Extracellular and intracellular activities of quinupristin-dalfopristin (Synercid) against *Staphylococcus aureus* with different MSSA, MRSA, VISA and MLS_B resistance phenotypes

P. Baudoux, Y. Glupczynski, P. M. Tulkens, F. Van Bambeke
Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Brussels, Belgium

Mailing address:
F. Van Bambeke
UCL 73.70 av. Mounier 73
1200 Brussels - Belgium
vanbambeke@facm.ucl.ac.be

25th ICC
17th ECCMID
P2058

ABSTRACT

Objectives
S. aureus survives and thrives in mild acidic pH environments, such as found intracellularly in phagolysosomes. Synercid ® commercial, a semi-synthetic streptogramin antibiotic composed of quinupristin and dalfopristin (30:70 w/w ratio), displays a synergistic antibacterial activity against *S. aureus* and other Gram-positive bacteria *in vitro*. Synercid also displays activity against *S. aureus* phagocitized by murine J774 macrophages (JAC, 1992; 30 Suppl A:107-15), but this has been examined after short term incubation (2h) and at a single fixed concentration (10 x MIC) only. Our objectives was to assess the activity of Synercid in a newly developed model of *S. aureus*-infected human THP-1 macrophages that allows longer exposure periods and a detailed analysis of dose-responses for extracellular and intracellular activities (AAC 2006; 50:841-851), and using strains of *S. aureus* with resistance phenotypes of clinical significance.

Methods
We used an erythromycin-susceptible MSSA strain (ATCC 25923) and two erythromycin-resistant (constitutive MLS_B) MRSA (ATCC 33591) and VISA (NRS 126) strains. MICs were determined by arithmetic microdilution in Mueller-Hinton (MH) broth adjusted to pH 7.4 and 5.4. Change in CFU, compared to controls, were examined for bacteria incubated in MH broth (extracellular activity) or phagocitized by THP-1 macrophages (intracellular activity) after 24h exposure to concentrations from 0,01 to 100 x the MIC. Key microbiological and pharmacological parameters (static concentration [Cs]; concentration yielding 50% of the maximal effect [EC50]; and maximal effect for drug concentration at infinity [Emax]) were determined by non-linear regression (Hill equation; slope factor = 1).

Results
Results are shown in the Table.

Strain	MIC at pH		Extracellular activity			Intracellular activity		
	7.4	5.4	EC_{50} [a]	C_s [a]	E_{max} [b]	EC_{50} [a]	C_s [a]	E_{max} [b]
MSSA	0.45	0.40	0.58	0.68	-2.96	3.95	0.43	-3.45
MRSA (MLS_B)	0.40	0.40	0.40	0.49	-2.94	0.71	0.16	-1.40
VISA (MLS_B)	0.30	0.30	0.31	0.43	-2.36	0.22	0.27	-1.19

Conclusion
Synercid (a) shows no increase in MIC at acidic pH; (b) shows comparable activities against extracellular erythromycin-susceptible MSSA and erythromycin-resistant MRSA and erythromycin-resistant VISA; (c) shows also activity against their intracellular forms (but a bactericidal effect [> 3 log] is only observed with erythromycin-susceptible MSSA).

Background and Aim

- *Staphylococcus aureus* is a widespread pathogenic bacterium capable of surviving and multiplying in hostile environments, showing a high tolerance to variations in pH. This confers an advantage for colonizing body sites characterized by a mild acidic pH or for thriving intracellularly in acid compartments such as phagolysosomes of phagocytic cells.
- Synercid, a synergic combination of the semi-synthetic water-soluble streptogramins quinupristin and dalfopristin (30:70 w/w ratio), displays antibacterial activity against Gram-positive bacteria including *S. aureus*.
- Streptogramins activity towards *S. aureus* might be affected by resistance mechanisms common to macrolides and lincosamides (MLS_B), among which ribosome methylation is the most widespread.[1,2]
- In the present work, we assessed the extracellular and intracellular activity of Synercid against *S. aureus* strains with various methicillin-, MLS_B- or vancomycin-resistance phenotypes of clinical relevance. We used a model[3] in which bacteria and cells are exposed to a wide range of drug concentrations for up to 24 h, allowing us to draw pharmacological as well as potentially clinically-meaningful conclusions.

