Activity of Oritavancin (LY333328), an Investigational Glycopeptide, Compared to That of Vancomycin against Multidrug-Resistant *Streptococcus pneumoniae* in an In Vitro Pharmacodynamic Model

ELIZABETH A. COYLE\(^1,2\) AND MICHAEL J. RYBAK\(^1,2,3,\*)

The Anti-Infective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital and University Health Center, \(^1\) College of Pharmacy and Allied Health Professions, \(^2\) and School of Medicine, \(^3\) Wayne State University, Detroit, Michigan 48201

Received 21 June 2000/Returned for modification 5 October 2000/Accepted 22 November 2000

In the past 2 decades, multidrug-resistant *Streptococcus pneumoniae* has been encountered with increasing frequency around the world. This has led to the need for newer agents in the treatment of *S. pneumoniae* infections. Oritavancin (LY333328) is a new glycopeptide antibiotic with activity against gram-positive organisms; however, there is limited information on the pharmacodynamics of oritavancin with respect to the treatment of *S. pneumoniae*. We utilized an in vitro pharmacodynamic model to compare the activity of oritavancin to that of vancomycin against two penicillin-, macrolide-, and ciprofloxacin-resistant *S. pneumoniae* isolates (R919 and R921) over a 48-h period. Both oritavancin and vancomycin achieved 99.9\% (3-log) kill, with oritavancin achieving the limit of detection (10\(^5\) CFU/ml) within 1 h and vancomycin achieving this limit at 24 h for both isolates. Detection of resistance was not observed for oritavancin or vancomycin during the 48-h experiments. The key pharmacodynamic parameter for oritavancin has not been well defined. In our experiment, the ratios of the area under the curve from 0 to 24 h to the MIC of oritavancin, oritavancin plus albumin, and vancomycin for both isolates were greater than 944.5, and the ratios of the maximum concentration of drug in serum to the MIC ranged from 73.7 to 7188.5. T>MIC was 100\% for oritavancin and vancomycin for both isolates. Oritavancin is a unique and potent antimicrobial that warrants further investigation against multidrug-resistant *S. pneumoniae*.

Over the last 20 years, penicillin-resistant *Streptococcus pneumoniae* has been encountered with increasing frequency. Similarly, resistance is increasing to other antimicrobials such as the macrolides, cephalosporins, and more recently the fluoroquinolones (4, 7). The glycopeptide vancomycin still remains a viable option for resistant *S. pneumoniae*; however, vancomycin tolerance has recently been reported (11). New agents with activity against resistant *S. pneumoniae* are needed. Oritavancin (LY333328) is an investigational, semisynthetic glycopeptide with good activity against most sensitive and resistant gram-positive pathogens. Recent information suggests that its mechanism of action may be different from that of vancomycin, which would explain its activity against vancomycin-resistant organisms such as enterococci (1).

**MATERIALS AND METHODS**

**Bacterial strains.** The study organisms consisted of two strains of *S. pneumoniae*, R919 and R921, that were derived from a clinical isolate, R79, by serial passage on agar plates containing various concentrations of ciprofloxacin (The Anti-Infective Research Lab), Detroit Receiving Hospital. The MIC of penicillin for both isolates is 2 \(\mu\)g/ml, that for erythromycin is 12 \(\mu\)g/ml, and the MICs of ciprofloxacin for R919 and R921 are 4 and 8 \(\mu\)g/ml, respectively.

**Antimicrobial agents and medium.** Vancomycin (lot no. 2MU91M) was commercially purchased from Eli Lilly and Company (Indianapolis, Ind.). Oritavancin (LY333328; lot no. S107316) was supplied by Eli Lilly and Company. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium (25 mg/ml) and magnesium (12.5 mg/ml) (SMHB) and 5\% lysed horse blood (LHB) (Rockland, Inc., Gilbertsville, Pa.) was used for susceptibility testing. Todd-Hewitt Broth (THB) (Difco Laboratories) supplemented with 0.5\% yeast extract (Difco Laboratories) was used in the in vitro pharmacodynamic models. To simulate the protein binding of oritavancin in vivo, human albumin (Baxter Healthcare Corporation, Glendale, Calif.) was added to all oritavancin in vitro pharmacodynamic models at the concentration of 4 g/dl.

**Susceptibility testing.** MICs and minimum bactericidal concentrations (MBCs) of both oritavancin and vancomycin were determined by broth microdilution in SMHB plus 5\% LHB, according to National Committee for Clinical Laboratory Standards guidelines for vancomycin (10). Samples (5 \(\mu\)l) from clear wells were plated on tryptic soy agar (TSA) plates with 5\% sheep blood (SB) to determine MBCs. All cultures were incubated in candle jars with approximately 3\% CO\(_2\) at a temperature of 37°C for 24 h. Oritavancin was additionally tested with the addition of albumin (4 g/dl).

