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Assessment of Oritavancin Serum Protein Binding across Species[▽]

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Biophysical methods to study the binding of oritavancin, a lipoglycopeptide, to serum protein are confounded by nonspecific drug adsorption to labware surfaces. We assessed oritavancin binding to serum from mouse, rat, dog, and human by a microbiological growth-based method under conditions that allow near-quantitative drug recovery. Protein binding was similar across species, ranging from 81.9% in human serum to 87.1% in dog serum. These estimates support the translation of oritavancin exposure from nonclinical studies to humans.

Estimates of serum protein binding are essential to translate drug exposure from nonclinical species to humans during assessments of toxicology, pharmacokinetics, and pharmacodynamics since the free fraction dictates drug activity (3, 7, 17, 18). Recent evidence supports the concept of an "active fraction" that offers insight into the pharmacodynamic behavior of highly protein-bound drugs, such as daptomycin (20).

Oritavancin is a late-stage investigational lipoglycopeptide under study for treatment of serious Gram-positive infections (6). Nonspecific binding of oritavancin to labware surfaces (1, 2) and to dialysis membranes has called into question the accuracy of previous oritavancin human serum binding estimates (85.7% to 89.9% [16]). We therefore used conditions that minimize nonspecific oritavancin binding (4, 5) to estimate its binding to serum by a single *in vitro* methodology for three nonclinical species (mouse, rat, and dog) and humans. Protein binding estimates were derived from serum-induced increases in oritavancin MICs (9, 10, 21). To control for any impact of serum components on bacterial growth and antibiotic activity, oritavancin activity in serum was compared to its activity in serum ultrafiltrate, which is devoid of albumin, the protein responsible for the majority of oritavancin serum binding (23). The method was benchmarked using daptomycin and ceftriaxone (8, 18, 22). (Part of this work was previously presented at the 19th European Congress of Clinical Microbiology and Infectious Diseases as a poster [12].)

Pooled serum from humans, mice, and rats was from Equitech-Bio (Kerrville, TX); pooled serum from beagle dogs was from Bioreclamation (Liverpool, NY). Serum ultrafiltrate was prepared using Centricon Plus-50 ultrafilters (Millipore, Billerica, MA), whose molecular mass cutoff (50 kDa) excludes albumin. MICs against *Staphylococcus aureus* ATCC 29213 were determined by broth microdilution (4) using arithmetic drug dilutions in 95% serum or 95% serum ultrafiltrate, each supplemented with 5% cation-adjusted Mueller-Hinton broth (CAMHB). Serum protein binding for each drug was calculated using the following formula: % bound = (1 − [mean MIC in serum ultrafiltrate/mean MIC in serum]) × 100.

The MICs for each condition, serum source, and test agent were precise (Table 1), with a mean coefficient of variation of 17%. MICs as determined under CLSI M7-A8 conditions (Table 1) (4) were within the quality control ranges (5).

Increases in the oritavancin MICs in serum compared to its MICs in serum ultrafiltrate, by species, were similar across species (5.5- to 7.8-fold) (Table 2). Such shifts yielded similar mean values of oritavancin serum protein binding for the four species tested (81.9% to 87.1%) (Table 2). The 81.9% human serum protein binding estimate from this study falls within the 79% to 89.9% range of previously reported values from growth-based or biophysical approaches (summarized in Table 3). Our finding supports the premise that growth-based methods can complement biophysical methods in the estimation of the free fraction of antibiotics.

Oritavancin was found to bind rat serum at 82.4% in the present study; this concurs with the >80% binding to rat plasma using a broth microdilution approach (Table 3) (23). Oritavancin binding to serum of beagle dogs, a species which had not been evaluated prior to the present study despite its importance in nonclinical toxicology assessments, was estimated at 87.1% (Table 2). Our results showing a similar extent of oritavancin protein binding to human, mouse, rat, and dog serum should facilitate the translation of drug exposure between these species since the free fraction of oritavancin is likely to be equivalent across species, within the error of measurement of any single assay.

Comparison of the assessment of the area under the bacterial kill curves (10) for oritavancin determined in the presence of serum and in the presence of serum ultrafiltrate yielded protein binding values of 67.4, 63.9, and 61.7% for human serum (at 0.5, 1, and 2 μg/ml oritavancin, respectively) and of 66.5, 68.3, and 68.8% for mouse serum (at 0.5, 1, and 2 μg/ml oritavancin, respectively) (12). While these estimates are lower than those derived from the analysis of arithmetic MIC shifts in human and mouse serum noted above, they may be ex-

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TABLE 1. Oritavancin, ceftriaxone, and daptomycin MICs against *S. aureus* ATCC 29213 in cation-adjusted Mueller-Hinton broth and 95% serum ultrafiltrate and 95% serum from human, mouse, rat, and dog

Species	MIC (μg/ml)								
	Ceftriaxone[b]			Oritavancin[a]			Daptomycin[c]		
	CAMHB	Ultrafiltrate	Serum	CAMHB[d]	Ultrafiltrate[e]	Serum[f]	CAMHB	Ultrafiltrate	Serum
Human[g]									
Mean	4.88	2.88	38.8	0.084	0.140	0.775	0.975	0.513	3.00
SD	0.835	0.354	11.0	0.005	0.038	0.324	0.046	0.125	0.535
Mouse[h]									
Mean	5.00	3.75	6.00	0.105	0.079	0.538	0.975	3.00	12.5
SD	0.816	0.500	1.16	0.030	0.004	0.052	0.05	0	2.89
Rat[g]									
Mean	3.50	3.88	5.88	0.086	0.055	0.313	1.25	0.538	1.56
SD	0.535	0.354	0.641	0.007	0.005	0.099	0.267	0.052	0.32
Dog[g]									
Mean	5.25	1.09	1.38	0.080	0.061	0.475	1.00	0.638	2.50
SD	0.707	0.582	0.518	0	0.014	0.046	0	0.150	0.530

