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Efficacy of oritavancin alone and in combination against vancomycin-susceptible and -resistant enterococci in an in-vivo *Galleria mellonella* survival model

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ABSTRACT

Objective: The optimal therapy for serious enterococcal infections, especially vancomycin-resistant enterococci (VRE), remains unclear, although combination therapy is often recommended. Oritavancin has demonstrated in-vitro activity against VRE, but data evaluating oritavancin in combination with other agents and in in-vivo systems are lacking. The objective of this study was to evaluate the efficacy of oritavancin alone and in combination with ceftriaxone, daptomycin, gentamicin, linezolid and rifampin against vancomycin-susceptible enterococci and VRE in an in-vivo *Galleria mellonella* survival model.

Methods: Five enterococcal strains were used: three clinical isolates (VRE S38141, VRE H19570, VRE W21579), *Enterococcus faecium* ATCC 700221 and *Enterococcus faecalis* ATCC 29212. *G. mellonella* larvae were inoculated with the test strain followed by the test drug at humanized weight-based dose alone or in combination within 1 h of inoculation. After injection, larvae were incubated at 37°C and survival was measured daily for 7 days. Survival was plotted using the Kaplan–Meier method, and differences between groups were determined via the log-rank test. Mean survival times were also determined.

Results: Each single agent improved survival significantly compared with the untreated control strain. Oritavancin was the most efficacious single agent, and led to a significant increase in survival compared with ceftriaxone, gentamicin and daptomycin. Compared with oritavancin alone, none of the oritavancin combinations tested were significantly better, and mean survival times were comparable.

Conclusions: Oritavancin monotherapy had the highest survival rate at 7 days, and none of the combinations tested showed improved survival over oritavancin alone. These data add to the body of literature rebutting the routine use of combination therapy with oritavancin for the treatment of infections due to VRE.

1. Introduction

Serious infections due to *Enterococcus* spp. are associated with significant morbidity, mortality and excess healthcare costs [1]. Vancomycin-resistant enterococci (VRE) are of particular concern given their prevalence among healthcare-associated infections and the limited number of available treatment options. Furthermore, monotherapy is often only bacteriostatic against enterococci, even with typically bactericidal agents such as the β-lactams. As such, combination therapy is the mainstay of treatment against serious infectious due to enterococci [2,3].

Oritavancin, a novel semisynthetic lipoglycopeptide antimicrobial, is the first agent approved by the Food and Drug Administration with in-vitro activity against *vanA*-type VRE since the introduction of daptomycin. The multiple mechanisms of action, bactericidal activity and novel pharmacokinetic profile of oritavancin make it an exciting addition to the antimicrobial armamentarium against Gram-positive pathogens. In-vitro activity against VRE makes oritavancin a potential therapeutic option for this difficult-to-treat pathogen, and clinical reports of its use for serious Gram-positive infections are already available [4,5]. As combination

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therapy is often recommended for serious enterococcal infections, it is important to evaluate the efficacy of combination therapy with oritavancin against VRE. To date, studies evaluating oritavancin combination therapy against enterococci have been limited primarily to in-vitro models [6–8].

While in-vitro systems are useful in elucidating the rate and extent of bactericidal activity, they lack an immune system and host response to infection. Mammalian systems provide important efficacy information but are time consuming, expensive and require full ethical consideration. Pre-mammalian models, such as the *Galleria melonella* wax worm model, possess innate immune systems similar to that of mammals [9] and have demonstrated utility as a model of bacterial virulence and survival in response to antimicrobial treatment [10–13]. *G. mellonella* also have advantages over other invertebrate models in that they can be incubated at human body temperature, and both the inoculum and antibiotic dose can be delivered directly to the host body [14,15]. These bridging models may 'derisk' drug development and provide data to inform both further research investigations and the clinical usage of antibiotics [16]. Evaluating the efficacy of oritavancin and other antimicrobials alone and in combination may provide initial data to help determine regimens that can be used effectively in practice to improve clinical outcomes.

The objective of this study was to evaluate the efficacy of oritavancin alone and in combination with ceftriaxone, daptomycin, gentamicin, linezolid and rifampin against vancomycin-susceptible enterococci and VRE in an in-vivo *G. mellonella* survival model. Additionally, this study sought to correlate in-vivo efficacy with in-vitro activity as determined in previous time-kill analyses [17].

