Enhanced antibiotic activity through complexation with metal ions: evaluated via ITC and biological studies

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Introduction

The growing problem of multi-drug resistant bacteria reduces the efficacy of antibacterial agents requiring alternative solutions to be developed. Some metal ions are known to provide antimicrobial effects and have previously been shown to be effective on open wounds and injured skin where uncontrolled microorganism growth can delay the normal process of wound healing. An effective antibacterial agent is required to improve the wound healing process in order to avoid open wound complications for patients.

Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) was used to examine complex formation between antibiotics and metal cations, such as Cu(II), Zn(II) or Ag(I). The ITC experiments performed revealed interactions between Ag(I) or Cu(II) and β-lactam antibiotics, these complexes showed an increase in activity of the antibiotic in the presence of the metal ions. Zn(II) complexes showed an endothermic binding by ITC, and negligible antimicrobial effect. Microbiological experiments revealed the highest synergistic effects of the complexes tended to match the molar ratio determined by ITC. Indicating that Ag(I) is synergistically working with β-lactam antibiotics by inhibiting and/or blocking the β-lactamase activity, allowing the antibiotics to be active against bacteria and a potential lactam/lactamase inhibitor combination for clinic use.

Antimicrobial Topical Skin Treatment

Silver (I) complexes of nitrate and acetate, as well as colloidal silver, were assessed as potential antimicrobial agents in an ex vivo experiment on rat skin. Samples were incubated on rat skin for twelve hours. Scrapings were then taken from the rat skin samples and streaked onto agar plates and incubated for twenty four hours. The resulting microbial growth was observed with reduced levels of microbial growth present on rat skin scrapings after treatment with Silver Nitrate and on one replicate of Silver Acetate compared to positive and negative controls.

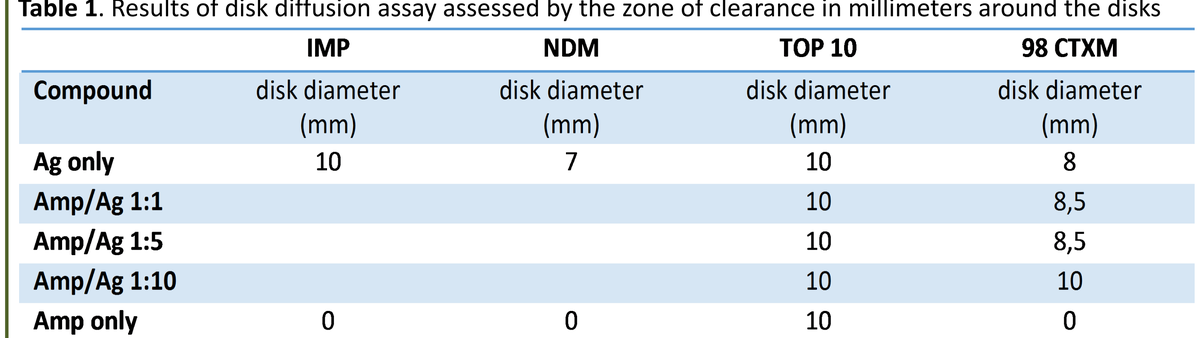

Figure 1. a) Microbial growth of rat skin scrapes after initial 16 hour treatment with compounds on blood agar plates (duplicate samples on left/right) after incubation for 24 hours. **b)** Microbial growth of rat skin scrapes on blood agar plates taken two hours after treatment (left/ and 12 hours after treatment (right)

Disk Diffusion Assay

Agar plates containing lawns of four different E.coli strains were prepared. In this experiment only the silver complexes were tested by inoculation onto disks and their area of inhibition of growth was monitored. The strains assessed were;

- **IMP**, which expresses an IMP-type carbapenemase,
- **NDM** a new delhi metallo-β-lactamase which is able to cleave carbapenems
- **CTXM 98** A strain which expresses a β-lactamase and is able to cleave cefotaxime, a third generation of cephalosporin antibiotics and
- **TOP10**, which is a common *E.coli* strain often used in the lab for transformation purposes.

