In Vitro Evaluation of Bacteriostatic Activity of Metal Complexes of Amodiaquine and Primaquine

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Abstract

Aminoquinolines are effective antimalarial agents but have weak antibacterial activity. To study the effect of metal ions on the pharmacological behaviour of amodiaquine and primaquine – drugs derived from aminoquinolines – their complexes with 16 metal ions have been screened for their bacteriostatic activity against five human pathogenic bacterial strains. It has been found that complexes of soft acid metal ions are more effective antibacterial agents (lower MIC) than others.

Introduction

Drugs derived from aminoquinolines are effective in the treatment of malaria and other diseases [1–3] but have poor antibacterial activity [4]. Since their pharmacological behaviour, particularly microbial activity, is reported to increase on complexation with a metal ion [5–8], the present study was aimed at screening the bacteriostatic activity of the metal complexes of amodiaquine and primaquine in order to evaluate the role of the coordinated metal ion.

Experimental

Thirty-two complexes of amodiaquine and primaquine with toxic as well as non-toxic metal ions (Tables I and II) were prepared by methods reported earlier [9–11]. All the prepared complexes were screened against five human pathogenic bacteria, *viz.*, *E. coli*, *S. aureus*, *S. typhi*, *Ps. aeruginosa* and *V. cholera* by the minimum inhibitory concentration (MIC) method of Collins and Lyne [12] using DIFCO nutrient agar as the medium for the growth of bacteria.

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TABLE I. Minimum Inhibitory Concentrations (in ppm) of Amodiaquine and its Metal Complexes

Compound	*S. aureus*	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*V. cholera*
Amodiaquine[a]	500	125	125	250	125
VO–AQ	[b]	500	[b]	[b]	[b]
Co–AQ	250	[b]	[b]	500	125
Cu–AQ	125	250	125	250	125
Ni–AQ	250	62.5	62.5	62.5	250
Fe–AQ	[b]	[b]	250	[b]	250
Zn–AQ	500	62.5	125	125	125
Hg–AQ	250	62.5	31.75	15.88	31.75
Cd–AQ	250	125	250	250	250
Cr–AQ	[b]	125	500	[b]	125
Pt–AQ	[b]	500	[b]	[b]	250
Ag–AQ	31.75	15.88	31.75	125	31.75
Mn–AQ	[b]	125	250	[b]	125
Sn–AQ	[b]	62.5	62.5	[b]	62.5
Au–AQ	[b]	[b]	[b]	[b]	250
Pd–AQ	125	125	125	125	125
Rh–AQ	[b]	[b]	500	[b]	500

[a]AQ = Amodiaquine. [b]>500 ppm.

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TABLE II. Minimum Inhibitory Concentrations (in ppm) of Primaquine and its Metal Complexes

Compound	*S. aureus*	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*V. cholera*
Primaquine[a]	250	[b]	[b]	[b]	250
VO–PQ	[b]	250	[b]	[b]	250
Co–PQ	[b]	250	250	250	125
Cu–PQ	125	62.5	62.5	125	125
Ni–PQ	[b]	250	250	[b]	250
Fe–PQ	[b]	[b]	[b]	[b]	[b]
Zn–PQ	[b]	[b]	[b]	[b]	[b]
Cd–PQ	125	125	62.5	62.5	62.5
Hg–PQ	31.75	15.88	31.75	31.75	31.75
Cr–PQ	[b]	[b]	[b]	[b]	[b]
Pt–PQ	[b]	[b]	[b]	[b]	[b]
Ag–PQ	125	15.88	31.75	62.5	62.5
Mn–PQ	[b]	[b]	[b]	[b]	125
Sn–PQ	[b]	[b]	[b]	[b]	[b]
Au–PQ	[b]	[b]	[b]	[b]	[b]
Pd–PQ	[b]	125	250	[b]	125
Rh–PQ	[b]	[b]	[b]	[b]	250

[a]PQ = Primaquine. [b]>500 ppm.

