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

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RESULTS

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 vascular graft infection, and it displayed resistance to several antibiotics.

The SCV isolate 15283397 is a thymidine-auxotrophic MRSA, growing as an extracellular organism. 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 CMAX. Extracellular activity: Drugs administered to the patient. Intracellular activity: none 2.52

<table>
<thead>
<tr>
<th>Antibiotic (Cmax [mg/L])</th>
<th>MICa (mg/L)</th>
<th>IC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIN (5.1)</td>
<td>4</td>
<td>-0.92</td>
</tr>
<tr>
<td>GEN (18)</td>
<td>1</td>
<td>-3.08</td>
</tr>
<tr>
<td>TGC (1)</td>
<td>0.25</td>
<td>-2.46</td>
</tr>
<tr>
<td>ORI (25)</td>
<td>0.125</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td>VAN (2)</td>
<td>2</td>
<td>-2.03</td>
</tr>
<tr>
<td>LNZ (16)</td>
<td>1</td>
<td>-2.42</td>
</tr>
<tr>
<td>Q-D (3)</td>
<td>0.06</td>
<td>-0.35</td>
</tr>
<tr>
<td>CLI (11)</td>
<td>0.08</td>
<td>-0.13</td>
</tr>
<tr>
<td>CLI (11)</td>
<td>1</td>
<td>-0.10</td>
</tr>
<tr>
<td>LIN (15)</td>
<td>0.12</td>
<td>-0.75</td>
</tr>
<tr>
<td>RIF (48)</td>
<td>1</td>
<td>-1.99</td>
</tr>
<tr>
<td>DAP (41)</td>
<td>1</td>
<td>-0.82</td>
</tr>
<tr>
<td>COX (19)</td>
<td>0.5</td>
<td>-0.72</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

All antibiotics were essentially less active intracellularly as compared to broth, and none, including those administered to the patients, reached a bactericidal threshold (3 log 10 CFU decrease) in cells. This in vitro assay may help in selecting the most potentially useful agents against SCVs.

**REFERENCES**

2. Von Eiff C. and Becker K. 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 have a reduced susceptibility to antibiotics and have a propensity to persist intracellularly (3), which may contribute to the persistent or recurrent nature of these infections. 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.

**ABSTRACT**

Background: SCVs are commonly detected 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 vascular graft infection, and it displayed resistance to several antibiotics.

Methods: SCV: thymidine auxotrophic MRSA. Activity: change in CFU after 24 h incubation at a concentration corresponding to reported human CMAX. Extracellular activity: drugs administered to the patient. Intracellular activity: none.

Conclusions: Most antibiotics were considerably less active intracellularly as compared to broth, and none, including those administered to the patients, reached a bactericidal threshold (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 have a reduced susceptibility to antibiotics and have a propensity to persist intracellularly (3), which may contribute to the persistent or recurrent nature of these infections. 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 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 (cloxacillin [MDA/TMP], minocycline [MIN], vancomycin [VAN], tetracycline [TET], lincomycin [LIN], and moxifloxacin [MXF]).
- Other approved antistaphylococcal antibiotics (gentamicin [GEN], moxifloxacin [MXF], quinupristin-dalfopristin [Q-D], daptomycin [DAP], and telavancin [TLV]).
- New molecules in late stages of development (tigecycline [TGC] and oritavancin [ORI]).

**METHODS**

**Bacteria:** The SCV isolate 15283397 is a thymidine-auxotrophic MRSA, growing as a tiny, non-pigmented and non-hemolytic colonies on Columbia blood agar. This isolate is susceptible in vitro to OXVA, SAT, CLI, LIN, RIF, quinupristin and TET (MICs <2 mg/L). Intracellular activity: Measurement of MIC and killing curves were carried out in broth (related to 0.002 % tween-80 for oritavancin to prevent adsorption on plastic surfaces.

Intracellular activity: In vitro killing of 15283397 was performed as previously described (5), with 1 x phagocytosis (4 bacterialoid), 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 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 CMAX. Results are expressed as changes in post-infection inoculum (1 log CFU/mL cell protein; CFU counting determined after 48 h incubation of cell lysates plated on BH agar).

**CONCLUSIONS**

All antibiotics were essentially less active intracellularly than extracellularly against SCV.

When antagonized by pyridoxine-dependent SCVs (6), SMX/TMP was ineffective against both extracellular and intracellular forms. Extracellularly, only RIF, GEN, DAP, and G/Q caused a bactericidal effect (3 log decreases) at 24 h, and TUL and ORI allowed to reach the limit of detection (4.5 log decreases).

In infected macrophages, RIF, LNZ, DAP, 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.

**REFERENCES**