Synthesis of Metal Complexes of Antimalarial Drugs and *In Vitro* Evaluation of their Activity Against *Plasmodium falciparum*

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Abstract

Complexation behaviour of two well known antimalarials, amodiaquine and primaquine, with VO(II), Cr(III), Fe(III), Cu(II), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Rh(III), Pd(II), Au(III), Ag(I), Mn(II), Sn(II) and Pt(II) has been studied. The corresponding complexes have been synthesized, characterised and screened by an *in vitro* microtechnique for their schizonticidal activity with a view to search for a more effective agent. The observed activities of the parent drugs and their metal complexes have been discussed to explain their mode of action.

Introduction

Coordination metal complexes are gaining increasing importance particularly in the design of repository, slow release or long acting drugs, in nutrition and in study of metabolism [1]. Metal ions are also known to accelerate drug action. The efficacy of a therapeutic agent is known to be enhanced upon coordination with a metal ion [2]. Some metal complexes are also known to exhibit remarkable antitumor activity [3, 4]. The present studies were planned with reference to one of the commonest tropical diseases, malaria. The management of this disease involves administering a suitable antimalarial drug, the major portion of which is usually lost via urinary excretion and is not available to combat the parasite. In our search for a suitable metal complex of the drug as its substitute, 32 metal complexes of amodiaquine (a blood schizontocide) and primaquine (a tissue schizontocide) were synthesized and screened for their blood schizontocidal activity by the *in vitro* microtechnique [5]. The results obtained have been interpreted in light of the existing information on the subject.

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Experimental

Amodiaquine hydrochloride (Park-Davis) and primaquine diphosphate (Indian Drugs and Pharmaceuticals Ltd.) were complexed with oxovanadium(II), chromium(III), iron(III), copper(II), cobalt(II), nickel(II), zinc(II), cadmium(II), mercury(II), rhodium(III), palladium(II), gold(III), silver(I), manganese(II), tin(II) and platinum(II) by the methods described below.

Synthesis of metal complexes

Amodiaquine metal complexes

An aqueous or methanolic solution of the metal salt (all chlorides except $VOSO_4$, $AgNO_3$, K_2PtCl_4 and $KAuCl_4$) was mixed with an aqueous or methanolic solution of amodiaquine hydrochloride (dissolved in minimum amount of the solvent) in 1:1 stoichiometric ratio. Solid separated out immediately in case of tin and platinum but in other cases the reaction mixture had to be refluxed for 1/2–1 h on a water bath. During heating, solids separated out in the cases of Zn(II), Hg(II), Cd(II), Ag(I) and Au(III), while in the rest, the volume of the reaction mixture had to be reduced to about one third, when the solid complex separated out on cooling. It was filtered and washed with ice cold water and dried over P_4O_{10} *in vacuo*. The complexes were purified by recrystallization from ethyl alcohol. The yield of the complexes was found to be ~55–65%.

Primaquine metal complexes

To an aqueous ethanolic solution of the metal salt (all chlorides except $VOSO_4$, $AgNO_3$, $KAuCl_4$ and K_2PtCl_4) was added an aqueous solution of the ligand (3 mol in case of trivalent metal ions and 2 mol in the rest). The reaction mixture was refluxed on a water bath for about 1/2 h followed by reduction of the volume to about one third. Solid separated out immediately at room temperature in the case of Ag(I), Pt(II), Pd(II), Rh(III) and Au(III), whereas in the

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TABLE I. Elemental Analysis and Molecular Weight Data of Amodiaquine(L)–Metal Complexes (L = $C_{20}H_{22}N_3OCl$)

