Properties of a Chloroquine Adduct of Rhodium Acetate

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Complexation of DNA-binding ligands by metals can produce metal transport agents which may affect the biological activity of the individual species. Where the DNA-binding agents are selectively absorbed by an organism or particular cell type, a systematic approach to selective toxicity of metal agents is possible. Previous studies using platinum and rhodium complexes of Berenil, a DNA-binding agent active against *T. rhodesiense* (the causative agent of sleeping sickness), have demonstrated the utility of this concept and, in some cases, complexes with higher activity than the parent ligand were found [1, 2]. The applicability of this reasoning to the design of antitumor agents has also been demonstrated [1]. In an extension of these studies, we have studied a chloroquine adduct of rhodium acetate (7-chloro-4-quinolinyl-*N*-*N'*-diethyl-1,4-pentanediamine) and this paper reports on its chemical and biological properties.

The use of DNA-binding ligands to target metal complexes has gained further relevance recently with the demonstration of ternary complex formation between an intercalator such as ethidium bromide or proflavine and cisplatin–DNA adducts [3–5]. It is therefore axiomatic that the opposite of this reaction, i.e. reaction of a metal-intercalator (or metal–DNA binder) complex with the purine and/or pyrimidine bases of the polynucleotide can also occur. Besides the transport of metal complexes to DNA, complexes of metal–DNA-binding ligands are likely to produce potential lesions structurally different from those derived directly from, for instance, cisplatin.

Experimental

IR spectra were obtained as KBr discs on a Perkin-Elmer 1430 spectrophotometer. UV-Vis spectra were run in DMF on a Perkin-Elmer Lambda 4B instrument and NMR spectra were recorded in d_7-DMF at 250 MHz on a Bruker WM250. Elemental analyses were by Robertson Laboratories, New Jersey.

The complex was prepared by addition of an equimolar aqueous solution of chloroquine (as its diphosphate salt) to an aqueous solution of rhodium acetate, followed by addition of excess of triethylamine. The blue–purple complex precipitated immediately, and was filtered, washed with water and acetone and dried *in vacuo*.

The biological assays discussed in the text were performed as previously reported for both trypanosomiasis [1] and antitumor activity [6]. The parameters for these tests are loss of infectivity in trypanosomes and the ID_{50} (the dose required to reduce cell growth by 50%) for antitumor activity. Both values are reported in the text as $-\log M$, where M is the molar concentration. The complex was dissolved in DMSO immediately prior to dilution in medium.

Results and Discussion

The study of Berenil complexes of rhodium carboxylates suggested the study of a similar chloroquine system, since the ligand has well-defined DNA-binding properties [7] and should bind through the ring nitrogen ($pK_a = 8.1$) [8]. Reaction of the commercially available salt chloroquine diphosphate with $[Rh_2(OAc)_4]$ in the presence of a base gives a product the elemental analysis and characterisation data of which (Table 1) indicate the formula $[ChlRh_2(OAc)_4]$ with Chl occupying one of the axial positions. No reaction occurs in the absence of base. The presence of chloroquine is easily observed by both IR and NMR spectroscopy. The ^1H NMR integration shows the 1:1 stoichiometry of the quinoline moiety to the rhodium acetate core. The color is typical of N-bound adducts of rhodium carboxylates and the proposed structure is as in Fig. 1. The complex is formally five-coordinate, but it is possible that in the solid state polymer formation occurs through axial binding of the $-NEt_2$ terminal end of the chloroquine, as has been proposed for similar adenine and adenosine adducts [9].

