Improvement of antibiotic biopotency by complexing them with metal cations

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Introduction

There is an urgent demand for antimicrobial agents effective against multi-drug resistant bacteria (MDR). Careless use of antibiotics has led to untreatable infections, and this rise and spread of resistant bacteria is a serious threat for health systems across the globe. With the ongoing increase in drug resistance, the treatment of bacterial diseases by antibiotics is becoming less effective, and there are very few new antibiotics in the clinical pipeline, necessitating the development of alternative approaches. The possibility of modifying existing commercially available drugs to overcome MDR is a favourable approach, saving costs and time compared to the design and development of completely new drugs.

As one possible solution, we propose antibiotic complexation by silver ions to enhance antimicrobial effectiveness, and thereby potentially overcome MDR inactivation of existing antibiotics.

In this study, several antibiotics have been complexed with metal ions (zinc, copper and silver) and analytical techniques such as isothermal titration calorimetry and nuclear magnetic resonance were used to examine the structure and interactions between Zn(II), Cu(II), or Ag(I) and antibiotics. The complexes were also tested for their antimicrobial activity, by micro-broth dilution and disk diffusion methods. The metal-antibiotic complex concept was proven to be successful with the activity of the drugs enhanced against Gram-positive and Gram-negative bacteria. The highest synergistic effects were observed for complexes formed with Ag(I).

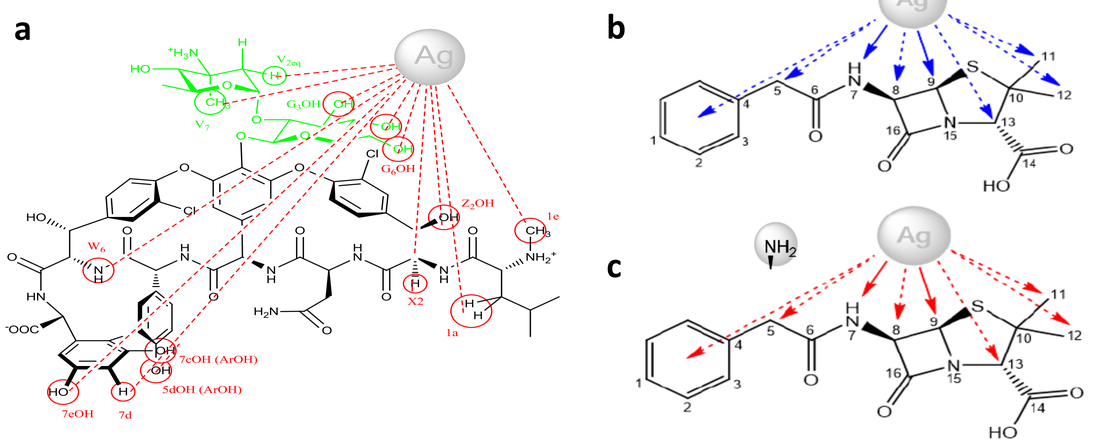

Figure 1: Binding sites determined by NMR experiments:
between silver ions and vancomycin **a**; penicillin G **b**; ampicillin **c**.

Silver ion complexes by ITC

ITC can determine thermodynamics and stoichiometry, important data when investigating interactions between metals and antibiotics.

Here we systematically study silver complexes with vancomycin at two pH conditions: 7.4 (blood) and 5.1 (skin); and three forms of metal salts to investigate the influence of condition on binding process.

Table 1. ITC results of $AgNO_3$, Ag_2SO_4 and AgAc to Vancomycin in 10 mM HEPES.

System	K [10^5 M^{-1}]	ΔG [10^{-3} cal/mol]	ΔH [10^{-3} cal/mol]	ΔS [cal/mol/K]	N
2.5 mM $AgNO_3$ to 0.15 mM Vancomycin, pH 7.5	2.91±0.39	-7.4	-8.1±0.1	-2.3	1.43±0.01
1.5 mM Ag_2SO_4 to 0.15 mM Vancomycin, pH 7.5	1.73±0.30	-7.7	-5.2±0.0	8.42	2.5±0.03
2.5 mM AgAc to 0.15 mM Vancomycin, pH 7.5	2.16±0.32	-7.3	-7.3±0.1	0.07	1.74±0.02
2.5 mM $AgNO_3$ to 0.15 mM Vancomycin, pH 5.1	6.60±0.35	-7.9	-14.6±0.2	-22.4	1.20±0.02
1.5 mM Ag_2SO_4 to 0.15 mM Vancomycin, pH 5.1	4.02±0.29	-7.8	-8.8±0.0	-3.21	2.11±0.01
2.5 mM AgAc to 0.15 mM Vancomycin, pH 5.1	7.71±0.65	-8.0	-13.5±0.0	-18.6	1.49±0.0

