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Comparative Intracellular Activity of 11 Antistaphylococcal Antibiotics (AABS) against a Stable, Thymidine-dependent Small Colony Variant (SCV) and its Normal Phenotype Isolate (NP) Counterpart from a Cystic Fibrosis Patient

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Abstract

Objectives:
SCVs are able to persist more efficiently within host cells making infections difficult to eradicate. We therefore compare the intracellular activities of AABs against a SCV and its NP counterpart.

Methods:
Mec A negative SCV and NP were isolated from the same patient and showed identical PFGE profiles. MICs were measured by microdilution in MHB. Intracellular activities were determined after phagocytosis by human THP-1 macrophages (changes in post-phagocytosis inoculum [delta log CFU] after 24 h in cells incubated with AABs at an extracellular concentration corresponding to their respective reported Cmax).

Results:

AAB (C_{max} [mg/L])	SCV		NP	
	MIC (mg/L)	delta log CFU	MIC (mg/L)	delta log CFU
control		0.22 ± 0.07[a]		0.81 ± 0.06[a]
vancomycin [VAN] (50)	0.5	-0.17 ± 0.05	0.5	-1.18 ± 0.05
quinupristin-dalfopristin [Q-D] (11)	0.5	-0.22 ± 0.03	0.25	-1.66 ± 0.02
oxacillin [OXA] (64)	0.125	-0.23 ± 0.04	0.5	-1.30 ± 0.04
clindamycin [CLI] (4)	0.125	-0.27 ± 0.10	0.125	-0.88 ± 0.05
fusidic acid [FA] (30)	0.03	-0.33 ± 0.07	0.125	-1.11 ± 0.01
daptomycin [DAP] (57)	0.125	-0.39 ± 0.02	0.125	-1.32 ± 0.03
linezolid [LNZ] (16)	2	-0.39 ± 0.01	4	-0.28 ± 0.06
gentamicin [GEN] (18)	0.125	-0.44 ± 0.09	0.5	-0.84 ± 0.05
moxifloxacin [MXF] (4)	0.125	-1.20 ± 0.06	0.25	-1.76 ± 0.04
rifampicin [RIF] (18)	0.0005	-1.24 ± 0.03	0.002	-1.68 ± 0.01
oritavancin [ORI] (25)	0.015	-2.71 ± 0.07	0.03	-2.82 ± 0.10

[a] with GEN (1xMIC) to prevent extracellular growth

At 24 h, intracellular growth was minimal for SCV but significant for NP. Against SCV, most AABs, except ORI were poorly active (1.2 log decr. max.). Activity was improved for all but LNZ towards NP. ORI was as active against SCV and NP, approaching a bactericidal effect (3 log decrease).

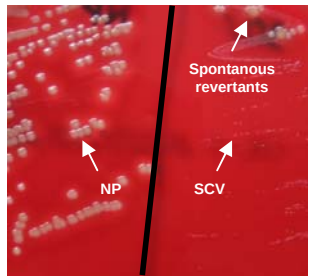
Conclusion:
Most AABs act poorly on intracellular SCV compared to NP, which may explain difficulty in eradicating these organisms in CF patients. Evaluation of AAB activity against intracellular SCV to select most appropriate therapies is needed.

Introduction

Colonization and recurrent infections of the airways with *S. aureus* occur in many paediatric cystic fibrosis patients, and lead to increased morbidity and mortality (1). Once a patient has been colonized, *S. aureus* cannot be cleared from the bronchial system despite the use of antimicrobials with high staphylocidal activity. This persistence has been recently found to be associated with the isolation of a *S. aureus* subpopulation displaying a small colony variant (SCV) phenotype (2).

SCV are slowly growing organisms that exhibit a growth as tiny, nonpigmented, nonhemolytic colonies, are auxotrophy towards different substrates (thymidine, hemin, menadione), and produce greatly reduced amounts of α-toxin. Compared with their normal phenotype counterpart, SCVs persist more easily within host cells, where they are protected from host defences and antibiotics (3).

It seems therefore important to evaluate antibiotic activity against intracellular forms of SCVs in order to select appropriate therapy.