2. Materials and methods

2.1. Bacteria and susceptibility testing

Five enterococcal strains were used for all experiments: three vancomycin-resistant VanA- type *E. faecium* clinical bloodstream isolates obtained from patients at the University of Illinois at Chicago (VRE S38141, VRE H19570, VRE W21579), vancomycin-resistant VanA-type *E. faecium* ATCC 700221 and vancomycin-susceptible VanA-negative *E. faecalis* ATCC 29212. Identification and VanA detection for clinical isolates were performed via Vitek 2 (bioMérieux, Durham, NC, USA) and Verigene Gram-positive blood culture assay (BC-GP, Nanosphere, Northbrook, IL, USA), respectively. Isolates were maintained at -80°C in cation-adjusted Mueller-Hinton broth (CAMHB) with 20% glycerol, and subcultured on tryptic soy agar with 5% sheep's blood twice prior to use. Analytical grade ceftriaxone, daptomycin, gentamicin, linezolid and rifampin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and oritavancin was provided by The Medicines Company (Parsippany, NJ, USA). Minimum inhibitory concentration (MIC) tests were performed in triplicate on the same day via broth microdilution at standard inoculum according to the guidelines of the Clinical Laboratory Standards Institute (CLSI), with 0.002% polysorbate-80 added to assays containing oritavancin and the Ca^{2+}content of CAMHB increased to 50 mg/L for assays containing daptomycin [18]. Non-tissue-culture-treated microtitre plates were used for all oritavancin assays to prevent any loss of drug potency [19]. Modal MIC values are reported.

2.2. Galleria mellonella survival model

G. mellonella larvae at final instar stage were acquired overnight from the wholesaler (Vanderhorst Wholesale, Inc., Saint Marys, OH, USA), stored in the dark at room temperature, and used within 7 days of receipt. Models proceeded in a stepwise fashion to ensure the lack of toxicity of each antimicrobial to healthy larvae, and to establish a lethal bacterial inoculum in the absence of antibiotic treatment. Healthy larvae weighing at least 250 mg and free of any gray markings were selected at random and administered 10 µL of each antimicrobial alone and in combination, delivered to the last left proleg via tuberculin syringe. Ten larvae were included in each test group along with at least two control groups: one injected with phosphate-buffered saline to assess needle trauma, and one untouched group for attrition. Experiments were performed in duplicate and repeated in cases of discordance, while the results of any experiment in which two or more larvae died in any control group were discarded. A starting bacterial inoculum of approximately 1×10^6 colony-forming units/mL was used for each strain, and this inoculum was titrated until 80% of the larvae were killed within 72 h. This lethal inoculum was determined for each of the five strains and used in survival experiments.

For survival experiments, *G. mellonella* larvae were inoculated with the test strain (10 µL) at the predetermined inoculum followed by the test drug alone or in combination (10 µL) within 1 h of inoculation. The same two control groups were included as described above. Humanized weight-based doses of each antibiotic were utilized as follows: oritavancin 15 mg/kg, ceftriaxone 25 mg/kg, daptomycin 10 mg/kg, gentamicin 1.3 mg/kg, rifampin 3.75 mg/kg and linezolid 7.5 mg/kg. After injection, larvae were incubated at 37°C and survival was measured daily for 7 days.

2.3. Statistical analysis

Larvae survival data were plotted using the Kaplan–Meier method, and differences between groups were determined using the log-rank (Mantel–Cox) test with Bonferroni's correction for multiple comparisons. Mean survival times for each pathogen–drug combination were also determined. All statistical analyses were performed using SPSS Version 24 (IBM Corp., Armonk, NY, USA).

3. Results

The MICs and interpretive category of each individual agent against the five enterococcal strains are displayed in Table 1. All five isolates were susceptible to linezolid and daptomycin according to CLSI breakpoints [18]. The MIC of *E. faecalis* ATCC 29212 to ceftriaxone was 128 mg/L, whereas the MICs to all VRE isolates were >256 mg/L. All VRE strains displayed high-level resistance to gentamicin with the exception of VRE H19570. *E. faecalis* ATCC 29212 was intermediate to rifampin while all VRE isolates were resistant. The oritavancin MIC to *E. faecalis* ATCC 29212 was 0.03 mg/L and considered susceptible. No interpretative criteria exist for oritavancin against VRE, although MICs ranged from 0.03 to 0.125 mg/L and were consistent with previous values [20].