Complex	%Found (calculated)				Molecular weight: Found (calculated)
	C	H	M	Cl	
$VOLSO_4$	46.92 (46.30)	4.35 (4.27)	10.58 (9.82)	5.11 (6.84)	528.50 (518.80)
$CrLCl_3$	45.67 (45.12)	4.33 (4.17)	9.18 (9.77)	25.75 (26.67)	533.45 (532.37)
$MnLCl_2$	46.68 (49.86)	4.74 (4.60)	11.92 (11.40)	21.50 (22.10)	519.59 (517.81)
$FeLCl_3 \cdot H_2O$	45.15 (44.72)	4.77 (4.13)	10.66 (10.40)	26.94 (26.43)	540.55 (537.22)
$CuLCl_2$	49.00 (48.95)	5.12 (4.52)	13.55 (12.96)	22.32 (21.72)	494.85 (490.41)
$CoLCl_2$	49.76 (49.45)	4.50 (4.57)	12.94 (12.13)	22.83 (21.92)	488.24 (485.78)
$NiLCl_2 \cdot 2H_2O$	45.98 (46.06)	5.56 (5.02)	11.88 (11.26)	21.61 (20.42)	525.71 (521.58)
$CdLCl_2$	45.21 (44.55)	4.74 (4.11)	21.47 (20.84)	20.25 (19.75)	541.42 (539.27)
$ZnLCl_2$	48.32 (48.80)	4.95 (4.51)	13.32 (13.28)	22.04 (21.64)	491.57 (492.24)
$HgLCl_2$	38.45 (38.29)	3.34 (3.53)	32.38 (31.97)	15.58 (16.97)	632.55 (627.46)
$SnLCl_2$	44.45 (44.03)	4.81 (4.07)	22.39 (21.76)	20.01 (19.52)	
$AgLNO_3$	46.00 (45.69)	4.51 (4.22)	20.77 (20.52)	7.00 (6.75)	
$AuLCl_3 \cdot H_2O$	35.80 (35.47)	3.32 (3.57)	30.15 (29.08)	20.23 (20.96)	
$RhLCl_3 \cdot H_2O$	41.87 (41.18)	4.35 (4.15)	17.93 (17.64)	25.18 (24.35)	588.52 (583.28)
$PdLCl_2$	45.42 (45.05)	4.73 (4.16)	19.11 (19.95)	19.25 (19.97)	536.30 (533.27)
$PtLCl_2$	38.57 (38.62)	3.22 (3.57)	31.58 (31.37)	16.93 (17.12)	

other cases the mixture had to be left in a refrigerator for about 12 h for the solid to separate out, with yield ~60–65%. All the complexes are soluble in water, alcohol, acetone and DMSO.

Characterization of Metal Complexes

All the metal complexes were characterized on the basis of elemental analysis, molecular weight determination, UV–Vis and IR spectra, magnetic and conductance measurement, and thermogravimetric studies. The elemental analysis and molecular weight data (Tables I and II) suggested a 1:1 (metal:ligand) ratio for metal–amodiaquine complexes. In the case of primaquine complexes, the general formula for trivalent and other metal ions appears to be $[ML_3]X_3$ and $[ML_2]X_n$, respectively, where X is an anion and n is the oxidation state of the central metal atom. Infrared spectra suggested the bonding of the metal in the two cases as in Figs. 1 and 2, respectively.

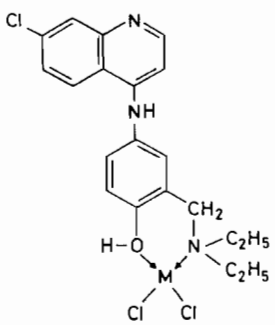

Fig. 1. Amodiaquine–metal complexes. M = Cu(II), Co(II), Zn(II), Sn(II), Pt(II), Pd(II), Hg(II) and Cd(II).