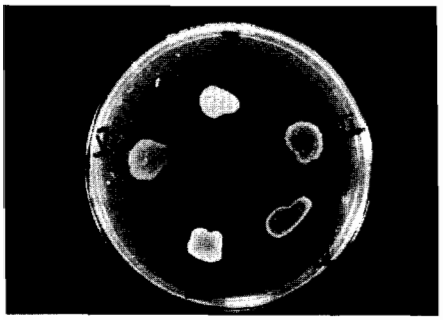
Fresh culture of selected microorganisms was grown by adding stock culture to the fresh nutrient agar medium and incubating it for 24 h to ensure proper growth. The testing was carried out in sterilized glass Petri dishes (diameter 6 cm) with covers. Sterilized 2.3% agar solution (19.0 ml) was added to each Petri dish, followed by addition of 1.0 ml of standard solution of the compound in alcohol/sterilized water. Insoluble complexes were finely powdered and their homogenised suspension in water was added. After thorough mixing of solutions, the Petri dishes were left in a sterilized chamber for about 3 h for solidification of agar. Thereafter, 0.01 ml of organism suspension of 4-h growth was inoculated on the surface of the medium and the dishes were incubated at 37 °C for 24 h. Each compound was tested at seven different concentrations from 500 to 7.94 ppm. From the results of the above microbiological experiments, the minimum inhibitory concentration of each compound for each organism was determined.

Results and Discussion

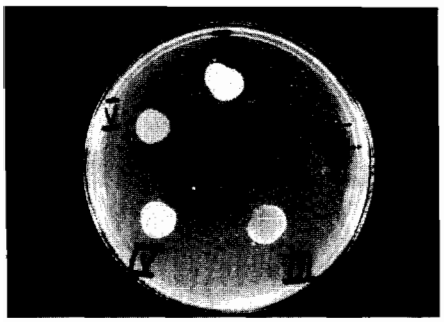
The minimum inhibitory concentrations (MIC) of the drugs and synthesized metal complexes are given in Tables I and II. Although a number of complexes listed in the tables have been found to be more active than the present chelating drug, the activity seems to be significantly enhanced on chelation with silver, mercury and cadmium, as shown by the decreased MIC values of their complexes. The observed relative activity order of these complexes has been found to be: silver > mercury > cadmium.

However, amongst complexes of metal ions of the first transition series, those of nickel, copper and zinc are far more active than chromium, iron and vanadium complexes. The order of activity for the complexes of various metal ions in this case has been found to be almost identical with the well-known Irving–Williams [13] order of the stability of the metal complexes $Zn^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Cr^{3+} > VO^{2+} > Fe^{3+}$. Since the activity of the complex was found to be dependent on the nature of the metal chelated, various parameters of metal ions have been examined in order to explain the observed activity order: coordination number; ease of binding with the available donor atom; structure of the resultant complex formed; oxidation state; size; charge density; position in the HSAB (hard and soft acid and base) series, etc.

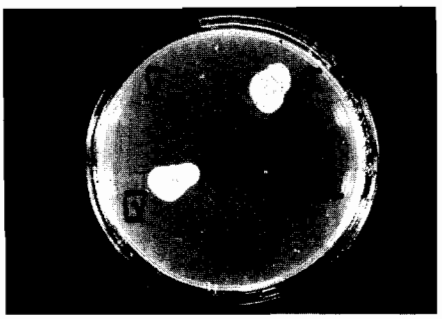
Antibacterial agents are known to affect the cell in a variety of ways, one such mode being the binding of the sulfhydryl groups of the cell enzymes [14]. As heavy metal ions preferentially bind to –SH groups, it is logical to assume that the complexes screened are involved in competitive equilibria involving the –SH group of the cell enzyme on one hand and the coordinated ligands amodiaquine or primaquine on the other. If this were the case, complexes of softer acids which are expected to bind to the –SH group of the cell enzymes more strongly than the nitrogen donor atom should have lower MIC values than complexes of relatively harder acids. As expected, complexes of Ag^{+}, the softest acid amongst the chosen metal ions because of the lowest charge density, exhibits the highest activity (Figs. 1b and 1c). Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} are borderline acids, whereas Cr^{3+}, Fe^{3+} and VO^{2+} are hard



(a)



(b)



(c)

Fig. 1. Microorganism: I, *S. aureus*; II, *E. coli*; III, *S. typhi*; IV, *Ps. aeruginosa*; V, *V. cholera*. (a) Control; (b) Ag–amodiaquine, 15.88 ppm; (c) Ag–primaquine, 31.75 ppm.

acids. Consequently, the observed MIC value pattern closely follows the suggested mechanism of bacteriostatic action.

On the basis of the above studies, it may be inferred that poor bacteriostatic properties of a compound may be significantly enhanced on chelation with a metal ion; the softer the metal ion coordinated, the more effective is the resulting complex as a bacteriostatic agent.

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