Telavancin accumulates in cultured macrophages and is active against intracellular *S. aureus*

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Background: TLV is a bactericidal lipoglycopeptide with multiple mechanisms of action against Gram positive organisms, including *S. aureus.* The latter is known to cause recurrent infections, associated with its ability to survive within eukaryotic cells. We have therefore examined TLV accumulation and activity against intracellular *S.aureus,* using a previously described quantitative model of infected macrophages (AAC 47:2283-92., 2003), in comparison with its activity in broth.

Methods: *S. aureus* ATCC 25923 (TLV MIC 0.5 mg/L) was used for all experiments. Infected and uninfected J774 mouse macrophages were incubated with TLV at extracellular concentrations (Ce) up to the human Cmax (90 mg/L). Cell content of TLV (Cc) was determined using 14C TLV. Activity was assessed by CFU counting in broth (extracell. activity) or in cells (intracell. activity).

Results: TLV displayed rapid (> 5 log decrease in 6 h at 20 mg/L), concentration-dependent bactericidal activity in broth. TLV accumulation in macrophages was linear over time (0.86 ± 0.04 µg/mg protein [prot] per h for cells incubated with 90 mg/L; R2= 0.988), leading to an apparent accumulation ratio of 45.6 ± 5.8 fold after 24 h. Cc was linearly related to Ce (up to 90 mg/L; R2=0.985) at a level of 0.24 ± 0.02 µg/mg prot in cells per mg/L in the medium (corresponding to an accumulation ratio of 54 ± 4). Efflux was slow (t½ of approx. 24 h). Intracellular CFU decreases were observed from 3 h and at Ce as low as 0.5 mg/L; and progressed on a time-(3-24 h) and concentration- (Cc=0.5-90 mg/L) dependent fashion to reach an approx. 2.8 log decrease after 24 h.

Conclusion: TLV accumulates slowly but steadily in macrophages, and displays significant bactericidal activity over 24 h against intracellular *S. aureus* at clinically relevant extracellular concentrations. Similar to all other antibiotics tested in this model so far (AAC 47:2283-92, 2003; 48: 2853-60, 2004), intracellular activity is less than that observed extracellularly.

INTRODUCTION

- *S. aureus* cause recurrent and persistent infections, probably in relation with its capacity to survive and multiply within eucaryotic cells (2). An optimized therapeutic choice for this type of infection would therefore require the use of antibiotics with bactericidal activity against both extracellular and intracellular bacteria.
- Telavancin is a new lipo-glycopeptide characterized by a highly bactericidal activity against *Staphylococcus aureus* and other Gram-positive organisms (1). This suggests that it may become a drug of choice for treating Gram-positive infections, especially those for which eradication may be critical.
- A previous study from our laboratory (3) showed that oritavancin, another highly bactericidal glycopeptide towards extracellular bacteria, accumulates in in macrophages and display a marked bactericidal effect against intracellular *S. aureus* (3). We therefore examined telavancin in this context.

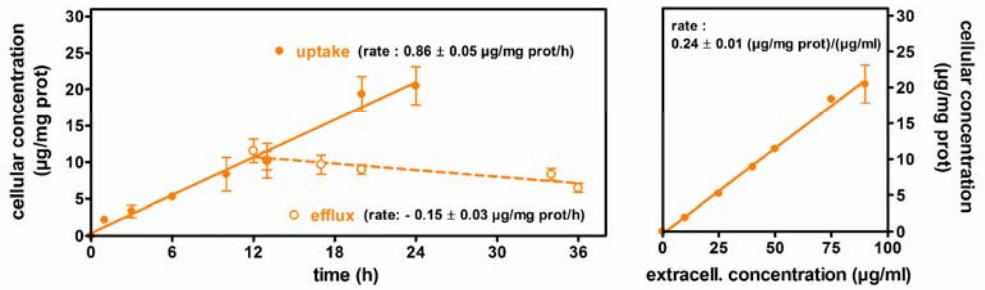
AIM OF THE STUDY

- To study the capacity of telavancin to accumulate in phagocytic cells (macrophages) → CELLULAR PHARMACOKINETICS
- To evaluate the activity of telavancin against intracellular *S.aureus* (as compared to its activity against extracellular bacteria) → CELLULAR PHARMACODYNAMICS