Biological Activity

Preliminary studies of the biological activity of the chloroquine–rhodium complex indicate properties distinct from either component. The *in vitro* biological activity was studied to examine both trypanocidal and antitumor activity, see Table 2. The correlation between these two effects is known for a wide variety of agents [10] and has been demon-

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TABLE 1. Chemical characterisation data for [(chloroquine)-$Rh_2(OAc)_4$]

Elemental analysis:	
Calc. (Found) (%)	C, 40.95 (41.4); H, 4.98 (5.46); N, 5.51 (5.72)
IR (cm^{-1})	ν(COO) = 1580, 1435 ν(NH) = 3360
^1H NMR[a] (δ)	δ(acetate): 1.8 δ(chloroquine): 1.0(t), 1.43(d), 1.72(m, br), 2.5(q), 3.56(br), 6.95(br), 7.58(d), 8.80(m, br)
UV–Vis (λ_{max}, nm; log ϵ)	270.5 (3.68), 322.0 (3.65) 345.0 (3.64), 573.6 (2.0)

[a]Relative to TMS: d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

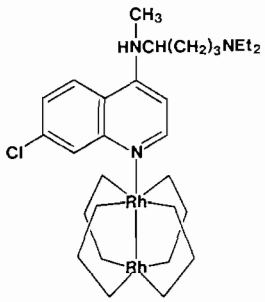

Fig. 1. Proposed structure of the chloroquine adduct of rhodium acetate.

strated previously for a range of metal complexes [11]. The trypanocidal effects of the chloroquine adduct have been briefly mentioned previously [1]. Interestingly, the *in vitro* studies on *T. rhodesiense* show the adduct to be more active ($-\log M = 5$) than either individual component, neither of which showed inhibition of infectivity up to 10^{-3} M ($-\log M = 3$). The activity of the adduct is therefore unusual as the properties of antimalarials and trypanocides are not in general coincident. The chloroquine adduct is also active *in vivo* in *P. falciparium* (malaria) at doses equivalent to the free ligand [12]. This is certainly of relevance because of the increasing incidence of chloroquine-resistant strains of malaria and further studies are warranted.

The strong correlation between antitrypanosomal and antitumor activity, along with the known antitumor properties of rhodium carboxylates [13], prompted us to examine the properties of the chloroquine–rhodium acetate complex in some murine tumor screens. The results in a standard screen such as L1210 leukemia showed the complex to be moderately active ($-\log M = 4.33$) and essentially equivalent to the simple rhodium acetate ($-\log M = 4.64$). Chloroquine itself showed no activity in L1210.

An increasing area of interest for the biological activity of metal complexes is the question of how tumor-selective or tumor-specific complexes may be designed. The selective uptake of chloroquine by melanin-containing cells [14] suggested the possibility that a selective antitumor response might be obtained, and to examine this aspect the activity of the complex was tested in B16 melanoma. In the B16 case there was a larger difference between the chloroquine adduct and either the free ligand or free complex. The rhodium complex itself showed no apparent selectivity between L1210 and B16. Free uncomplexed chloroquine was slightly more cytotoxic to B16 cells but the rhodium–chloroquine adduct was significantly more cytotoxic in the B16 system (Table 2). The molar ratio refers to the ratio of ID_{50} in μM for the two tumor lines. The nature of this difference needs to be examined and these preliminary results should be corroborated *in vivo* and also correlated with the melanin content of the B16 line. The results do indicate, however, that carrier molecules may be used to produce selective effects of metal complexes in tumors. The biological data further emphasise the correlation in antitumor and antitrypanosomal action of metal complexes and reveal a rewarding area for further research.

TABLE 2. Biological activity *in vitro* of [(chloroquine)$Rh_2(OAc)_4$] [a]

	Trypanocidal activity (Loss of infectivity, $-\log M$) *T. rhodesiense*	Antitumor activity (ID_{50}, $-\log M$)		
		L1210	B16	Molar ratio[b] (L1210/B16)
Chloroquine	<3	<4.5	5.2	>5
$[Rh_2(OAc)_4]$	<3	4.64	4.85	1.68
$[ClRh_2(OAc)_4]$	5	4.33	6.13	63

[a]For details see references in Experimental. [b]Molar ratio is the ratio of ID_{50} in μM.

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