TABLE II. Elemental Analysis and Molecular Weight Data of Primaquine (L)–Metal Complexes (L = $C_{15}H_{21}N_2O$)

Complex	% Found (calculated)				Molecular weight: Found (calculated)
	C	H	Cl	M	
VOL_2SO_4	53.02 (52.86)	6.55 (6.21)		9.98 (9.82)	685.20 (681.71)
CrL_3Cl_3	58.13 (57.71)	6.67 (6.78)	12.00 (11.37)	5.84 (5.55)	939.81 (936.56)
$MnL_2Cl_2 \cdot 2H_2O$	52.71 (52.93)	6.77 (6.81)	10.74 (10.43)	8.68 (8.07)	681.14 (680.65)
FeL_3Cl_3	57.86 (57.48)	6.92 (6.75)	11.97 (11.32)	5.81 (5.94)	942.98 (940.41)
CuL_2Cl_2	55.43 (55.16)	6.62 (6.48)	10.62 (10.87)	9.95 (9.73)	658.79 (653.25)
CoL_2Cl_2	48.45 (48.78)	7.26 (7.09)	9.85 (9.61)	7.73 (7.98)	730.23 (738.63)
$NiL_2Cl_2 \cdot 2H_2O$	52.72 (52.65)	6.95 (6.77)	10.19 (10.37)	8.23 (8.58)	686.61 (684.42)
$ZnL_2Cl_2 \cdot 2H_2O$	52.81 (52.13)	6.35 (6.13)	10.53 (10.27)	9.88 (9.46)	699.52 (691.08)
CdL_2Cl_2	51.73 (51.32)	6.51 (6.03)	10.71 (10.11)	16.44 (16.01)	707.98 (702.11)
HgL_2Cl_2	45.83 (45.59)	5.57 (5.36)	8.26 (8.98)	25.51 (25.38)	792.87 (790.30)
SnL_2Cl_2	51.10 (50.87)	5.52 (5.98)	10.65 (10.02)	16.78 (16.75)	713.99 (708.40)
AgL_2NO_3	53.03 (52.33)	6.64 (6.15)		15.91 (15.67)	692.68 (688.58)
AuL_3Cl_3	50.03 (49.98)	5.73 (5.87)	9.18 (9.85)	19.08 (18.21)	1083.98 (1081.53)
RhL_3Cl_3	54.88 (54.74)	6.91 (6.43)	11.32 (10.79)	10.95 (10.42)	989.92 (987.47)
PdL_2Cl_2	51.57 (51.76)	6.72 (6.08)	10.10 (10.20)	14.67 (15.28)	699.25 (696.11)
PtL_2Cl_2	45.42 (45.91)	5.58 (5.39)	9.88 (9.05)	25.21 (24.86)	787.54 (784.40)

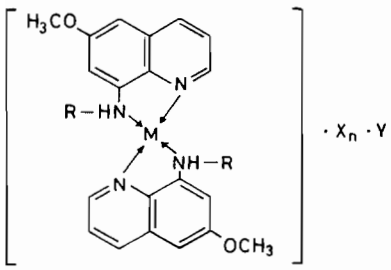

Fig. 2. Primaquine–metal complexes. R = $-\underset{\displaystyle CH_3}{\underset{|}{CH}}-(CH_2)_3-NH_2$

M = Cu(II), Co(II), Zn(II), Sn(II), Ag(I), Cd(II), Hg(II) and Pd(II); X = univalent anion; n = oxidation state of metal ion; Δ = $2H_2O$ in case of Zn, $5H_2O$ for Co and nil in all other cases.

In Vitro Assay

The *in vitro* microtechnique [5] was employed to test the inhibitory activity of amodiaquine, primaquine and their metal complexes. In all the studies, a culture-adapted strain of *P. falciparum* (FAN-5) at different subculture levels was used. Millimolar stock solutions were prepared in a suitable solvent, filtered through 0.45 μm membrane and stored at 4 °C until used. Further serial dilutions were prepared in RPMI-1640 complete medium and used in the tests. Appropriate solvent controls were included. Salt solutions of toxic metal ions, Hg(II), Ag(I), Sn(II), and Cd(II) were also tested by the aforesaid method.

