SCV Phenotype and Reduced Intracellular Activity of Antibiotics: a cause for Persistent Staphylococcal infection ?

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ABSTRACT

Background: SCVs are commonly observed in chronic, recurrent infections and are difficult to eradicate, probably due to their intracellular localization. We have isolated a SCV from a patient with complicated prosthetic vascular graft infection and relapsing MRSA bacteraemia who was unsuccessfully treated with SMX/TMP, MIN, VAN/RIF and then LNZ/RIF over a period of 3 months. Our aim was to assess the activity of these ABs towards extracellular and intracellular forms of the isolated SCV, in comparison with other antistaphylococcal antibiotics.

Methods: SCV: thymidine auxotrophic MRSA. Activity: change in CFU after 24 h incubation at a concentration corresponding to reported human C_{max} (i) in broth (EC) or (ii) after phagocytosis by THP-1 macrophages (IC, see AAC 2006; 50: 841-51 for model description).

Results:

Antibiotic (C_{max} [mg/L]) *	MIC[a] (mg/L)	Delta log_{10} CFU from time 0	
		EC	IC
none		2.52 ± 0.04	-0.35 ± 0.06 [b]
SXT/TMP (38/2)	*>304/16*	*2.88 ± 0.09*	*0.99 ± 0.13*
VAN (50)	*2*	*-2.03 ± 0.11*	*-0.72 ± 0.05*
LNZ (16)	*1*	*-2.42 ± 0.11*	*-1.35 ± 0.09*
RIF (18)	*0.002*	*-3.27 ± 0.20*	*-1.61 ± 0.04*
TGC (1)	0.25	-2.46 ± 0.22	-0.74 ± 0.06
DAP (57)	2	-3.20 ± 0.10	-0.75 ± 0.04
MIN (5.1)	4	-0.92 ± 0.05	-0.78 ± 0.10
TLV (90)	0.125	> 4.5	-0.79 ± 0.01
MXF (4)	1	-2.73 ± 0.22	-0.82 ± 0.04
GEN (18)	1	-3.08 ± 0.01	-0.84 ± 0.02
ORI (25)	0.125	> 4.5	-1.33 ± 0.07
Q-D (11)	0.5	-2.97 ± 0.11	-1.99 ± 0.06

* AB shown in italics are those received by the patient

[a] microdilution in BHI broth
[b] with GEN (1xMIC) to prevent extracellular growth

Conclusion: Most antibiotics were considerably less active intracellularly as compared to broth, and none, including those administered to the patients, reached a bactericidal theshold (3 log_{10} CFU decrease) in cells. This in vitro assay may help in selecting the most potentially useful agents against SCVs.

INTRODUCTION

Foreign body-associated infections are one of the most common health care-associated infections resulting in increased patient's morbidity and costs (1). Over the past few years, several reports have documented the implication of staphylococcal SCVs in these infections (2). Compared to the normal phenotype counterpart, SCVs often show a low susceptibility to antibiotics and have a propensity to persist intracellularly (3), which may contribute to the persistent or recurrent character of infections. In this context, it is quite difficult to select antibiotics based only on MIC as determined in broth. *In vitro* models evaluating activity against intracellular SCVs may be useful not only to better understand the causes for treatment failure, but also to help in selecting appropriate antibiotic regimens.

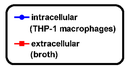
AIM OF THE STUDY

- To examine the extracellular and the intracellular activity of a series of antistaphylococcal drugs against a stable SCV isolated from a patient with complicated prosthetic vascular graft infection and recurrent MRSA bacteraemia and treated unsuccessfully with a series of antibiotics.
- Specifically, to compare
  - antibiotics unsuccessfully used to treat the patient (cotrimoxazole [SMX/TMP], minocycline [MIN], vancomycin [VAN], rifampin [RIF], linezolid [LNZ])
  - other approved antistaphylococcal antibiotics (gentamicin [GEN], moxifloxacin [MXF], quinupristin-dalfopristin [Q-D], tigecycline [TGC], and daptomycin [DAP]
  - new molecules in late stages of development (telavancin [TLV] and oritavancin [ORI]).

