Bactericidal Activity of Ceragenin CSA-13 Against Intracellular MSSA, Hospital-acquired (HA) and Community-acquired (CA) MRSA, and VISA in THP-1 macrophages: relation to cellular toxicity?

P.M. Tulkens
Pharmacologie cellulaire et moléculaire
UCL 73.70 av. Mounier 73
1200 Brussels - Belgium
tulkens@facm.ucl.ac.be

Abstract

Objectives: Recurrent infection may be related to intraphagocytic bacterial persistence. S. aureus is the causative agent of chronic and relapsing infections, probably in relation with its ability to survive and multiply within eucaryotic cells. An adequate therapeutic choice would therefore require the use of antibiotics active against both extracellular and intracellular forms.

Methods: MICs were determined broth micro-dilution method, in MHB (supplemented with NaCl 2 % for MRSA). MICs of CSA-13 for all strains were 1-2 mg/L. CSA-13 caused a concentration-reduction of the post-phagocytosis inoculum for all strains with an apparent static effect at 1-5 mg/L and an almost complete eradication (5 log CFU decrease) at 100 mg/L. This effect was largely paralleled by a release of LDH from untreated cells exposed to CSA-13 (cells with most cells stained by trypan blue and displaying chromatin membrane damages by EM when incubated 5 h with 20 mg/L CSA-13).

Conclusions: CSA-13 is highly and quickly bactericidal against intracellular S. aureus, but this effect could be mediated by membrane-destabilizing effects towards macrophages that may give it direct access to intracellular bacteria. Animal studies are needed to assess if such increased accessibility allows for a faster and more extensive S. aureus eradication without causing undue toxicity.

Background

S. aureus is the causative agent of chronic and relapsing infections, probably in relation with its ability to survive and multiply within eucaryotic cells. An adequate therapeutic choice would therefore require the use of antibiotics active against both extracellular and intracellular forms.

Cationic Steroid Antimicrobials (CSAs) constitute a new class of antibiotics which, like antimicrobial peptides, are bactericidal by destabilizing bacterial membranes. Unlike the latter, these compounds are active against multi-resistant S. aureus (ICAAC 2005, abstract no. F-1233) and not expected to readily to cause stable resistance.

Aim of the study

• To evaluate the intracellular activity of CSA-13 against intracellular MSSA, HA- and CA-MRSA, and VISA.
• To assess its potential toxicity against eucaryotic cells.

Results

<table>
<thead>
<tr>
<th>Strains</th>
<th>MICs (mg/L)</th>
<th>Intracellular activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>ATCC 25923</td>
<td>1</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td>ATCC 33591</td>
<td>1</td>
</tr>
<tr>
<td>CA-MRSA S.a. 310</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>S.a. 310</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>VISA</td>
<td>NRS 126</td>
<td>1</td>
</tr>
</tbody>
</table>

Intracellular activities of CSA-13

Change in cfu (log₁₀) from time 0 h of infected macrophages exposed to an extracellular concentration of 20 mg/L.

Cellular toxicity of CSA-13

(a) Comparison of intr. activity and toxicity for macrophages

Killing of intracellular bacteria is observed for CSA-13 concentration causing a cytotoxic effect (≥20 mg/mL)

Comparison of the dose responses of the cell toxicity (assessed by trypan blue staining (Figures show the proportion of strained cells in % of the total count)) and of the apparent intracellular activity against intracellular S. aureus (MSSA ATCC 25923) in infected-THP1 macrophages (ratio of [Δ log cfu drug – ctl] to the post-phagocytose inoculum [Time 0h] : a figure of 60 for activity means, therefore, a reduction of the inoculum of 3 log10 CFU). Exposure was for 5 h in each case.

(b) Electron microscopy

Macrophages incubated for 5h with CSA-13 (20 mg/L) show no more intracellular bacteria but clear-cut signs of destruction.

References


A copy of this poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters.htm