Overall, monotherapy with any agent produced only modest improvements in 7-day survival (30–40%) compared with the control groups when all five strains were aggregated (Fig. 1). Kaplan–Meier plots for each individual isolate are provided in Fig. S1 (see online supplementary material). Log-rank analysis revealed that each single agent significantly improved survival compared with the control strain (Table 2), although increases in mean survival time were marginal for some agents (i.e. daptomycin and ceftriaxone) (Table 3). Compared with the control group, oritavancin was the most efficacious single agent, improving mean survival time by almost 2 days (Table 3). Oritavancin alone led to a significant increase in survival compared with ceftriaxone, gentamicin and daptomycin, but was similar to linezolid and rifampin (Table 2).

Combination therapy with oritavancin improved overall 7-day survival to approximately 50% (Fig. 2). Kaplan–Meier plots for each individual isolate are provided in Fig. S2. Log-rank analyses again demonstrated that each oritavancin combination improved survival

Table 1
Minimum inhibitory concentration (MIC) values (μg/mL) and interpretive category[a] of enterococcal strains to tested antimicrobials

Antibiotic	VRE S38141		VRE H19570		VRE W21579		VRE ATCC 700221		*E. faecalis*[b] ATCC 29212	
	MIC	Interpretive category	MIC	Interpretive category	MIC	Interpretive category	MIC	Interpretive category	MIC	Interpretive category
Ceftriaxone	>256	NC	>256	NC	>256	NC	>256	NC	128	NC
Daptomycin	1	S	2	S	1	S	1	S	1	S
Gentamicin	>500	HLAR	8	NC	>500	HLAR	>500	HLAR	16	NC
Linezolid	2	S	2	S	2	S	2	S	2	S
Rifampin	>256	R	64	R	>256	R	>256	R	2	I
Oritavancin	0.06	NC	0.125	NC	0.06	NC	0.03	NC	0.03	S

VRE, vancomycin-resistant enterococci; *E. faecalis, Enterococcus faecalis*; CLSI, Clinical Laboratory Standards Institute; NC, no CLSI interpretive criteria; S, susceptible; I, intermediate; R, resistant; HLAR, high-level aminoglycoside resistant.
[a] According to CLSI M100-S27.
[b] Vancomycin-susceptible.

Table 2
Log rank (Mantel–Cox) pairwise comparisons of each agent alone and in combination with oritavancin against the infected untreated control strain and oritavancin monotherapy[a,b]

Antibiotic	Ceftriaxone		Daptomycin		Gentamicin		Linezolid		Rifampin		Oritavancin	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Control	11.61	0.001	7.18	0.007	17.27	<0.001	32.97	<0.001	32.93	<0.001	45.31	<0.001
Oritavancin	12.28	<0.001	14.25	<0.001	9.06	0.003	0.89	0.345	0.54	0.462	-	-
					Oritavancin +							
Antibiotic	Ceftriaxone		Daptomycin		Gentamicin		Linezolid		Rifampin			
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*		
Control	82.9	<0.001	86.0	<0.001	90.5	<0.001	66.6	<0.001	82.6	<0.001		
Oritavancin	1.86	1.73	0.487	0.485	2.21	0.137	0.749	0.387	2.32	0.128		

[a] $\alpha \leq 0.008$ after Bonferroni's correction for multiple comparisons.
[b] Adjusted for isolate.