TABLE III. Minimum Inhibitory Concentration of Antimalarial Drugs, their Metal Complexes and Metal Salts

Compound	MIC	Activity
Amodiaquine	10^{-7} M	1
Complexes of amodiaquine	10^{-7} M	1
Primaquine	10^{-6} M	1
Complexes of primaquine	10^{-6} M	1
$HgCl_2$	10^{-10} M	1000
$CdCl_2$	10^{-10} M	1000
$SnCl_2$	10^{-8} M	10
$AgNO_3$	10^{-8} M	10
$KAuCl_4$	10^{-7} M	1
K_2PtCl_4	10^{-7} M	1
$CuCl_2$	10^{-7} M	1

Results and Discussion

The antimalarial activity of the synthesized metal complexes, whether of toxic or nontoxic metal ions, was found to be of the same order as the corresponding parent drug (Table III). The minimum inhibitory concentration of amodiaquine and its metal complexes was 10^{-7} M while that of primaquine and its metal complexes was 10^{-6} M. Since from *in vitro* studies only toxicity to the plasmodia can be studied, the results do not relate to toxicity to the host. Further, it may be inferred that antiparasitic activity of the two selected antimalarial drugs is independent of their coordination to any metal atom. However, it was interesting to note that some toxic metals in their salt form (e.g., $HgCl_2$ and $CdCl_2$) exhibited higher inhibitory activity (Table III) than their coordination complexes with drugs. It suggests that the metals in ionic form are more toxic to the parasite than in the complexed form.

In order to explain the above observations it is appropriate to examine the mode of action of antimalarial drugs. The mode of action is an intraerythrocytic performance. The erythrocyte is known to transport oxyhaemoglobin complexes and carbon dioxide from lungs and tissue. In the case of erythrocytes infected with plasmodium, degradation of haemoglobin takes place. It has been shown that chloroquine, quinine and related drugs are effective antimalarial agents against parasites that digest haemoglobin [6]. Ferriprotoporphyrin IX (FP), a product of intraerythrocyte haemoglobin degradation, is known to play an important role in the activity of the aforesaid drugs because of its affinity for nitrogenous bases [7, 8]. Studies have proved that FP is a high affinity drug receptor of malaria parasites [9, 10]. Recently Banyal and Fitch [11] have shown that these drugs act by diverting FP from non-toxic complexes with soluble intracellular haem binders into a toxic drug–FP complex which affects the parasite.

The mode of interaction between ferriprotoporphyrin and drugs has been found to be different from that of coordination complexes between the iron of metalloporphyrin and organic bases [10], and it has been suggested that the interaction probably involves the aromatic ring portions of reacting molecules through π-orbital overlap or charge transfer. The identical activity of the drug and its metal chelates in the present case suggests that chelating centres of the drug are not responsible for its antimalarial action because blocking the chelating centres does not affect the drug activity. The present study, therefore, lends support to the FP theory for the mechanism of action of the drugs of this class.

The fact that metal salts have a higher toxicity than the corresponding complexes may be attributed to the higher permeability of red cells to the former than the latter. An erythrocyte has a definite concentration of essential cations like Ca^{2+}, Na^+ and K^+. Under normal conditions, these cations pass across the membrane slowly, but this rate may increase in presence of a non-penetrating –SH inhibitor [12]. The –SH groups of erythrocyte membrane proteins may play an important role in the maintenance of the functional and structural integrity of erythrocytes; 75% of the membrane sulfhydryl groups [13] do not react with organic and organometallic complexes including metal chelates, but do react with free metal ions [14] resulting in increased Na^+ permeability, tanning of the membrane and denaturation of the protein. This affects the erythrocyte and, consequently, plasmodia. On the other hand, a metal ion in a coordinately saturated complex (as in the present case, Figs. 1 and 2) is unable to react with –SH groups, particularly when the groups already bound are more basic than –SH. Besides, the metal ions, being charged, are known to complex with donor –SH groups [12–14]. Hence the activities of the former against plasmodia may be directly related to their chelation with the latter. This explains the decreased MIC of metal complexes as compared to free metal ions.

It may thus be inferred that introducing substituents in the proper positions to facilitate π-orbital involvement may result in a more efficacious antimalarial products. Further studies in this reaction are in progress.

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