Efficacy of Oritavancin in a Murine Model of *Bacillus anthracis* Spore Inhalation Anthrax

A-101

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Abstract

Background: *Bacillus anthracis* (BA), the causative agent of anthrax, principally causes disease in herbivores but also causes infections in humans of which the respiratory form is often fatal. Anthrax as an agent of biowarfare and bioterrorism could exhibit multi-drug resistance, further complicating treatment. Ciprofloxacin (CIP) therapy for 60 days is the current standard for anthrax postexposure prophylaxis. Oritavancin (ORI) is a semi-synthetic lipoglycopeptide with activity against gram-positive bacteria, including BA *in vitro* (ORI MIC_{90} with polysorbate-80, 0.12 µg/mL; n=30). To investigate potential new therapies, we tested the efficacy of ORI in a mouse aerosol-anthrax model. **Methods/Results:** Mice were challenged with 75 LD_{50} of spores of BA (Ames strain). Control animals received phosphate-buffered saline (PBS) or CIP 30 mg/kg intraperitoneally (i.p.) each 12 h (q12h) for 14 d. Postexposure prophylaxis in ORI dose-ranging studies (0.1-30 mg/kg i.p. q48h for 14 d or alternatively 5-50 mg/kg as a single intravenous [i.v.] dose) began 24 h after challenge. Thirty-day postchallenge survival proportions were 0/10 (PBS), 9/10 (CIP), 5/10 (1 mg/kg ORI i.p.), 10/10 (≥3 mg/kg ORI i.v.), 7/10 (15 mg/kg ORI i.v.) and 10/10 (50 mg/kg ORI i.v.). A follow-up experiment showed that a single 50 mg/kg i.v. dose of ORI given 24 h prechallenge also provided 100% survival (n=10) at day 30. Delay of therapy (10 mg/kg i.p. q48h for 14 d) to 36 and 48 h postchallenge to simulate treatment yielded 30-day results similar to CIP (9/10 ORI-36 h, 7/10 CIP-36 h; 5/10 ORI-48 h, 8/10 CIP-48 h). **Conclusions:** The *in vivo* efficacy data suggest that ORI could serve as an alternative therapy for anthrax prophylaxis or treatment. Due to its extended efficacy *in vivo*, fewer doses of ORI vs. CIP may be required. In addition, multiple mechanisms of action for ORI may allow it to retain activity against drug- (including vancomycin)-resistant BA.

Introduction

Bacillus anthracis, the causative agent of anthrax, primarily causes disease in animals. In humans it can produce a fatal disease if inhaled or ingested. With the added concern of engineered resistance in a biological threat setting, it is important to test new agents for potential utility in treatment. The objective of these studies was to assess the efficacy of oritavancin against inhalational anthrax, using an accepted small animal model.

Methods

Oritavancin (ORI) minimal inhibitory concentrations (MICs) were determined by broth micro-dilution (M7-A7, Clinical and Laboratory Standards Institute)[1] with the addition of 0.002% polysorbate-80 throughout all steps of the assay to minimize oritavancin binding to surfaces[2, 3]. § For injection, oritavancin diphosphate was dissolved in 5% dextrose. Negative control animals received either no treatment or vehicle alone. A positive control of ciprofloxacin (CIP) 30 mg/kg intraperitoneally (i.p.), starting 24 h postchallenge, q12h for 14 d, was also included. Mice were evaluated daily for clinical signs and survival. § For ORI efficacy trials, Ames spores of *B. anthracis* were used for an aerosol challenge of 50-75 LD_{50} (LD_{50} = 3.4 x 10^4 spores)[4]. § A dose-ranging study in the post-exposure prophylaxis anthrax model tested ORI i.p. at 0.1, 0.3, 1, 3, 10, and 30 mg/kg q48h for 14 d. Alternatively, ORI was given as a single intravenous (i.v.) dose of 5, 15, or 50 mg/kg. Treatments were initiated 24 h after challenge. § A delay of therapy study to simulate post-symptomatic treatment was initiated either 36 or 48 h post-challenge with ORI at 10 mg/kg i.p. q48h or CIP at 30 mg/kg i.p. q12h for 14 days and followed to day 30. § A follow-up experiment was conducted with a single 50 mg/kg i.v. dose of ORI administered 24 h pre-challenge and followed to day 30. § Kaplan-Meier curves were compared by the log rank test for significance over controls. § Tissue-bacterial burdens were determined from dead or moribund animals. Surviving mice from each group were euthanized at Day 30. Lungs were aseptically removed, weighed and homogenized in 1 ml of sterile water. Homogenates were serially diluted 1:10 in water and 100 µL aliquots were plated on sheep blood agar plates (SBAP). For anthrax spore quantitation, homogenates were "heat shocked" for 15 minutes at 65ºC to kill vegetative cells then serially diluted and plated on SBAP.

