

Intracellular activity of daptomycin (DAP) against Methicillin-Sensitive (MSSA), Methicillin-Resistant (MRSA) and Vancomycin-Intermediate (VISA) *S. aureus*

S. Lemaire, F. Van Bambeke, M.-P. Mingeot-Leclercq and P.M. Tulkens,

^aUnité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Brussels;

Mailing address:



Sandrine Lemaire
Pharmacologie cellulaire et
moléculaire
UCL 73.70 av. Mounier 73
1200 Brussels - Belgium
sandrine.lemaire@facm.ucl.ac.be

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Abstract

Objectives: Relapsing and chronic *S. aureus* infections has been ascribed to intracellular bacterial persistence and emergence of resistance. The activity of antibiotics may differ markedly between the extracellular and intracellular milieu (AAC 2006, 50:841-851). Intracellular activity needs, therefore, to be assessed in specific models. In this context, we have examined the activity of DAP, a cyclic lipopeptide with fast and extensive bactericidal activity against *S. aureus*.

Methods: MSSA (ATCC 25923), HA-MRSA (ATCC 33591), and VISA (NRS 23) were used. MICs were determined in MH broth (supplemented with 50 mg/L of Ca²⁺) by micro-dilution method. Infection of THP-1 macrophages was performed as described previously (JAC 2005, 55:897-904) and the intracellular localization of the bacteria was ascertained by confocal and electron microscopy. Activity was measured after 24 h exposure to a drug concentration corresponding to reported human C_{max} (77 mg/L) (controls cells: gentamicin [0.5 x MIC] to prevent extracellular growth).

Results: In controls, phagocytosed bacteria were seen in membrane-bounded vacuoles (phagolysosomes). MICs and intracellular activities are shown in the table.

Strains	DAP	
	MIC (mg/L)	THP-1 Δ Log cfu ± SD
MSSA	0.125	-1.7 ± 0.1
HA-MRSA	0.125	-1.5 ± 0.1
VISA	0.5	-1.3 ± 0.1

Conclusions :
DAP shows intracellular activity against MSSA, HA-MRSA and VISA.

Background

Intracellular survival is often considered as an important determinant in the relapsing and recurrent character of staphylococcal infections.¹ We have shown that the activity of antibiotics towards phagocytized *S. aureus* developing in phagolysosomes is often considerably weaker than their extracellular activity.² Yet, major differences exist among antibiotics with respect of their capacity to decrease the intracellular inoculum. It should, therefore, be possible to optimize therapy by selecting antibiotics active against both extracellular and intracellular forms of *S. aureus*.

Daptomycin (DAP) is a cyclic lipopeptide³ which shows extensive bactericidal effect towards extracellular *S. aureus*. Because there is evidence that DAP could localize in lysosomes of eukaryotic cells.⁴ We have, therefore, examined its intracellular activity against *S. aureus* with different resistance phenotypes (MSSA, HA-MRSA and VISA).

Results

1 Dose-effect relationship against MSSA

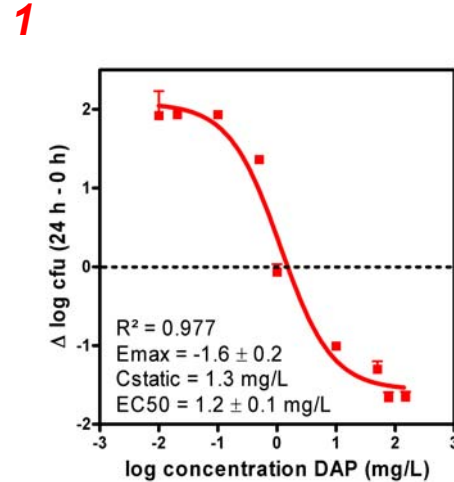


Fig.1. Dose-response curves of DAP over a wide range of extracellular concentrations against intracellular MSSA ATCC 25923. The ordinate shows the change in cfu per mg of cell protein at 24 h compared to the post-phagocytosis inoculum. A sigmoidal function was used for the regression, with the pertinent regression parameters (50 % effective concentration [EC₅₀], static concentration [C_{static}], maximal efficacy [E_{max}]).

2 Comparative activity of DAP against MSSA, HA-MRSA & VISA

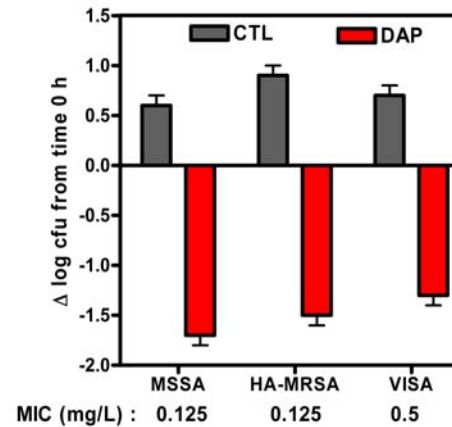


Fig.2. Comparative intracellular activity of DAP against intracellular MSSA ATCC 25923, HA-MRSA ATCC 33591 and VISA NRS 23. Infected cells were incubated with a drug concentration equivalent to its human C_{max} (77 mg/L) for 24 h.

Conclusions

DAP, known to be highly bactericidal extracellularly, displays intracellular concentration-dependent activity with

- EC₅₀ and C_{static} values close to 9-10 x its MIC in broth (denoting a loss of intrinsic activity similar to that of gentamicin²) and E_{max} value of about -1.6 log cfu (denoting a limit in the extent of its intracellular activity similar to that of ciprofloxacin²)
- is not markedly influenced by the resistance phenotype (towards beta-lactams or vancomycin)

Methods

MICs :

Susceptibility testing was performed by micro-dilution in Mueller-Hinton broth supplemented with 50 mg/L CaCl₂.

Intracellular infection and determination of the intracellular activity of daptomycin

THP-1 macrophages were infected with opsonized bacteria (1 h; 37°C), washed with phosphate-buffered saline, and incubated for 45 minutes with gentamicin (50 mg/Liter) to eliminate non-adherent and non-internalized bacteria.

Infected cells were then exposed for 24 h to antibiotics while control cells were maintained in the continuous presence of gentamicin ([0.5 x MIC]) to prevent the extracellular growth of bacteria released from dead cells (this method is described in details in refs. 3 and 5)

References

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