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Activity of antibiotic combinations towards intracellular small colony variants (SCVs) of *S. aureus* in a model of THP-1 macrophages

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ABSTRACT

Background: In a model of human THP-1 macrophages intracellular infection, SCVs of *S. aureus* have been shown to be poorly susceptible to most antibiotics (ICAAC 2007, A1437), except ORI or MXF, RIF (- 2 log CFU in 24 or 72 h). In the present study, we have examined whether combining these drugs could further enhance intracellular efficacy in comparison with conventional (RIF-OXA, RIF-VAN, RIF-FA) as comparators.

Methods: We used a stable thymidine-auxotrophic *mecA* negative SCV of *S. aureus* isolated from a cystic fibrosis (CF) patient. MICs were determined in MH broth after 48 h incubation. Intracellular activities were measured as the change in post-phagocytosis inoculum [delta log CFU] after 24 h or 72 h of incubation with ABs at extracellular concentrations (Ce) corresponding to their respective serum peak concentration (Cmax) when administered at standard doses.

Results:

Antibiotic(s)	MIC (mg/L)	Cmax (mg/L)	Delta log CFU from initial inoculum at 24 h	Delta log CFU from initial inoculum at 72 h
Oxacillin [OXA]	0.125	64	-0.06 ± 0.04	-1.31 ± 0.06
Vancomycin [VAN]	0.5	50	-0.11 ± 0.04	-1.35 ± 0.08
Fusidic acid [FA]	0.03	30	-0.33 ± 0.07	-0.87 ± 0.04
Rifampicin [RIF]	0.0005	18	-1.40 ± 0.18	-3.39 ± 0.18
Moxifloxacin [MXF]	0.125	4	-1.20 ± 0.05	-2.53 ± 0.04
Oritavancin [ORI]	0.015 [a]	25	-2.53 ± 0.10	-2.98 ± 0.11
RIF-OXA			-1.28 ± 0.05	- 2.81 ± 0.01
RIF-VAN			-1.42 ± 0.04	-2.75 ± 0.01
RIF-FA			-1.06 ± 0.01	-2.44 ± 0.11
RIF-MXF			-1.22 ± 0.06	-2.95 ± 0.07
RIF-ORI			-3.19 ± 0.10	> 5 [b]
MXF-ORI			-3.26 ± 0.13	-4.18 ± 0.16

[a] determined in the presence of 0.002 % P80; [b] below the limit of detection (- 5 log)

Combination of RIF with OXA, VAN, FA, or MXF was indifferent (same activity as RIF alone at 24 h, slight reduction at 72 h). In contrast, combination of ORI with RIF or MXF resulted in synergistic effects both at 24h and 72 h (higher activity as observed for the most active drug given alone), with RIF-ORI reaching the limit of detection at 72 h.

Conclusions: The data suggest that combining oritavancin with other bactericidal antibiotics may prove useful for eradicating intracellular SCVs, and plead for the further evaluation of such combinations in *in vivo* pertinent models of recurrent staphylococcal infections that imply SCVs

INTRODUCTION

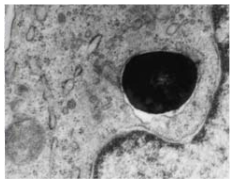
Persistence of *S. aureus* infection has been associated with the presence of Small Colony Variants (SCVs) in various clinical situations including cystic fibrosis, osteomyelitis, or device-associated infections (Procter *et al,* 2006). Furthermore, these SCVs can persist intracellularly, which contributes to protect them from antibiotic activity (Von Eiff, 2008).

In a previous study using a model of human THP-1 macrophages, we found that most antibiotics act only poorly on intracellular SCVs, with the noticeable exceptions of rifampin, moxifloxacin, and oritavancin (Nguyen *et al*, 2007). While a bactericidal effect could be achieved after 24 h or 72 h of incubation, none of these antibiotics, however, was able to sterilize the infected macrophages when used alone, suggesting the need of combinations (Von Eiff, 2008).

AIM OF THE STUDY

To assess and compare the intracellular activities of antistaphylococcal antibiotics when used alone or in combination.