Results

Oritavancin demonstrated potent activity (MIC_{90} with polysorbate-80, 0.12 µg/mL) against 30 strains of *B. anthracis* representing all eight genotype clades identified by Keim[5]. As seen in previous *in vitro* studies with staphylococci and enterococci[2,3], the ORI MIC_{90} for *B. anthracis* was 16-fold higher in the absence of polysorbate-80, likely as a result of drug binding to surfaces of test vessels (Figure 1).

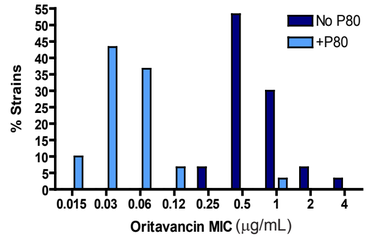

Figure 1. Oritavancin Susceptibility Distribution for *B. anthracis* (n=30).

Abbreviations: No P80, no polysorbate-80; +P80, with 0.002% polysorbate-80.

Dose ranging studies (Figure 2) showed that i.p. doses of ORI at 3 mg/kg q48h i.p. for 14 d offered effective post-exposure prophylaxis against a *B. anthracis*, Ames strain aerosol challenge. ORI doses of 10 and 30 mg/kg administered q48h i.p. for 14 d also provided 100% protection (not shown).

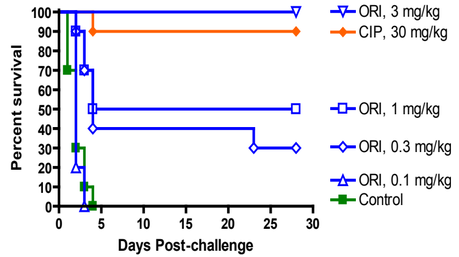

Figure 2. Multiple Dose ORI i.p. Dose Ranging in Post-exposure Prophylaxis.
Mice in the "CIP" group received ciprofloxacin at 30 mg/kg q12h i.p. for 14 d. Oritavancin ("ORI") doses are indicated in the legend and were administered q48h i.p. for 14 d. Treatments began 24 h post-challenge.

A single i.v. dose of 50 mg/kg oritavancin 24 hours after challenge offered 100% protection; further, 7/10 mice survived to 30 days with a single i.v. dose of 15 mg/kg oritavancin. Late deaths in the 15 mg/kg treatment group are most likely due to outgrowth of residual spores still present in the lung tissue, after the antibiotic levels dropped. At 50 mg/kg, oritavancin levels may have persisted until after spores were cleared to below the infection threshold (4; Figure 3).

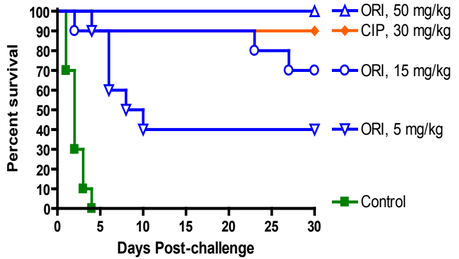

Figure 3. Single-dose ORI i.v. Dose Ranging in Post-exposure Prophylaxis.
Mice in the "CIP" group received ciprofloxacin at 30 mg/kg q12h i.p. for 14 days. Single-dose oritavancin ("ORI") doses are indicated in the figure legend and were administered i.v. Treatments began 24 h post-challenge.

Delayed treatment studies, in which treatment was initiated post-symptom development, showed that ciprofloxacin offered significant protection (70-80% survival) when administered 36 or 48 h after challenge (Figure 4). This finding is consistent with that reported by Heine et al. (4) in the same model.

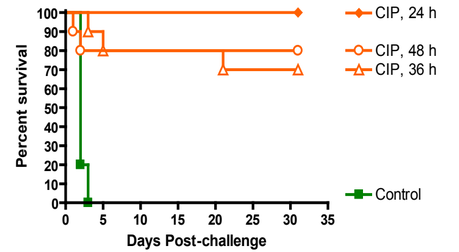

Figure 4. Proportional Survival from CIP Post-exposure Treatment.
Mice in the ciprofloxacin (CIP) groups received ciprofloxacin at 30 mg/kg q12h i.p. for 14 days. Treatment was initiated at either 24 h ("CIP, 24 h"), 36 h ("CIP, 36 h") or 48 h ("CIP, 48 h") post-challenge.

