow) within the mitochondrial tubules of trypanosomes after exposure for 2½ hr *in vitro* to 1 mM [$PtCl_2$(dac)] (×44,200).

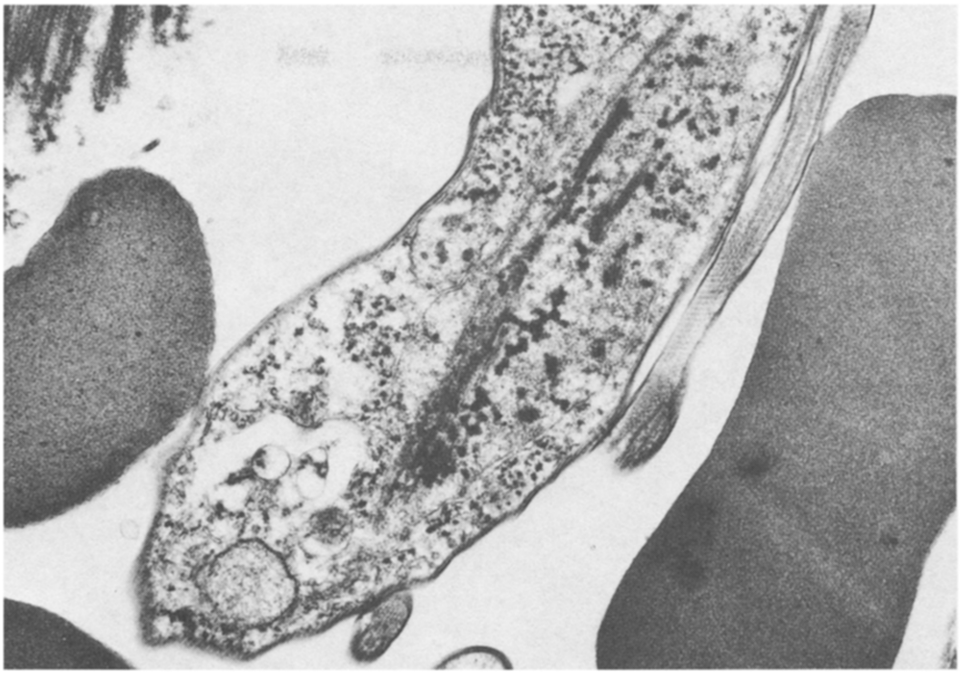


Fig. 8. Trypanosomes exposed for $2\frac{1}{2}$ hr *in vitro* to 1 mM [$PtCl_2$(Berenil)] show enlarged ribosomes and prominent microtubules/microfilaments in the nucleoplasm (×18,000).

samples were examined after both *in vitro* and *in vivo* treatment with platinum- and rhodium-Berenil complexes.

In vitro, after $2\frac{1}{2}$ hr at 37°, 1 mM [$PtCl_2$(Berenil)] produced two lesions typical of the parent drug and other related diamidines [39–41], i.e. nucleolar fragmentation and kinetoplast DNA condensation. In addition, the nuclear chromatin sometimes became marginated (Fig. 10), ribosomes were enlarged and aggregated, dense (spindle?) microtubule/microfilament elements appeared in the nucleoplasm (Fig. 8), and dense elongated bodies resembling the elongated microbodies in *Leptomonas samueli* [42] appeared in the cytoplasm (Fig. 9). One mM[Rh_2

Fig. 9. Arrays of elongated dense bodies seen in the cytoplasm of trypanosomes after exposure for $2\frac{1}{2}$ hr *in vitro* to 1 mM [$PtCl_2$(Berenil)] (×44,000).

Fig. 10. Abnormally marginated nuclear chromatin in trypanosomes exposed for $2\frac{1}{2}$ hr *in vitro* to 1 mM [$PtCl_2$(Berenil)] (×27,300).