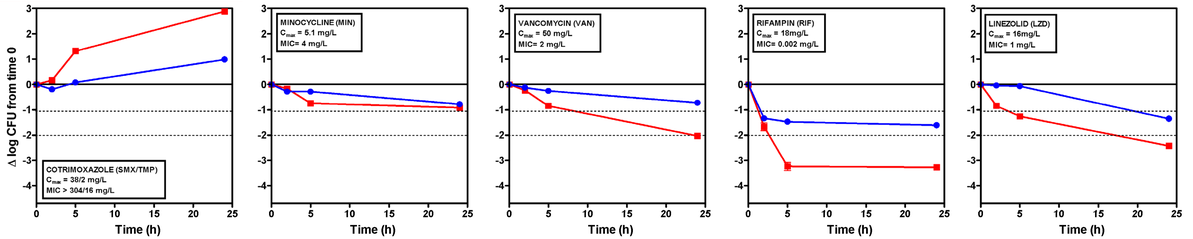
RESULTS

Extracellular *versus* intracellular activity of antibiotics against *SCV*

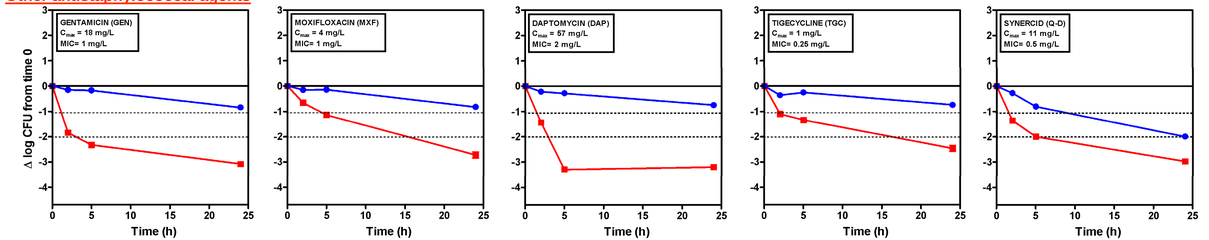
- Incubation for up to 24 h incubation with antibiotics at a concentration corresponding to their C_{max} in humans
- Limit of detection set at –4.5 log; dotted lines pointing to –1 and –2 log effects to faciltate comparisons between drugs



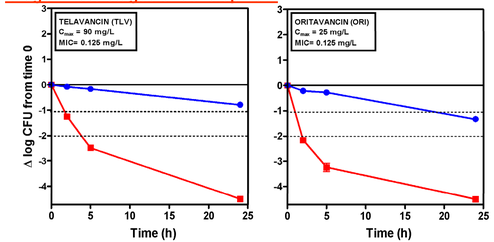
→ **Drugs administered to the patient**



→ **Other antistaphylococcal agents**



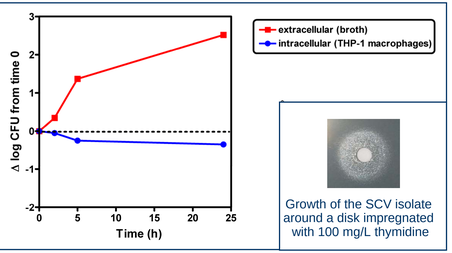
→ **Drugs in late stages of development**



Growth curves for SCV 15283397

- **in BHI broth (extracellular)**
- **in THP-1 macrophages (intracellular)**

In infected macrophages, a small reduction in bacterial counts was evidenced at 24 h.



Growth of the SCV isolate around a disk impregnated with 100 mg/L thymidine

METHODS

- **Bacteria:** The SCV isolate 15283397 is a thymidine-auxotrophic MRSA, growing as tiny, non-pigmented and non-hemolytic colonies on Columbia blood agar. This isolate is resistant to OXA, SXT, CLI, LIN, ERY, quinupristin and TET (MIC > CLSI Bkpts).
- **Extracellular activity:** Measurement of MICs and kill-curves were carried out in BHI broth (added by 0.002 % tween-80 for oritavancin to prevent adsorption on plastic surfaces [4]).
- **Intracellular activity:** Infection of THP-1 macrophages was performed as previously described (5), with 1 h phagocytosis (4 bacteria/cell), followed by a washing with 100 mg/L gentamicin (to eliminate extracellular bacteria) and reincubation in fresh medium containing either the tested antibiotic or gentamicin at its MIC (1 mg/L; control). Intracellular activity was measured after 2 h, 5 h and 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 BHI agar).

CONCLUSIONS

- All antibiotics were considerably less active intracellularly than extracellularly against SCV.
- As anticipated for thymidine-dependent SCVs (6), SMX-TMP was ineffective against both extracellular and intracellular forms.
- Extracellularly, only RIF, GEN, DAP, and Q-D caused a bactericidal effect (3 log decrease) at 24 h, and TLV and ORI allowed to reach the limit of detection (4.5 log decrease).
- In infected macrophages, RIF, LNZ, Q-D, and ORI were the only drugs capable of reducing the intracellular counts of more than 1 log at 24 h.
- Nevertheless, none of the tested antibiotics, including those administered to the patient, reached a bactericidal effect against intracellular within the 24 h time frame of these experiments. This may explain the observed failure of antibiotic treatment in this patient and the difficulty of eradicating these organisms in general.
- Our cellular model may serve to evaluate antibiotic susceptibility in infections for which intracellular reservoirs and SCVs could play an important role.

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