Table 1. Results of disk diffusion assay assessed by the zone of clearance in millimeters around the disks

	IMP	NDM	TOP 10	98 CTXM
Compound	disk diameter (mm)	disk diameter (mm)	disk diameter (mm)	disk diameter (mm)
Ag only	10	7	10	8
Amp/Ag 1:1			10	8,5
Amp/Ag 1:5			10	8,5
Amp/Ag 1:10			10	10
Amp only	0	0	10	0
Me/Ag 1:1	9	9	20	15
Me/Ag 1:10	11	9	18	15
Me only	0	0	19	15
PenG/Ag 1:0.5	8	7	8	8
PenG/Ag 1:5	11	7	10	9
PenG/Ag 1:10	11,5	9	10	10
PenG only	0	0	0	0

Analysis of Synergistic Enhancement

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. The MIC of silver in the silver:compound combinations was calculated by the following formula:

$$MIC_{silver\ in\ complex} = MIC_{antibiotic\ in\ complex} \times \left(\frac{starting\ concentration\ of\ silver\ salt}{starting\ concentration\ of\ antibiotic} \right)$$

Synergistic effects were quantified by calculating the fractional inhibitory concentration index (FICI), where a FICI ≤0.5 indicates synergy:

$$FICI = \frac{MIC_{antibiotic\ in\ complex}}{MIC_{antibiotic\ alone}} + \frac{MIC_{silver\ in\ complex}}{MIC_{silver\ alone}}$$

Table 2. Minimum inhibition concentrations of various antibiotics and complexes.

		E. coli	K. pneumoniae	A. baumannii	P. aeruginosa	S. aureus
Compound/ Complex	Metal salt Starting Conc.	ATCC 25922 FDA control	ATCC 700603 MDR	ATCC 19606	ATCC 27853 QC strain	ATCC 43300 MRSA
	[mg/mL]	MIC [µg/mL] (FICI)				
Colistin		0.03/0.06	0.03/0.06	0.06	0.06/0.125	>50
Vancomycin		>128	>128			1
Ampicillin		4	>128	>128	>128	16.0/8.0
Penicillin G		32/64	>128	>128	>128	16.0/8.0
Gentamicin		1				>128
Colloid Silver A	0.005	0.125/0.063				0.25
Colloid Silver B	0.01	0.5/0.25				>0.5/0.5
$AgNO_3$	2.56	2	4	2	4.0/2.0	16
Van/Ag 1:2	2.34	16 (>7.4)	32/16 (>7.6/>3.8)			-1.1 (1.1)
Van/Ag 1:10	11.7	4 (>9.2)	8.0/4.0 (>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)	2.0/4.0 (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	4.0/2.0 (0.30/0.53)	8 (<0.54)	2 (<0.25)	4.0/2.0 (<0.25/<0.27)	4 (0.56/0.30)
PenG/Ag 1:5	3.05	4.0/2.0 (1.2/2.4)	4 (<1.22)	1 (<0.60)	2.0/1.0 (<0.60/<0.61)	2 (0.40/0.27)
Gen/Ag 1:1		0.5/0.25				0.216/0.108 (0.171/0.139)
Gen/Ag 1:10		0.572/0.536				0.717 (0.725)
Amp/Cu 1:5		>128	>128	>128	>128	4.0/2.0 (0.143/0.536)
Pen/Cu 1:1		>128	>128	>128	>128	1.0/1.0 (0.066/0.129)
Pen/Cu 1:10		>128	>128	>128	>128	4.0/4.0 (0.403/0.653)
Amp/Zn 1:10		>128	>128	>128	>128	4.0/2.0 (0.348/0.229)

Conclusion

The Cu(II) and Zn(II) complexes assessed showed greater activity than antibiotic alone only in the case of Methicillin-resistant *S. aureus*, except for the Zn(II) complex with penicillin G which did not. Most of the antibiotic complexes of Ag(I) demonstrated synergistic effects, when the complex exhibits better potency than antibiotic or metal alone against tested bacterial strains.

Further investigations are underway, to confirm these preliminary results about the mode and stoichiometry of complexation. This study also demonstrated that the counterion influenced the binding process, hence future plans also include testing of different metal salts, as well as different oxidation and physical states of metals including nanoparticles, organoparticles and colloidal forms.

The combination of existing antibiotics complexed with various metal ions shows enhanced antimicrobial activity and avenues for future studies. Additional research is planned to assess the microbiome of rat skin to understand the microbial population present prior to treatment and how this is affected by treatment with various complexes.

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