(acetate)$_4$(Berenil)$_2$] caused intracellular damage which was too extensive for detailed localization of specific lesions, but chromatin margination was apparent in trypanosomes with recognizable nuclei.

In vivo, both at 6 and 23 hr after a single curative dose of [$PtCl_2$(Berenil)] (5 mg/kg), trypanosomes showed all three major lesions typical of diamidine drugs (nucleolar segregation and fragmentation, kinetoplast DNA condensation and cytoplasmic cleft formation) but, in addition, there was abnormal distribution and margination of chromatin as *in vitro* (Fig. 10), autophagic vacuole formation and in unstained sections, deposition of fine dense granules in lysosomal vesicles. No elementary platinum or rhodium was detected by X-ray emission probe analysis in thick sections of parasites from either the *in vitro* or *in vivo* experiments.

A general feature of all sections of trypanosomes exposed to metal or metal-drug complexes was an apparent intensification of heavy metal staining, as noted by Sodhi and Sarna [35]. The appearance of pronounced nuclear microfilament/microtubules after [$PtCl_2$(Berenil)] treatment *in vitro* and after ($PtCl_2$ (dac)) treatment *in vivo,* may be related to the *cis*-platin-microfilament interaction observed in sarcoma cells [34], where production of multinucleate cells was ascribed to inhibition of cytokinesis following depolymerization of microfilaments by Pt. This interpretation has also been applied to the inhibition of chemotactic activity of macrophages by platinum complexes [38].

CONCLUSIONS

Our results show that metal-drug complex formation gives products which are, in general, less toxic than simple platinum species. An exception is [Pt(dac)$(DMSO)_2]^{2-}$ whose low toxicity is comparable to that of metal-drug complexes. Improved water-solubility is an important asset of the latter, and this property and lower toxicity are functions of the ligand (i.e. drug) portion of the complex. The therapeutic indices of a number of metal-drug complexes are higher than that of the free drug, in some cases due to lower toxicity but in others because of increased activity, where they represent viable curative agents.

Unlike the free drug, the metal-drug complexes react with simple bases, and produce effects on trypanosome ultrastructure typical of the parent drug. Extra lesions which are not found on treatment with the parent drug alone, can be attributed to the effect of the metal; the ligands may thus be considered to be metal-transport agents. The practical utility of this kind of 'low toxicity metal-carrier' is likely to be more evident in transition-metal-sensitive tumour treatment, and preliminary experiments (Table 5) suggest that metal substitution may confer some anti tumour activity on Berenil and Ethidium.

We were unable to detect platinum or rhodium by X-ray emission probe analysis in drug-treated trypanosomes. As the detection limits of this method have been estimated as 0.1–0.001 pg [36], an approximate calculation shows that in the *in vitro* experiment with 10^7 trypanosomes in 1 mM [$PtCl_2$(dac)], uptake of at least 1% of the available platinum should have been detectable. Despite the abnormally high concentrations of platinum-metal complex used here and in experiments on tumour cells [37] where intracellular platinum was detected, it may be that the reduced uptake of platinum-metal complexes in trypanosomes reflects their relative insensitivity to the complexes compared to tumour cells. In the case of the metal drug complexes where effective drug concentrations are much lower, metal uptake is likely to be below the detectable limits.

The good correlation which exists between antitumour and trypanocidal activity has been demonstrated by a survey where a group of 66 compounds with the former activity was found to contain 25 with trypanocidal activity, a 'hit rate' of 22.7%, which is twice as high as that found when other selection methods are applied [37]. This correlation, which probably extends also to anti-viral activity, may reflect the susceptibility of template-DNA-directed RNA synthesis as a common target in all three types of disease agent [2]. The results on the complexes described here and the similarity of many of the morphological effects on trypanosomes to those described in tumour cells, allow us to state that metal complexes fall into this category and, apart from their intrinsic interest for trypanosomiasis treatment, confirm the therapeutic utility of parallel trypanosome-tumour models.

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