Bacterial strains and resistance phenotypes

Strain	Methicillin	Macrolides	Lincosamides	Streptogr. B	Vancomycin
ATCC25923	S	S	S	S	S
ATCC33591	R	R	R	R	S
NRS126	R	R	R	R	I

S = susceptible, I = intermediate, R = resistant, ND = not determined

Results

Table 1 :
MICs and MBC (mg/L) of Synercid for all the strains tested.

Strain	MIC at pH		MBC
	7.4	5.4	
ATCC25923	0.45	0.40	1
ATCC33591	0.40	0.40	1
NRS126	0.30	0.30	1

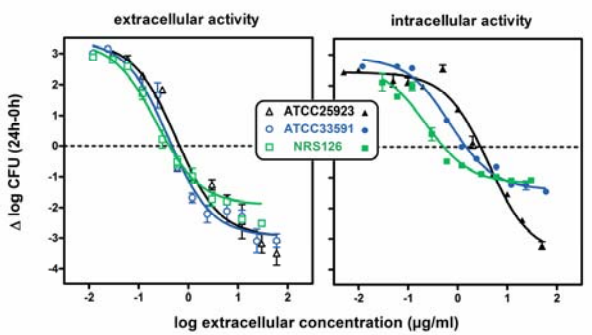

Figure 1 :
Left : Change in the number of CFU/ml (in log scale) after 24h incubation of S. aureus in broth.
Right : Change in the number of CFU/mg of cell protein (in log scale) after 24h incubation of infected THP-1 macrophages with antibiotic.
Data are plotted as a function of the drug concentration expressed in mg/L for each strain

Strain	Extracellular activity			Intracellular activity		
	EC_{50}*	C_s*	E_{max}**	EC_{50}*	C_s*	E_{max}**
ATCC25923	0.58	0.68	-2.96	4.22	2.88	-3.54
ATCC33591	0.40	0.47	-2.94	0.71	1.45	-1.40
NRS126	0.31	0.43	-2.36	0.23	0.52	-1.19

Table 2 :
Key pharmacological parameters of Synercid extracellular and intracellular activity against the *S. aureus* strains.
EC_{50} : concentration yielding 50% of the maximal effect;
C_s : static concentration;
E_{max} : maximal effect for drug concentration at infinity).
* : mg/L; ** : change in $\log_{10}$CFU from initial inoculum (10^6 CFU/ml).

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Methods

Extracellular activities of antibiotics

MICs and MBCs were determined by arithmetical microdilution in MH broth. Killing curve experiments were performed by incubating bacteria in MH broth for 24h in presence of antibiotic.

Cell infection and determination of intracellular activity

All experiments were conducted with THP-1 macrophages, infected with opsonized *S. aureus*, and incubated for 24 h in presence of antibiotic.[3]

Conclusions

- MICs of Synercid were neither affected by acidic pH, nor by the resistance phenotype of the strains tested.
- Extracellularly, a static effect was reached for all strains at a drug concentration close to its MIC. Reachable maximal effects were similar for all, irrespective to their resistance phenotype.
- Intracellularly, static effects were obtained for all strains upon exposure to extracellular concentrations of 2-8 x MIC.
  - Towards the macrolide-susceptible strain, Synercid reached a higher maximal effect than extracellularly.
  - Towards the macrolide-resistant strains, Synercid was not bactericidal intracellulalry, reached only a –1.5 log decrease from the initial inoculum as maximal effect.
- The data suggest that Synercid could be useful for eradicating macrolide-susceptible *S. aureus* in situations where persistence of intracellular foci is suspected

References

1. Leclercq *et al.*, JAC, 1992; 30 Suppl A:67-75.
2. Leclercq, CID, 2002; 34:482-92.
3. Barcia-Macay *et al.*, AAC, 2006; 50: 841-51.

Supported by the Belgian *Fonds de la Recherche Scientifique Médicale* and by the "STAPHAUR" programme of the *Région Wallonne*.