ΔG =ΔH – TΔS, where K is the binding constant, T = 298 K, ΔG is Gibbs energy, ΔH is enthalpy, ΔS is entropy, and N is the stoichiometry.

- Free Gibbs energy values are similar regardless of salts form or pH conditions
- The binding affinity is the weakest and the ratio of silver cations to Vancomycin is the largest for complexes obtained from silver sulfate
- The K values are larger at pH 5.1 then at pH 7.5, with the same trend observed between silver salts
- At the pH 5.1, larger values of enthalpy are nicely balanced by the increase in entropy for complex formation from silver nitrate and acetate

Synergistic effect

For several complexes between antibiotics and metal ions synergistic effects were observed. Synergy occurs if the combined effect of two agents is greater than the sum of their individual effects. For the quantification of synergism the fractional inhibitory concentration index (FICI) was calculated with the following formula, where an FICI is ≤ 0.5 a synergistic effect is indicated:

$$FICI = \frac{MIC_{antibiotic\ in\ the\ complex}}{MIC_{antibiotic\ alone}} + \frac{MIC_{metal\ in\ the\ complex}}{MIC_{metal\ alone}} \leq 0.5$$

Table 2: Micro-broth dilution results for the silver complexes with vancomycin, ampicillin and penicillin G. MIC (Minimum Inhibitory Concentration) and FICI (Fractional Inhibitory Concentration Index) values were calculated.

Compound	Metal salt Starting Conc.	*E. coli*	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	*S. aureus*
		ATCC 25922	ATCC 700603	ATCC 19606	ATCC 27853	ATCC 43300
		FDA control	MDR	Type	QC strain	MRSA
	[mg/mL]	MIC [μg/mL]* FICI				
colistin		0.03/0.06	0.03/ 0.06	0.06	0.06/0.125	
Vancomycin		>128	>128			1
Ampicillin		4	>128	>128	>128	8/16
Penicillin G		32/64	>128	>128	>128	8/16
AgNO3	2.56	2	4	2	2/4	16
Van/Ag 1:2	2.34	16 (>7.4)	32/16 (>7.6/>3.8)			1 (1.1)
Van/Ag 1:10	11.7	4 (>9.2)	8/4 (>1.9/>0.9)			1 (1.3)
Amp/Ag 1:1	1.17	2 (0.96)	4 **<0.48**	2 **0.47**	2 **<0.47/<0.24**	2/4 **0.31/0.36**
Amp/Ag 1:10	11.7	1/0.5 (2.5/1.3)	1 (<1.1)	0.125/0.25 (**<0.28**/<0.57)	0.25 (<0.57/**<0.28**)	1 **0.41/0.12**
PenG/Ag 1:0.5	0.61	2/4 (**0.30**/0.53)	8 (<0.54)	2 **<0.25**	2/4 **<0.25/<0.27**	4 **0.56/0.30**
PenG/Ag 1:5	3.05	2/4 (1.2/2.4)	4 (<1.22)	1 (<0.60)	1/2 (<0.60/<0.61)	2 **0.40/0.27**

To obtain the values of $MIC_{metal\ in\ complex}$ the following formula was applied:

$$MIC_{metal\ in\ complex} = MIC_{antibiotic\ in\ complex} * \frac{starting\ concentration\ of\ metal\ salts}{starting\ concentration\ of\ antibiotic}$$

The starting concentration of the respective antibiotics was 2.56 mg/mL, while the concentrations of metal salts are provided in the table.

Conclusion

At the current stage, our results add to the preliminary evidence supporting the application of silver ion as an antibiotic adjuvant to develop more efficient and sophisticated therapies and potentially offers a new approach towards treating the threat of 'superbugs'.

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