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Intracellular activity of antibiotics against a stable small colony variant (SCV) isolated from a CF patient in a model Calu-3 human airway epithelial cells

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ABSTRACT

Background: Persistence of *S. aureus* infections in CF may be related to the isolation of SCV phenotype and to the capacity of bacteria to internalize and to survive within host cells. Using a stable thymidine-auxotrophic *mecA* negative SCV of *S. aureus* isolated from a CF patient, we showed that most antibiotics (AB) act only poorly on intracellular forms in a model of human THP-1 macrophages (ICAAC 2007, abstr. A1437). We have now compared the activity of commonly used (gentamicin, rifampicin, vancomycin, moxifloxacin) and newly developed AB (linezolid, tigecycline, oritavancin) against this SCV within Calu-3 cells as a model of airway epithelium.

Methods: MICs were determined in MH broth after 48 h incubation. Intracellular activities (IA) were measured as the change in post-phagocytosis inoculum [delta log CFU] after 24 h or 96 h in Calu-3 cells incubated with ABs at extracellular concentrations (Ce) corresponding to their respective peak (Cmax), free peak (fCmax), trough (Cmin) and free trough (fCmin) in plasma when administered at standard doses.

Results:

AB	MIC (mg/L)	delta log CFU measured at 24 h for Ce (mg/L) corresponding to Cmax		fCmax		Cmin		fCmin	
		Ce	IA	Ce	IA	Ce	IA	Ce	IA
TGC	0.125	1	-0.54 ± 0.05	0.2	0.17 ± 0.04	0.13	0.18 ± 0.12	0.026	0.55 ± 0.14
LNZ	2	16	-0.98 ± 0.07	8	-0.19 ± 0.04	4	-0.16 ± 0.08	2	0.21 ± 0.09
RIF	0.0005	18	-0.59 ± 0.08	2.7	-0.28 ± 0.08	1.2	-0.23 ± 0.11	0.18	-0.14 ± 0.09
MXF	0.125	4	-1.74 ± 0.18	2	-1.34 ± 0.05	0.4	-0.20 ± 0.08	0.2	0.17 ± 0.02
GEN	0.125	18	-1.72 ± 0.10	18	-1.72 ± 0.10	1.5	-0.62 ± 0.11	1.5	-0.62 ± 0.11
VAN	0.5	50	-1.59 ± 0.01	22.5	-1.28 ± 0.04	10	-0.93 ± 0.12	4.5	-0.89 ± 0.09
ORI 200 mg	0.015 [a]	32	-1.91 ± 0.02	4	-1.27 ± 0.05	2	-0.85 ± 0.05	0.5	-0.22 ± 0.02
ORI 800 mg		128	-2.53 ± 0.53	16	-1.61 ± 0.05	16	-1.81 ± 0.05	2	-0.85 ± 0.05

[a] in the presence of 0.002% P80

Over 24 h, the intracellular inoculum remains constant (-0.14 ± 0.01 log CFU with 10 mg/L lysostaphin to prevent extracellular growth). All AB show a concentration-dependent effect, with a significant decrease in inoculum obtained at Cmax for TGC, LNZ and RIF, fCmax for MXF, Cmin for ORI low dose, and fCmin for GEN, VAN and ORI high dose. At 96 h, the activity of all drugs (except LNZ and TGC) markedly progressed at Cmax, approaching or reaching a bactericidal effect, while regrowth was observed at Cmin for all drugs but VAN, MXF and RIF.

Conclusions: Prolonged incubation with high concentrations of bactericidal drugs is needed to durably act on intracellular SCVs, suggesting the importance of AB selection and PK/PD optimisation to avoid failure in eradicating these bacteria in CF patients.

INTRODUCTION

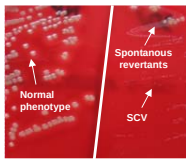
Small colony variants (SCVs) of *S. aureus* has been associated with persistence of infection in cystic fibrosis (CF) patients (Kahl *et al*., 2003). SCVs are able to persist within non-professional phagocytes and this intracellular location may protect them from host defenses and action of antibiotics (Schröder *et al.,* 2006). It seems, therefore, important to evaluate the activity of antibiotics against intracellular forms of SCVs in order to select the most appropriate therapy (Vaudaux *et al.,* 2006). In a previous study, we evaluated the intracellular activity of 10 antistaphylococcal antibiotics against a stable SCVs in a model of THP-1 macrophages (Nguyen *et al.,* 2007).

Respiratory epithelial cells play an important role in pathogenesis of lung infections in patients with CF. Recent data demonstrates that *S. aureus* can invade and persist inside epithelial cells (Jarry and Cheung, 2006).

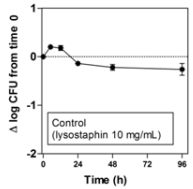
AIM OF THE STUDY

To examine the activity of selected antitaphylocoocal agents on intracellular SCV *of S. aureus* in a model of Calu-3 bronchial epithelial cells.

RESULTS



SCV used in this study
Colonies on Columbia blood agar with SCV phenotype, as compared to normal and revertant isogenic strains



Setting-up the model of Intracellular infection

- **Adherence of SCVs to Calu-3** :
  20% of initial inoculum at the end of the 2 h phagocytosis
- **Internalization of SCVs by Calu-3:**
  1.7% of initial inoculum after elimination of adherent bacteria
- **Intracellular replication of SCVs**:
  no marked change in intracellular inoculum over a 96 h post-infection.