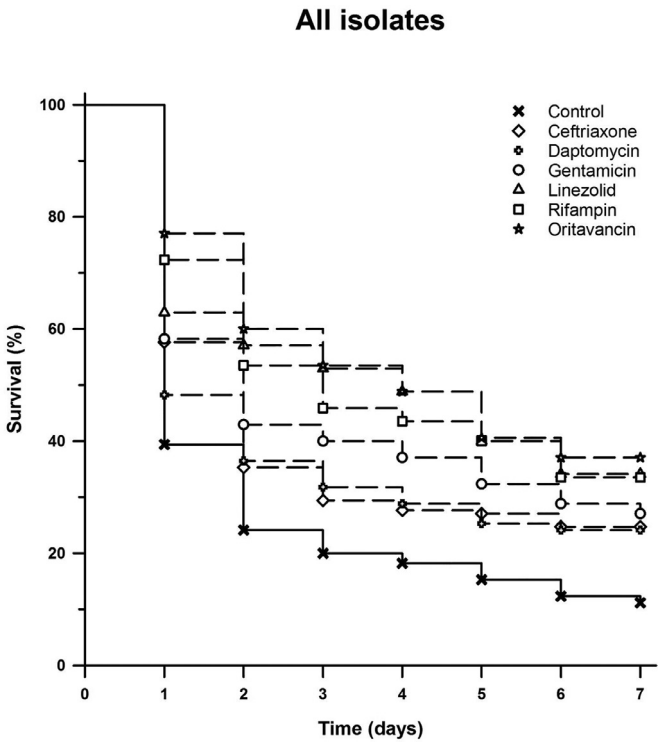

Fig. 1. Kaplan–Meier survival curves displaying efficacy of each agent alone against all tested enterococcal strains aggregated together. Curves represent average counts for triplicate experiments.

significantly compared with the control group (Table 2). All combinations improved mean survival time by at least 2 days (Table 3), with oritavancin plus gentamicin demonstrating the largest increase in mean survival time and the most significant difference

Table 3
Mean survival time for each agent alone and in combination with oritavancin compared with the infected untreated control strain[a]

Antibiotic	Survival time (days)	SE	95% CI
Control	2.29	0.16	1.98–2.61
Ceftriaxone	3.02	0.19	2.65–3.39
Daptomycin	2.95	0.19	2.57–3.33
Gentamicin	3.39	0.20	3.00–3.79
Linezolid	3.96	0.20	3.56–4.36
Rifampin	3.89	0.20	3.50–4.28
Oritavancin	4.17	0.19	3.79–4.55
Oritavancin +	Survival time (days)	SE	95% CI
Control	2.05	0.11	1.84–2.26
Ceftriaxone	4.22	0.23	3.76–4.67
Daptomycin	4.19	0.22	3.75–4.63
Gentamicin	4.32	0.23	3.88–4.76
Linezolid	4.19	0.23	3.75–4.64
Rifampin	4.27	0.24	3.81–4.73

SE, standard error; CI, confidence interval.
[a] Aggregate values for all five enterococcal strains.

compared with the control strain. Conversely, oritavancin in combination with linezolid or daptomycin produced the least increase in survival time, with oritavancin + linezolid being the least significantly different from the control. When compared with oritavancin alone, none of the combinations were significantly different and mean survival times were comparable.

4. Discussion

To the authors' knowledge, this is the first study to evaluate the efficacy of oritavancin alone and in combination in an in-vivo *G. mellonella* survival model. Each agent alone afforded significantly improved survival compared with untreated controls, with oritavancin monotherapy providing the highest rate of survival. In comparison with the other agents, oritavancin improved survival

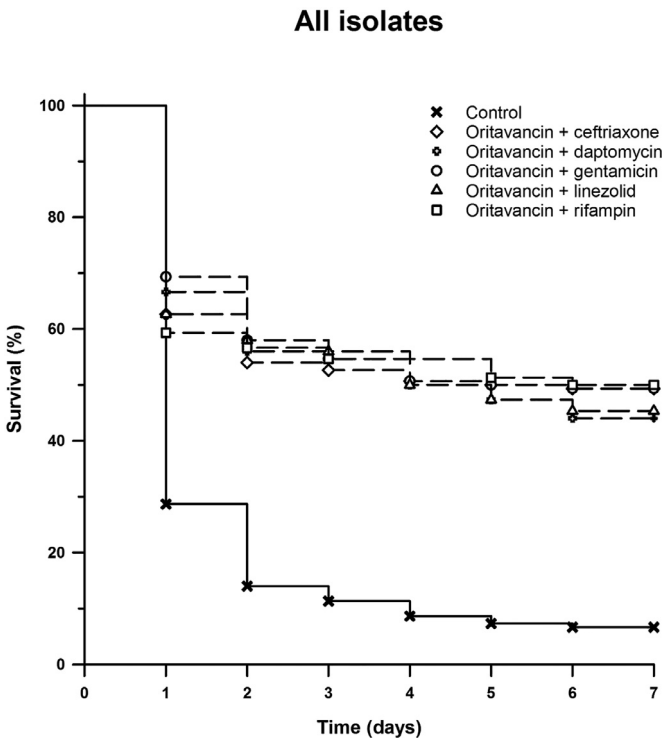

Fig. 2. Kaplan–Meier survival curves displaying efficacy of each agent in combination with oritavancin against all tested enterococcal strains aggregated together. Curves represent average counts for triplicate experiments.