Oritavancin treatment post-symptom development also resulted in significant protection. While ORI treatment resulted in lower proportional survival (50%) than did ciprofloxacin (80%) when initiated at 48 h post-challenge, the difference was not statistically significant (Figure 5).

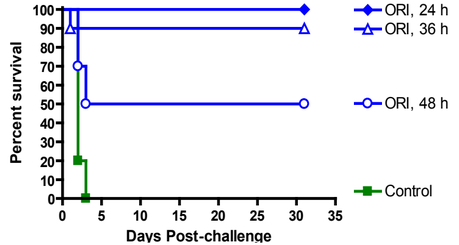

Figure 5. Proportional Survival from ORI Post-exposure Treatment.
Mice in the oritavancin (ORI) groups received oritavancin at 10 mg/kg q48h i.p. for 14 days. Treatment was initiated at either 24 h ("ORI, 24 h"), 36 h ("ORI, 36 h") or 48 h ("ORI, 48 h") post-challenge.

The complete protection that was provided by a single i.v. dose of 50 mg/kg ORI 24 h prior to challenge further supports the 24 hour post-exposure data and highlights the extended half-life of oritavancin. This finding suggests that ORI concentrates in those cellular compartments where spores may be germinating in the early stages of the infection (Figure 6).

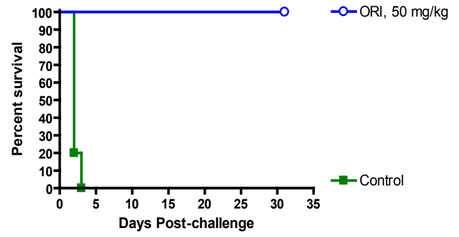

Figure 6. Proportional Survival from ORI Pre-exposure Prophylaxis.
Mice in the "ORI, 50 mg/kg" group received a single 50 mg/kg i.v. dose of oritavancin 24 hours prior to aerosol challenge.

Table 1. Efficacy and Post-treatment Spore Counts in Mouse Lung Tissue.

Agent	Regimen	Start of therapy (h post challenge)	Proportional survival at 30/31 d (%; n=10)	Spore load in lung (CFU/g)
None	-	-	0 *	n.d.
CIP	30 mg/kg q12h x 14 d	24 h	90	5.20×10^4
ORI	30 mg/kg i.p. q48h x 14 d	24 h	100	1.82×10^4
	10 mg/kg i.p. q48h x 14 d	24 h	100	4.79×10^4
	3 mg/kg i.p. q48h x 14 d	24 h	100	3.68×10^4
	1 mg/kg i.p. q48h x 14 d	24 h	50	2.95×10^4
	0.3 mg/kg i.p. q48h x 14 d	24 h	30	2.80×10^4
	50 mg/kg i.v. single dose	24 h	100	3.61×10^4
	15 mg/kg i.v. single dose	24 h	70	2.03×10^4
	5 mg/kg i.v. single dose	24 h	40	3.50×10^4
CIP	30 mg/kg q12h x 14 d	24 h	100	1.09×10^3
		36 h	70	2.88×10^3
		48 h	80	3.08×10^3
ORI	10 mg/kg i.p. q48h x 14 d	24 h	100	3.50×10^3
		36 h	90	2.52×10^3
		48 h	50	1.68×10^3
ORI	50 mg/kg i.v. single dose	– 24 h	100	1.98×10^3

*100% dead at 4d

Conclusions

§ *In vitro* and *in vivo* data suggest that ORI could serve as a therapy for prophylaxis or treatment after exposure to *B. anthracis*.

§ The ability of ORI to protect mice when administered 24h pre-exposure suggests that ORI may accumulate in those cellular compartments where spores may germinate, in the early stages of *B. anthracis* infection.

§ Due to the persistence of spores in the lungs and tissues of individuals exposed to *B. anthracis*, current therapies last 60 days. The extended efficacy of ORI *in vivo*, as demonstrated here, predicts that infrequent dosing of ORI may be sufficient for protection in humans.

§ Furthermore, multiple mechanisms of action for oritavancin may allow it to retain activity against drug- resistant isolates of *B. anthracis*, including those resistant to vancomycin, β-lactams, quinolones, and macrolides.

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