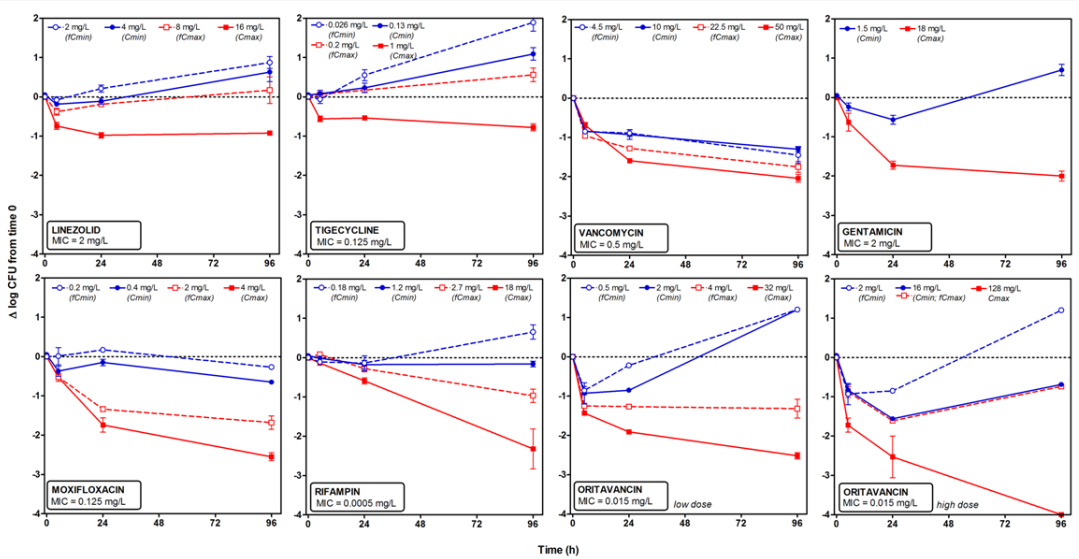
Selection of antibiotic concentrations

drug	Conventional unitary dose	Cmax (mg/L)	Free fraction
LZD	600 mg	16	~ 25 %
TGC	50 mg	1	~ 20 %
VAN	1000 mg	50	~ 50 %
GEN	6 mg/kg	18	~ 100 %
MXF	400 mg	4	~ 50 %
RIF	600 mg	18	~ 15 %
ORI	200 mg (low)	32	~ 12 %
	800 mg (high)	128	~ 12 %

Intracellular activity of antibiotics

■ **At 24 h,**
- **at equivalent of human C_{max}:**
  - modest decrease in bacterial counts for LZD, TGC, RIF
  - 1-2 log decrease for VAN, GEN, MXF, ORI low dose
  - > 2 log decrease for ORI high dose.
- **at equivalent of human free C_{min}:**
  - static effect for most drugs
  - 0.5-1.5 log decrease for VAN, GEN, ORI high dose

■ **At 96 h,**
- **at equivalent of human C_{max}:**
  - increase in activity for all drugs except TGC and LZD
  - very marked time-effect for RIF and ORI high dose
- **at equivalent of free C_{min} :**
  - increase in bacterial counts for all drugs, except VAN and MXF



METHODS

- **Bacteria:** we used a CF clinical isolate with stable thymidine auxotrophic SCV phenotype (Vergison *et al,* 2007), growing as tiny, non-pigmented and non-hemolytic colonies on sheep blood agar. This strain was grown aerobically on Muller Hinton II medium with low and controlled content of thymidine.
- **Susceptibility testing:** MICs were determined by microdilution method in Muller Hinton broth (in the presence of 0.002 % polysorbate 80 for ORI) with readings made after 48 h of incubation.
- **Intracellular infection:** Phagocytosis of bacteria by Calu-3 epithelial cells (ATCC HTB-55) was allowed during 2 hours, with an inoculum of 25 bacteria/cell. Cells were then washed with 50 mg/L gentamicin for 1 h (to eliminate non-internalized bacteria) and reincubated in fresh medium containing the tested antibiotic or lysostaphin at 10 mg/mL (control) to prevent the extracellular growth of bacteria. The post-phagocytosis inoculum typically ranged from 0.8 to $1.0x10^6$ CFU/mg protein and the extracellular bacteria contamination was minimal (< 0.5 %).
- **Intracellular activity** was measured after 5 h, 24 h and 96 h of exposure to antibiotics at a fixed concentration corresponding to their respective peak (C_{max}), free peak (fC_{max}), trough (C_{min}) and free trough (fC_{min}), as found in the serum of patients receiving conventional dosages. Results are expressed as changes in post-infection inoculum (Δ log CFU/mg cell protein). CFU counting determined after 48 h incubation of cell lysates plated on brain heart infusion agar.

CONCLUSIONS

- Most currently available anti-staphylococcal agents are only modestly active against intracellular SCVs, probably in relation with the quiescent character of the intracellular infection.
- Prolonged incubation with high concentrations of common bactericidal drugs (rifampin, moxifloxacin, and to a lower extent, gentamicin and vancomycin) is needed to reach marked intracellular effect.
- When used at high concentrations, the novel lipoglycopeptide oritavancin shows the most rapid and substantial effect.
- The intracellular regrowth seen at low but nevertheless clinically meaningful concentrations underlines the importance of optimization of antibiotic dosages and regimens to avoid failure in eradicating SCVs.

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