significantly over ceftriaxone, gentamicin and daptomycin, but was similar to rifampin and linezolid. The combination of oritavancin and gentamicin was the most efficacious of the combination therapies evaluated, although none of the oritavancin combination regimens tested offered a significant improvement in survival compared with oritavancin monotherapy.

These findings expand on the authors' previous work evaluating the same isolates and antimicrobial combinations in in-vitro time-kill analyses [17]. It was demonstrated that combination therapy with oritavancin did not consistently improve bactericidal activity or produce synergy *in vitro*. Synergy was only observed between oritavancin and gentamicin against the two enterococcal strains without high-level aminoglycoside resistance (VRE H19570 and *E. faecalis* ATCC 29212). This correlates well with the present finding that oritavancin in combination with gentamicin provided the greatest increase in survival time and the most significant difference from the untreated control strain. Additionally, the authors' in-vitro work demonstrated antagonism between oritavancin with daptomycin against 50% of the VRE strains. This also correlates well with this combination having poor efficacy in the current survival model.

These results align well with previous data evaluating oritavancin combinations and *G. mellonella* models involving enterococci. In an in-vivo rabbit endocarditis model, the combination of oritavancin and gentamicin improved bacterial kill against vancomycin-susceptible and -resistant *E. faecalis* [8]. Luther *et al.* showed good correlation between their in-vitro pharmacodynamic model and their *G. mellonella* combination survival models against vancomycin-susceptible *E. faecalis* and vancomycin-resistant *E. faecium* [21]. Finally, Skinner *et al.* observed correlation between checkerboard synergy and survival in *G. mellonella* combination models against vancomycin-susceptible *E. faecalis* and vancomycin-resistant *E. faecium* [22]. Similar results have been observed in an in-vitro/in-vivo tandem *G. mellonella* model evaluating combination therapy against *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* [23].

These data suggest that there is good in-vitro/in-vivo correlation with this tandem experimental model, and that *G. mellonella* survival assays can be used to bolster in-vitro pharmacodynamic findings by providing an innate immune system and host response to infection. Taken together, these results can support further mammalian, and eventually human, studies on antimicrobial combinations against difficult-to-treat pathogens such as VRE.

Limitations to this study include the limited number of strains and combination concentrations tested. Inherent bacterial strain-to-strain and *G. mellonella* lot-to-lot variability is evident in the supplementary figures, although aggregated standard error values were very low. In-vivo efficacy did not seem to correlate with in-vitro susceptibility, which may be due to protein binding issues in *G. mellonella* haemolymph. The authors' future work will investigate the pharmacokinetic properties of antimicrobials within the *G. mellonella* larvae, and establish a more robust dose–exposure–response relationship. Finally, the *G. mellonella* survival model may not be representative of all clinical VRE infections, such as those occurring in immunocompromised patients or related to implanted prostheses.

To the authors' knowledge, this is the first study to explore the efficacy of oritavancin monotherapy and combination therapy against vancomycin-susceptible and -resistant enterococci in a *G. mellonella* survival model. Oritavancin monotherapy provided the highest rate of 7-day survival, and none of the combinations tested improved survival over oritavancin alone. These data, in addition to previous work and in the absence of clinical data, do not support the routine use of combination therapy with oritavancin for the treatment of infections due to VRE. Confirmation of these findings in in-vivo and clinical studies is warranted.

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Competing interests

EW serves on the speaker's bureau for Melinta Therapeutics, Astellas Pharma, and the advisory board for Shionogi and GenMark Diagnostics. LHD serves on the speaker's bureau for Melinta Therapeutics and Allergan, Plc. All other authors declare no potential conflicts of interest.

Ethical approval

not required.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.04.010.

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