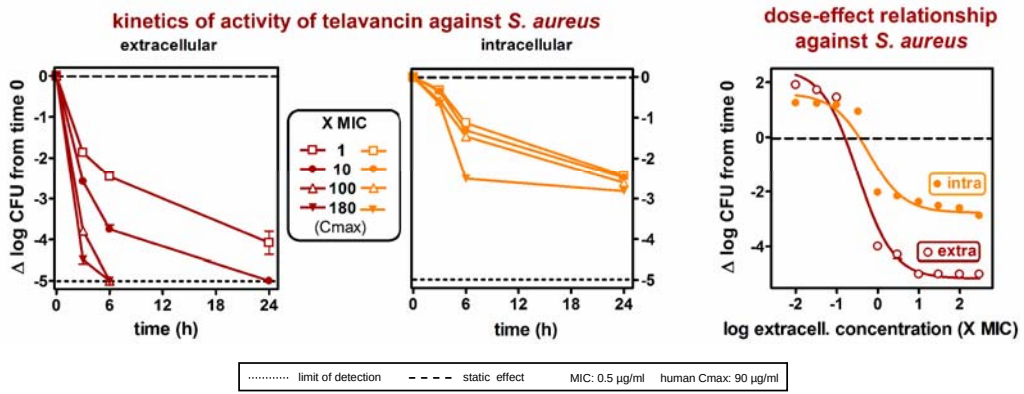
METHODS

- **Pharmacokinetics**: J774 mouse macrophages were exposed to ^{14}C-telavancin for up to 24 h, washed in ice-cold NaCl 0.9 %, collected by scraping, and lysed by sonication. Telavancin cell content was determined by scintillation counting, and expressed by reference to the protein content of the samples (3).
- **Pharmacodynamics:** Extracellular activity against *S. aureus* ATCC 25923 (fully susceptible strain) was evaluated by CFU counting after a 24 h incubation in culture medium. Intracellular infection was obtained by a 1 h incubation with serum-opsonized bacteria (0.5 bacteria/cell), washing with 50 mg/L gentamicin and reincubation in fresh medium containing either the tested antibiotic or 0.5 mg/L gentamicin (control), as described (3,4). CFU/mg cell protein were determined by plating cell lysates.

cellular pharmacokinetics



pharmacodynamics



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Cellular pharmacokinetics

- Telavancin accumulates linearly over time, reaching cellular concentrations about 50 times higher than the extracellular ones after 24 h (considering a cellular volume of 5 µl/mg cell prot.).
- Telavancin efflux proceeds at a slower rate than influx (half-life ~ 24 h for efflux from cells exposed to the drug during 12 h).
- Telavancin cellular concentration is proportional to its extracellular concentration, with accumulation ratios of 40 to 55 after 24 h incubation over the whole range of extracellular concentrations investigated.

Pharmacodynamics

- Telavancin displays a rapid and concentration-dependent bactericidal activity against extracellular *S. aureus.*
- Telavancin is also bactericidal against intracellular *S. aureus*, but to a level that is significantly lower than extracelularly.
- Both extracellular and intracellular activities develop on a concentration-dependent fashion, with the following pharmacological parameters:

	extra	intra
E_{max} (log decrease CFU)	– 5.2 ± 0.2	– 2.8 ± 0.3
EC_{50} (X MIC)	2	4
static effect (X MIC)	0.2	0.4

- This activity is comparable to what we previously observed for oritavancin (3) and superior to all other antibiotics tested in this model (4).

CONCLUSIONS

- Telavancin accumulates slowly but steadily in macrophages and expresses bactericidal activity against intraphgoyctic *S. aureus*
- Telavancin intracelular activity may be worthwile to be further explored in *in vivo* models and in appropriate clinical trials.