**In vitro pharmacodynamic model.** The in vitro pharmacodynamic model consists of a 250-ml one-compartment glass chamber with ports for the addition and removal of the THB with 0.5\% yeast extract with or without albumin, injection of antibiotics, and removal of samples. Prior to each experiment, colonies from an overnight growth of bacteria on TSA plates with 5\% SB were added to THB with 0.5\% yeast extract to obtain a concentration of 10\(^8\) CFU/ml. Fresh stock solutions of oritavancin and vancomycin were prepared daily and were stored at 2 to 8°C between dose administration times. Experimental regimens simulated antibiotic concentrations achieved in human plasma. Vancomycin was administered at a dose of 1 g every 12 h (four doses given) to achieve a peak concentration of 15 \(\mu\)g/ml. To achieve targeted concentrations of oritavancin in plasma during the first 48 h of dosing in humans, oritavancin was administered at a loading dose of 5 mg/kg of body weight at 0 h, followed by 4 mg/kg at 24 h, to achieve a peak concentration of 100 \(\mu\)g/ml and 24-h trough concentration of 15 \(\mu\)g/ml. Each antibiotic was administered as a bolus into the models over 30 s using a hypodermic syringe. Fresh medium (SMHB) was continuously supplied and removed from the model along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, Ill.) set to simulate the half-lives (1\(_t,2\)) of vancomycin (6.5 h) and oritavancin (2.1 h).
**TABLE 1.** MICs and MBCs for *S. pneumoniae* isolates R919 and R921

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (MBC) for isolate as determined by microdilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R919</td>
</tr>
<tr>
<td>Oritavancin</td>
<td>$\leq0.015$ ($\leq0.015$)</td>
</tr>
<tr>
<td>Oritavancin + albumin</td>
<td>0.03 (0.06)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.5 (1.0)</td>
</tr>
</tbody>
</table>

* MICs and MBCs are given in micrograms per milliliter.

and oritavancin ($t_{1/2}$ at α phase $[t_{1/2α}] = 2$ h); the pump ran in this manner for 8 h after dosing and then was changed to simulate a $t_{1/2}$ of 12.3 h for the remaining 16 h of the 24 h dosing period (Eli Lilly and Company, unpublished data). Each model apparatus was placed in a water bath and maintained at 37°C for the entire 48 h study period. The pharmacodynamic model experiments were performed in duplicate, simultaneously, in order to ensure reproducibility.

**Pharmacokinetic analysis.** Samples (0.5 ml) from each of the duplicate pharmacodynamic models were obtained at 0, 2, 4, 8, 24, 28, 32, and 48 h for determination of antibiotic concentrations. All samples were stored at −70°C until analysis. Vancomycin concentrations were determined using a fluorescence polarization immunoassay (Abbott Diagnostics TDx). This method has a sensitivity of 2.0 μg/ml for vancomycin. All vancomycin assays had a coefficient of determination of $\geq0.92$ and a between-day coefficient of variation ranging from 1.6 to 3.3%. Concentrations of oritavancin were determined using a microbioassay with *Micrococcus luteus* ATCC 9341 as the indicator organism. Wells (diameter, 4 mm) were made in AMA #11 agar plates with 150- to 200-μl samples placed in each well. Plates were inoculated with an even lawn of indicator organism and incubated for 18 to 24 h at 37°C. All plates achieved a 99.9% bacterial reduction at 37°C for 48 h. The potential for antibiotic carryover was evaluated by serial dilution (range, 1:10 to 1:10,000) for all samples prior to plating. Antibiotic carryover was also evaluated by the incorporation of cholestyramine (lot no. MK005A, Bristol-Myers Squibb Co., Princeton, N.J.), D-Ala–D-Ala (lot no. 77H1207; Sigma Chemicals, St. Louis, Mo.), polyanirole sulfonic acid (lot no. 104H05531; Sigma Chemicals), and polymeric absorbent beads (Amberlite; Rohm and Haas, Philadelphia, Pa.) into each serial dilution before plating.

**Results**

**Susceptibility testing.** MIC and MBC results for R919 and R921 are summarized in Table 1. There was no difference between the MICs of oritavancin, oritavancin plus albumin, and vancomycin for both isolates. Oritavancin alone demonstrated the greatest activity, with an MIC of $\leq0.015$ for R919 and R921, followed by oritavancin plus albumin and then vancomycin.

