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# Oritavancin Demonstrates Rapid and Sustained Bactericidal Activity in the Rat Granuloma Pouch Model of *Staphylococcus aureus* Infection

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## Abstract

**Background:** *Staphylococcus aureus* remains one of the most important pathogens causing nosocomial infections. Oritavancin (ORI) is a semi-synthetic glycopeptide with activity against Gram-positive cocci including methicillin-resistant *S. aureus* (MRSA) and vancomycin (VAN)-resistant *S. aureus*. Its long elimination half-life from tissues prompted us to study its pharmacokinetics (PK) and activity in the rat granuloma pouch model of *S. aureus* infection.

**Methods:** Granuloma pouches were induced with an injection of sterile air and croton oil into connective dorsal tissue of rats 7 days before inoculation of $10^9$ CFU of methicillin-sensitive *S. aureus* (MSSA) ATCC 13709 into each pouch. ORI was injected intravenously (i.v.) 2 h post-infection to rats at 7 doses ranging from 0.25 to 30 mg/kg (n=5/dose). A group of rats (n=5) received VAN at 100 mg/kg subcutaneously (s.c.) as a comparator. Blood and pouch exudates were recovered at time points 2, 4, 6, 24, 48, 72, 96, and 168 h post-infection for bacterial counts on tryptic soy agar plates and for determination of ORI levels by bioassay using *Enterococcus faecalis* ATCC 29212 as the indicator strain.

**Results:** Single-dose i.v. PK studies with infected rats showed linear relationships between dose versus maximum concentration of drug in serum ($C_{max}$) and between dose versus area under the concentration-time curve ($AUC_{inf}$). ORI was more rapidly bactericidal than VAN. Regrowth was delayed longer with ORI than with VAN (>72 h and 24 h, respectively).

**Conclusions:** ORI is highly active in the rat granuloma pouch model of MSSA infection. Its *in vivo* bactericidal activity is more rapid and is sustained longer than that of VAN. These efficacy studies support the idea that infrequent dosing of ORI may be applicable in treating infections caused by Gram-positive bacteria.

## Introduction

- *Staphylococcus aureus* remains one of the most important pathogens causing nosocomial infections.
- The continuing increase in antimicrobial resistance in hospitals remains a concern. The proportion of *S. aureus* isolates that are resistant to methicillin, oxacillin, or nafcillin continues to rise and is presently nearly 60% in the U.S. [1].
- Novel, potent antibacterial agents are needed to counteract drug-resistant organisms.
- Oritavancin (ORI) is a semi-synthetic glycopeptide with activity against most Gram-positive bacteria including methicillin-resistant *S. aureus* (MRSA) and vancomycin (VAN)-resistant *Enterococcus* (VRE) [2].
- A PK/PD study in a neutropenic mouse model of *S. aureus* thigh infection revealed a long plasma half-life for ORI, suggesting that accumulation in plasma or tissue may be possible over time and raising the possibility of infrequent dosing [3].
- The rat granuloma pouch model has previously been used in the evaluation of PK/PD properties of antibacterial agents [4, 5]. An advantage of this model is that it permits recovery of multiple samples from the same animal during the course of the experiment: drug concentrations can be followed in blood as well as in the granuloma pouch exudates while drug activity can be measured as bacterial titer per ml of pouch exudates at multiple time points.
- Here we studied ORI PK and antibacterial activity in the rat granuloma pouch model of *S. aureus* infection.

## Methods

***In vitro* activities:** *S. aureus* ATCC 13709 (Smith; MSSA) was used in this study. Minimum inhibitory concentration (MIC) was determined by the broth microdilution method in 96-well plates according to CLSI (formerly NCCLS) guidelines. Compounds were serially diluted twofold in cation-adjusted Mueller-Hinton broth (CAMHB). Both ORI and VAN have an MIC of 1 µg/ml for this strain.

***In vivo* activities:** Rat granuloma pouch model [4]: Female CD rats (Charles River, St-Constant, Canada) weighing 150-170 g were used. Granuloma pouches were induced with an injection into connective dorsal tissue of 30 ml sterile air followed by 0.5 ml of 1% croton oil in olive oil. Seven days later, each pouch was inoculated with 1 ml of a bacterial suspension containing $10^9$ cells of *S. aureus* ATCC 13709. ORI was prepared in 5% dextrose and injected intravenously (i.v.) 2 h post-infection to rats at 7 doses ranging from 0.25 to 30 mg/kg (n=5/dose). A group of rats received VAN subcutaneously (s.c.) at 100 mg/kg prepared in saline as a comparator. Blood and pouch exudates were recovered at time points 2, 4, 6, 24, 48, 72, 96, and 168 h post-infection for bacterial counts on tryptic soy agar plates and for determination of ORI levels by bioassay. Δ $Log_{10}$ difference CFU/ml of exudates was calculated by subtracting the mean Log CFU/ml at 72 h post-infection from the mean Log CFU/ml of the untreated group.

**Analytical methods for plasma and pouch exudates:** ORI levels in plasma and pouch exudates were determined by an MIC bioassay using *Enterococcus faecalis* ATCC 29212 as the indicator strain. Standards of ORI spiked into naïve plasma or pouch exudates were used as reference. The limit of detection of ORI in both plasma and exudates is 0.125 µg/ml. For calculation of free ORI concentration, protein binding of ORI in mouse plasma was assumed to be 85% [3].