[a] Arithmetic dilution steps of 0.5 μg/ml from 3 to 1 μg/ml, of 0.1 μg/ml from 1 to 0.3 μg/ml, of 0.05 μg/ml from 0.3 to 0.1 μg/ml, and of 0.01 μg/ml from 0.1 to 0.04 μg/ml were prepared in cation-adjusted Mueller-Hinton broth (CAMHB) containing 0.002% polysorbate-80.
[b] Arithmetic dilution steps of 10 μg/ml from 100 to 10 μg/ml and of 1 μg/ml from 10 to 1 μg/ml were prepared in cation-adjusted Mueller-Hinton broth.
[c] Arithmetic dilution steps of 5 μg/ml from 20 to 10 μg/ml, of 1 μg/ml from 10 to 2 μg/ml, of 0.5 μg/ml from 2 to 1 μg/ml, and of 0.1 μg/ml from 1 to 0.3 μg/ml were prepared in cation-adjusted Mueller-Hinton broth supplemented with 50 μg/ml $CaCl_2$.
[d] MICs determined by CLSI M7-A8 guidelines in cation-adjusted Mueller-Hinton broth, supplemented with 0.002% polysorbate-80 (oritavancin) or 50 μg/ml $CaCl_2$ (daptomycin) (5).
[e] MICs determined in 95% serum ultrafiltrate plus 5% cation-adjusted Mueller-Hinton broth.
[f] MICs determined in 95% serum plus 5% cation-adjusted Mueller-Hinton broth.
[g] Means were derived from 8 replicates per condition per drug.
[h] Means were derived from 4 to 8 replicates per condition per drug.

plained at least in part by the rapid killing kinetics of oritavancin (11) that cannot be surmised from the MIC shift endpoints of broth microdilution assays.

Ceftriaxone was highly bound to human serum (92.6%) (Table 2), in agreement with both Yuk et al. (22) and MIC shift assessments by Schmidt et al. (18) but substantially higher than the 76.8% binding estimate derived from *in vitro* microdialysis (18). Variability in ceftriaxone serum protein binding across species (15, 18) was also noted in the present study, with substantially lower binding estimates for serum from mouse, rat, and beagle dog (range, 20.9% to 37.5%) than for human serum. These differences may result from true species-specific binding affinity differences (15) or from methodological differences during the isolation or assay of serum from each species.

TABLE 2. Serum-induced increases in broth microdilution MICs against *S. aureus* ATCC 29213 and corresponding protein binding estimates for oritavancin, ceftriaxone, and daptomycin

Serum source	Oritavancin		Ceftriaxone		Daptomycin	
	Mean fold MIC increase[a]	% Bound[b]	Mean fold MIC increase	% Bound	Mean fold MIC increase	% Bound
Human	5.5	81.9	13.5	92.6	5.8	82.9
Mouse	6.8	85.3	1.6	37.5	4.2	76.0
Rat	5.7	82.4	1.5	34.0	2.9	65.6
Dog	7.8	87.1	1.3	20.9	3.9	74.5

[a] Ratio of the mean arithmetic MIC in 95% serum to the mean arithmetic MIC in 95% serum ultrafiltrate.
[b] Calculated from mean MICs using the following formula: percent protein bound = [1 − (MIC in ultrafiltrate/MIC in serum)] × 100.

Daptomycin binding to serum protein also varied across species in the present study, ranging from 65.6% (rat) to 82.9% (human) (Table 2). For human serum, this value falls between the values of 58% reported by Tsuji et al. (21) and 94% reported by Lee et al. (8). The implications of such variability are potentially important during the translation of nonclinical findings to humans, for example, in pharmacokinetic-pharmacodynamic target attainment studies to support susceptibility breakpoint proposals (13).

While it is difficult to assess the accuracy of serum protein binding estimates from any single method, the precision of our cross-species comparative study, the concordance of single-species data from different methods, and the similarity of binding estimates across different species suggest that oritavancin is approximately 85% bound to serum protein and that differences in oritavancin protein binding across species are negligible. This conclusion is similar to one from studies of telavancin, another lipoglycopeptide, in which plasma protein binding was approximately 90% across tested species (19), although this value was substantially higher than the 62 to 70% estimates determined using a growth-based assay (21). The approximately 65% protein binding estimates from time kill-based assays with oritavancin (12) support the idea that the active fraction (20) of oritavancin, namely, its bioactive concentration in the presence of serum protein, is greater than the free fraction as predicted from biophysical approaches. Whether this conclu-

TABLE 3. Oritavancin serum protein binding estimates for human, mouse, rat, and dog

Species	Matrix	Protein binding[a] (%)	Method	Oritavancin concn (μg/ml)	Reference
Human	Plasma	87.5	Broth microdilution	Various	23
	Plasma	85.7–89.9	DCC[b] adsorption	1–91	16
	Albumin	79 ± 0.2	Cantilever nanosensor[c]	0.2	R. A. McKendry, unpublished
	Serum	81.9	Broth microdilution	Various	This study
Mouse	Serum	85.3	Broth microdilution	Various	This study
Rat	Plasma	>80	Broth microdilution	Various	23
	Serum	82.4	Broth microdilution	Various	This study
Dog	Serum	87.1	Broth microdilution	Various	This study

[a] Standard deviation value is provided where available.
[b] Dextran-coated charcoal.
[c] See reference 14.

sion applies to other lipoglycopeptides remains to be determined.

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