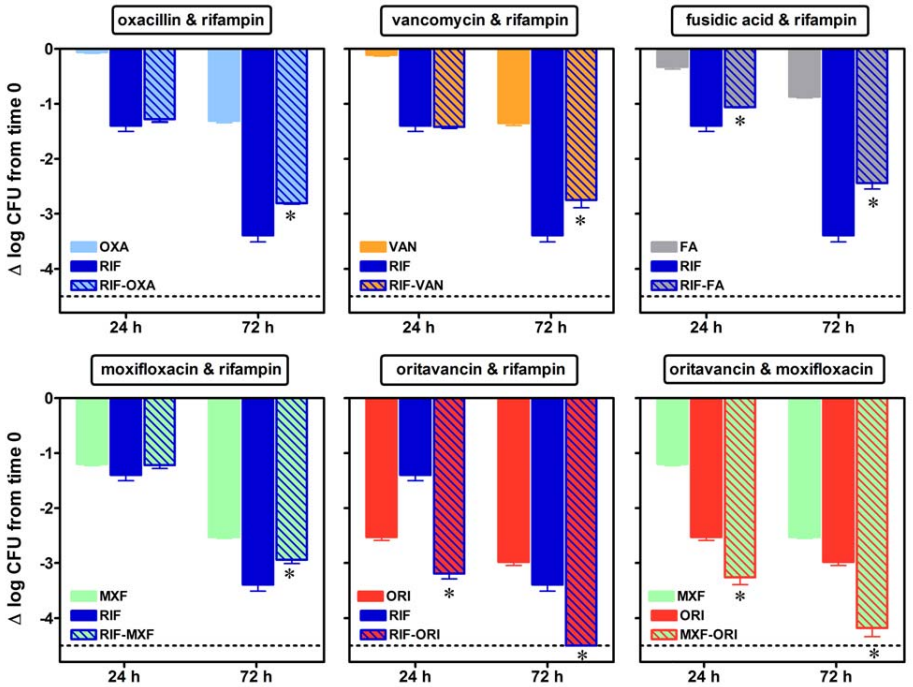
RESULTS



SCV used in this study as seen infecting THP-1 macrophages

MIC and C_{max} of antibiotics used in this study

Antibiotics	MIC (mg/L)	Cmax (mg/L)
Oxacillin [OXA]	0.125	64
Vancomycin [VAN]	0.5	50
Fusidic acid [FA]	0.03	30
Rifampicin [RIF]	0.0005	18
Moxifloxacin	0.125	4
Oritavancin [ORI]	0.015	25



Dotted line: limit of detection; statistical analysis (ANOVA): * combination different from drugs alone ($p < 0.05$)

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 activity:** Infection of THP-1 was performed as previously described (Barcia-Macay *et al*, 2006), with 1 h phagocytosis (4 bacteria/cell), followed by incubation with 50 mg/L gentamicin for 1 h (to eliminate non-internalized bacteria) and reincubation in fresh medium containing the tested antibiotic. Intracellular activity was measured after 24 h or 72 h exposure to antibiotics at a concentration corresponding to their respective human C_{max}. CFUs were counted after 48 h incubation of cell lysates plated on brain heart infusion agar. Results are expressed as changes in post-infection inoculum (Δ log CFU/mg cell protein).

DATA DESCRIPTION

- When used alone, only MXF, RIF and ORI achieve an intracellular bactericidal effect (3 $\log_{10}$ CFU decrease) at 72 h.
- Combinations of conventional antistaphylococcal agents (OXA, VAN, FA) with RIF results in indifferent effect at 24 h and marginal antagonism at 72h.
- The combination of moxifloxacin with rifampin is indifferent
- The combination of oritavancin with another bactericidal antibiotic (MXF or RIF) is synergistic.
- Apparent intracellular sterilization is achieved when oritavancin is combined with rifampin.

CONCLUSIONS

Combining bactericidal antibiotics (ORI-MXF, ORI-RIF) may prove useful for eradicating intracellular SCVs.

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