**Data analysis:** PK parameters were calculated using WinNonlin software (Pharsight). All parameters were calculated using the non-compartmental model. Differences in viable counts between the untreated and drug-treated groups were analyzed by Kruskal-Wallis's multiple-comparison procedure. *P* values below 0.05 were considered statistically significant.

## Results

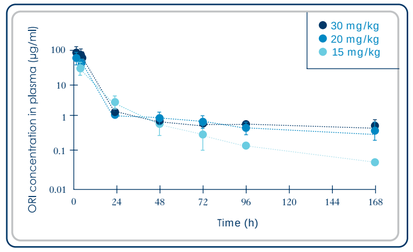


Figure 1.
Mean concentration of ORI in plasma up to 168 h following i.v. administration of a single 15, 20, or 30 mg/kg dose to infected rats.

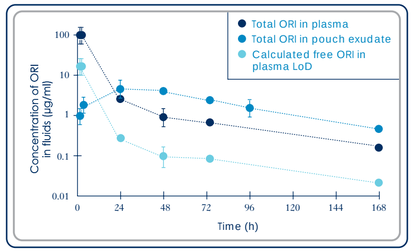


Figure 2.
Mean concentration of ORI in plasma and granuloma pouch exudates up to 168 h following i.v. administration of a single 30 mg/kg dose to infected rats.

Table 1.
$C_{max}$, $T_{max}$, $T_{1/2}$ and exposure in plasma and granuloma pouch exudates following a single i.v. dose of ORI at 30 mg/kg to infected rats.

| | Free ORI in plasma | Total ORI in | |
|---|---|---|---|
| | | Plasma | Exudate |
| $C_{max}$ (µg/ml) | 16 | 107 | 5.3 |
| $T_{max}$ (h) | 2 | 2 | 24 |
| $T_{1/2}$ (h) | 18 | 18 | 41 |
| $AUC_{inf}$ (h*µg/ml) | 255 | 1592 | 398 |

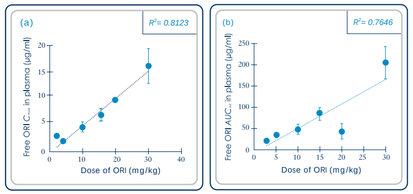


Figure 3.
Linear relationship of $fC_{max}$ (a) and $fAUC_{inf}$ (b) of ORI in plasma following i.v. administration of a single 30 mg/kg dose to infected rats.

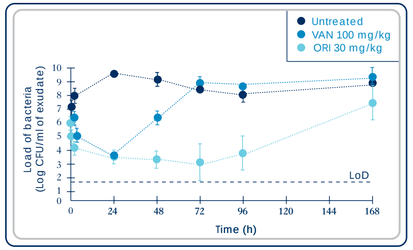


Figure 4.
Efficacy of a single 30 mg/kg i.v. dose of ORI compared with a 100 mg/kg s.c. dose of VAN in the rat granuloma pouch model.

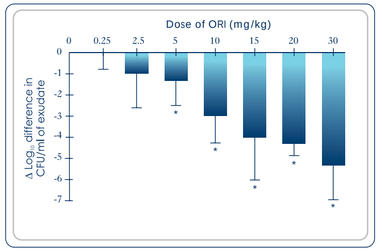


Figure 5.
Efficacies of single doses of ORI against *S. aureus* ATCC 13709 compared with the untreated group in the rat granuloma pouch model at 72 h post-therapy. *$P$<0.05

## Conclusions

Single-dose ORI PK studies with infected rats showed:

- Long half-life of ORI in both plasma and granuloma pouch exudates following i.v. administration
- Linear relationships between ORI dose and both $C_{max}$ and $AUC_{inf}$ in plasma

ORI is highly active in the rat granuloma pouch model against MSSA:

- *In vivo* bactericidal activity of ORI at 30 mg/kg is more rapid than that of VAN at 100 mg/kg (~2 log CFU decrease at 4 h vs between 4-24 h, respectively)
- Activity of ORI is sustained longer than that of VAN (regrowth prevented for >72 h and 24 h, respectively)
- ORI activity is dose-dependent
- The rat granuloma pouch model could also be used for further investigations to evaluate ORI efficacy against MRSA and to select an infrequent dose regimen

The pharmacokinetic properties and efficacy data obtained for ORI support the idea that it might retain efficacy when used in an infrequent dosing regimen (administered less frequently than other treatments)

## References

1. *National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004.* Am J Infect Control, 2004. **32**(8): p. 470-85.
2. Allen, N.E. and T.I. Nicas, *Mechanism of action of oritavancin and related glycopeptide antibiotics.* FEMS Microbiol Rev, 2003. **26**(5): p. 511-32.
3. Boylan, C.J., et al., *Pharmacodynamics of Oritavancin (LY333328) in a Neutropenic-Mouse Thigh Model of Staphylococcus aureus Infection.* Antimicrob Agents Chemother, 2003. **47**(5): p. 1700-6.
4. Dalhoff, A., *The granuloma pouch,* in *Experimental models in antimicrobial chemotherapy.*, O. Zak and M.A. Sande, Editors. 1986, Academic Press: London, UK. p. 123-137.
5. Jabes, D., et al., *Efficacy of dalbavancin against methicillin-resistant Staphylococcus aureus in the rat granuloma pouch infection model.* Antimicrob Agents Chemother, 2004. **48**(4): p. 1118-23.

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