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 ?

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Abstract

Objectives: Recurrent *S. aureus* infection may be related to intraphagocytic bacterial persistence and insuffisiant intracellular activity of antibiotics (AAC, 2006;50-841). Cationic Steroid Antibiotics (CSAs) are a novel class of anti-staphylococcal drugs with marked activity against multi-resistant *S. aureus* (ICAAC 2005, abstract no. F-1233). We have examined the activity of one member of this family (CSA-13) against intracellular *S. aureus*.

Methods: MSSA (ATCC 25923), HA-MRSA (ATCC 33591), CA-MRSA (clinical isolate 310 and 325) and VISA (NRS 126) were used. Intracellular activity on phagocytosed *S. aureus* was measured in THP-1 macrophages over a wide range of CSA-13 extracellular concentrations (0.01 – 100 mg/L). Cell viability and intactness of the pericellular membrane upon exposure to CSA-13 were assessed in uninfected cells by the release of LDH, trypan blue staining and electron microscopy (EM).

Results: 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 uninfected cells exposed to CSA-13 (with most cells stained by trypan blue and displaying obvious 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.

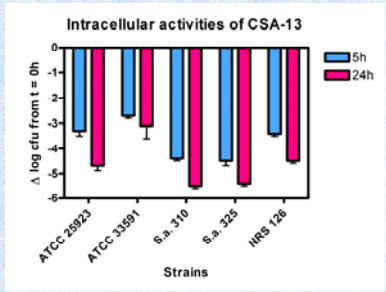
Results

MICs

Strains		MICs (mg/L)
MSSA	ATCC 25923	1
HA-MRSA	ATCC 33591	1
CA-MRSA	S.a. 310	2
	S.a. 325	2
VISA	NRS 126	1

Intracellular activity

CSA-13 is quickly bactericidal against intracellular *S. aureus* whatever the resistance phenotype

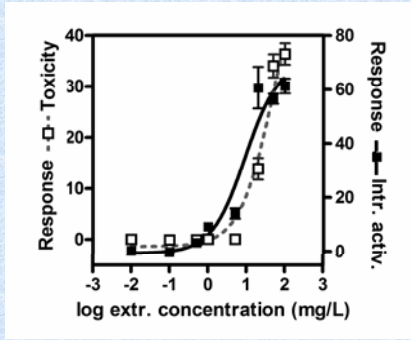


Change in cfu (log_{10}) from time 0h of infected macrophages exposed to an extracellular concentration of 20 mg/L

Cellular toxicity of CSA-13

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

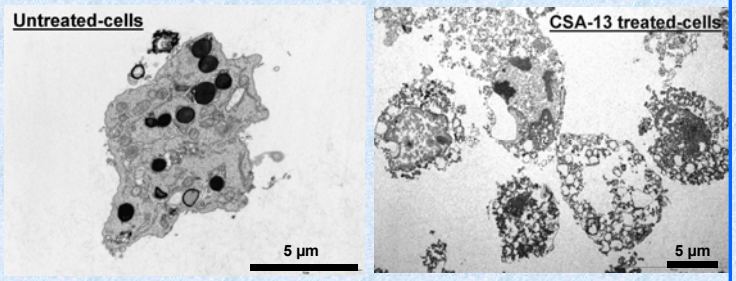
Killing of intracellular bacteria is observed for CSA-13 concentration causing a cytotoxic effect (≥ 20 mg/L)



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-phagocytosis 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



Methods

- MICs were determined broth micro-dilution method, in MHB (supplemented with NaCl 2 % for MRSA).
- Intracellular activity was studied in THP-1 macrophages.[1] Briefly, cells were infected with pre-opsonised bacteria (1h, 37°C), washed with PBS for the elimination of non-adherent and non-phagocytosed bacteria, and resuspended for 5 h or 24h in fresh medium supplemented with CSA-13 (extracellular concentration : 0.01 – 100 mg/L).
- Electron microscopy was performed as previously described on cells infected by ATCC 25923.[1]
- Toxicity studies was assessed by trypan blue exclusion assay.[2]

Conclusions

CSA-13 seems highly and quickly bactericidal towards intracellular *S. aureus*.

However, this effect is probably mediated by the drug induced-permeabilisation of macrophages, giving to CSA-13 a direct access to intracellular *S. aureus*.

References

[1] Lemaire et al., Activity of three ß-lactams (ertapenem, meropenem and ampicillin) against intraphagocytic *Listeria monocytogenes* and *Staphylococcus aureus,* J Antimicrob Chemother (2005) 55 (6): 897-904.

[2] Sanchez et al., Evaluation of Antibacterial Agents in a High-Volume Bovine Polymorphonuclear Neutrophil Staphylococcus aureus Intracellular Killing Assay; Antimicrob Agents Chemother. (1986) 29 (4) : 634 – 638.

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