S. aureus colonies on Columbia blood agar (24 h incubation) exhibiting the normal phenotype (left) and SCV phenotype (right)

Aim of the study

To compare the intracellular activity of a series of antibiotics against
- a stable, thymidine-dependent SCV variant of a CF mecA-negative isolate of *S. aureus*
- its normal phenotype (NP) counterpart.

To this effect, we used a model of infection in THP-1 human macrophages and antibiotic concentrations mimiking the C_{max} reached in patients undergoing therapy with conventional dosages.

Methods

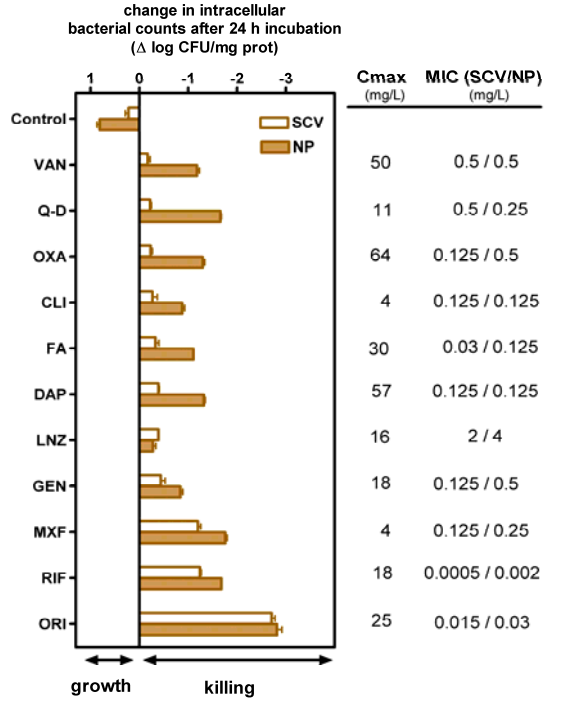
- **Bacteria:** *mecA* negative SCV and NP were isolated from the same patient and showed identical PFGE profiles (4). SCVs were maintained aerobically in Muller Hinton II medium with low and controlled content of thymidine.
- **Susceptibility testing:** MICs were determined by microdilution method in Muller Hinton broth and were read after 24h and 48 h of incubation, respectively for NP and for SCV.
- **Intracellular activity:** infection of THP-1 macrophages was performed as previously described (5), with one hour phagocytosis (4 bacteria/cell), followed by a washing with 50 mg/L gentamicin (to eliminate extracellular bacteria) and reincubation in fresh medium containing either the tested antibiotic or gentamicin at its MIC (control). Intracellular activity was measured after 24 h exposure to antibiotics at a concentration corresponding to their respective human C_{max}. 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 (β-lactams, glycopeptides, lipopeptides, lincosamides, oxazolidinones, streptogramins, aminoglycosides, fusidic acid) act only poorly on intracellular SCVs, as compared to their normal counterpart. This may explain the difficulty for eradicating these organisms in CF patients.
- The difference in intracellular activity between SCV *vs.* NP was most pronounced for vancomycin, quinupristin-dalfopristin, oxacillin, clindamycin, fusidic acid and daptomycin, and the activity of linezolid was low in both cases.
- Only oritavancin, a novel lipoglycopeptide in development that act both on the membrane and the cell wall (6), was bactericidal against SCVs and as active as against NP.
- Our results strongly suggest the interest of evaluating antibiotic activity against intracellular SCV to select most appropriate therapies. They also warrant further examining the interest of oritavancin and other new antibiotics in *in vitro* and *in vivo* models of difficult-to-treat staphylococcal infections (including CF) in which SCVs are involved.

Results

Intracellular activity of antibiotics against *S. aureus*



Change in intracellular counts of *S. aureus* (open bars: SCV; closed bars: NP) after 24 h of incubation in the presence of the indicated antibiotics at their human Cmax.
Controls : Gentamicin at its MIC was maintained during the whole incubation period; il allows to control extracellular contamination.
Abbreviations: VAN, vancomycin; Q-D, quinupristin-dalfopristin (synercid); OXA, oxacillin; CLI, clindamycin; FA, fusidic acid; DAP, daptomycin; LNZ, linezolid; GEN, gentamicin; MXF, moxifloxacin; RIF, rifampin; ORI, oritavancin.
ORI MIC: determined in the presence of 0.002 % of polysorbate.

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