**Pharmacodynamic and pharmacokinetic studies and resistance.** The activities of oritavancin and vancomycin are shown in Fig. 1 and 2. Oritavancin appeared to have more rapid bactericidal activity ($P < 0.001$) than vancomycin within the
first 8 h, with the limit of detection in bacteria (10^2 CFU/ml) being reached in ≤1 h and remaining over the 48 h period for both R919 and R921. Vancomycin also lowered the colony counts of R919 and R921 to the level of the detection limits, and this action was sustained for both isolates at ≥28 h. There was no observed or statistical difference in CFU per milliliter between oritavancin and vancomycin at 24 and 48 h. Mean AUC from 0 to 24 h (AUC_{0–24}/MIC and C_{\text{max}}/MIC ratios can be seen in Table 2. AUC_{0–24}/MIC and C_{\text{max}}/MIC ratios ranged from 944.5 to 36,505.8 and 72.7 to 7,188.5, respectively. Oritavancin alone showed the highest AUC_{0–24}/MIC and C_{\text{max}}/MIC ratios, followed by oritavancin plus albumin and then vancomycin. The mean C_{\text{max}}, τ_{1/2} and AUC_{0–24} can be seen in Table 3. No resistance to oritavancin or vancomycin was seen with either isolate.

Only serial saline dilution (1:10 or greater) of samples containing oritavancin was effective in carryover avoidance. Incorporation of cholestyramine, D-Ala–D-Ala, or polyanetholesulfonic acid was less effective in diminishing antibiotic carryover then was serial dilution alone.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>C_{\text{max}} (μg/ml)</th>
<th>τ_{1/2} (h)</th>
<th>AUC_{0–24}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oritavancin</td>
<td>107.8 ± 10</td>
<td>2.9 ± 0.9^b</td>
<td>547.6 ± 89</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>36.9 ± 1.5</td>
<td>11.6 ± 4.5^c</td>
<td>472.3 ± 41</td>
</tr>
</tbody>
</table>

^a Results reported as means ± standard deviations.
^b At 0 to 8 h.
^c At 8 to 24 h.

**DISCUSSION**

The incidence of multidrug-resistant *S. pneumoniae* continues to rise, with resistance to penicillin as high as 30% in some areas of the United States. Macrolide, cephalosporin, and trimethoprim-sulfamethoxazole resistance is also increasing. There is a need for newer agents active against resistant *S. pneumoniae*. Although the fluoroquinolones still remain viable options to treat resistant *S. pneumoniae* infections, the threat of resistance due to their increased use is evident. Reports from Canada and Hong Kong demonstrate an increase in *S. pneumoniae* fluoroquinolone resistance (4, 7).

*S. pneumoniae* continues to remain susceptible to vancomycin (MIC, 0.125 to 0.5 μg/ml); however, vancomycin-tolerant strains have been reported (5, 10). The new glycopeptide oritavancin may potentially be an alternative for treatment of multidrug-resistant *S. pneumoniae*. Previous studies have found MICs of oritavancin for *S. pneumoniae* to be around ≤0.01 to 0.25 μg/ml (5). Our study showed that the two multidrug-resistant *S. pneumoniae* isolates were very susceptible to oritavancin (MICs, ≤0.015 to 0.03 μg/ml) and displayed sig-

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Ratio (mean ± SD) for R919 and R921</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC_{0–24}/MIC</td>
</tr>
<tr>
<td>Oritavancina^a</td>
<td>36,505.8 ± 5902.4</td>
</tr>
<tr>
<td>Oritavancin + albumin</td>
<td>18,252.9 ± 2951.2</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>944.5 ± 81</td>
</tr>
</tbody>
</table>

^a Both ratios are based on a MIC of 0.015 μg/ml.
significant killing in the 48 h models, with the limit of detection (10^2 CFU/ml) being attained at ≤1 h.

Unfortunately, in our study, antibiotic carryover could not be ruled out for some of our sampling points. This would suggest that kill curve data from the models at early time points where the oritavancin concentrations were highest might have overestimated oritavancin’s activity. However, on the basis of the very low MICs obtained for isolates R919 and R921 and kill curve data from the pharmacodynamic models in which antibiotic carryover could be accounted for, oritavancin was capable of killing these organisms to the limits of detection early in the experiment, as is depicted by the data. Our data also suggested that there were no detectable effects of protein on oritavancin’s bactericidal activity. Although minimal changes in MIC were seen by the addition of protein to the media, concentrations simulated in the model representing human pharmacokinetics appeared to be high enough that the effect of protein was not observed or was minimized. This is consistent with data from previous reports for oritavancin and vancomycin resistance to glycopeptide LY333328 mediated by genes of the vanA and vanB clusters in enterococci. Antimicrob. Agents Chemother. 43:1875–1880.

1. Arthur, M. F., F. Depardieu, P. Reynolds, and P. Courvalin. 1999. Moderate resistance to glycopeptide LY333328 mediated by genes of the vanA and


