Experimental Study of LY333328 (Oritavancin), Alone and in Combination, in Therapy of Cephalosporin-Resistant Pneumococcal Meningitis

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Using a rabbit model of meningitis, we sought to determine the efficacy of LY333328, a semisynthetic glycopeptide, in the treatment of cephalosporin-resistant pneumococcal meningitis. LY333328 was administered at a dose of 10 mg/kg of body weight/day, alone and in combination with ceftriaxone at 100 mg/kg/day with or without dexamethasone at 0.25 mg/kg/day. The therapeutic groups were treated with LY333328 with or without dexamethasone and LY333328-ceftriaxone with or without dexamethasone. Rabbits were inoculated with a cephalosporin-resistant pneumococcal strain (ceftriaxone MIC, 2 μg/ml; penicillin MIC, 4 μg/ml; LY333328 MIC, 0.008 μg/ml) and were treated over a 26-h period beginning 18 h after inoculation. The bacterial counts in cerebrospinal fluid (CSF), the white blood cell count, the lactic acid concentration, the CSF LY333328 concentration, and bactericidal and bacteriostatic activities were determined at different time points. In vitro, LY333328 was highly bactericidal and its use in combination with ceftriaxone at one-half the MIC was synergistic. In the rabbit model, LY333328 alone was an excellent treatment for cephalosporin-resistant pneumococcal meningitis, with a rapid decrease in colony counts and no therapeutic failures. The use of LY333328 in combination with ceftriaxone improved the activity of LY333328, but no synergistic effect was observed. The combination of LY333328 with dexamethasone was also rapidly bactericidal, but two therapeutic failures were observed. The combination of LY333328 with ceftriaxone and dexamethasone was effective, without therapeutic failures.

The treatment of cephalosporin-resistant pneumococcal meningitis is a challenging issue. High doses of cefotaxime have been successfully used to treat infections caused by organisms with intermediate resistance to expanded-spectrum cephalosporins, but sporadic failures have also occurred (3, 6, 24). Experience with the treatment of adult patients with systemic vancomycin alone is very limited, and therapeutic failures have been reported (23), especially when vancomycin is used in combination with dexamethasone. Some experimental studies have suggested that vancomycin plus ceftriaxone would be synergistic against pneumococci (11), and most experts recommend use of this combination for the empirical therapy of pneumococcal meningitis (12, 16).

LY333328 is a semisynthetic glycopeptide antibiotic derived from LY264826, which is active in vitro against gram-positive pathogens including methicillin- and amoxycillin-resistant Staphylococcus aureus; coagulase-negative staphylococci; enterococci, including some enterococci resistant to vancomycin; and susceptible and penicillin- and cephalosporin-resistant Streptococcus pneumoniae (2, 10, 13, 20, 22). LY333328 has been demonstrated to have efficacy in animal models of S. pneumoniae septicemia, vancomycin-resistant enterococcal endocarditis, S. aureus endocarditis, S. aureus catheter-related infections, S. aureus soft tissue infections, and S. aureus foreign body-related skin infections (1, 17). Also, recent work by Gerber et al. (14) showed that the antibiotic has good activity against a penicillin-susceptible strain of S. pneumoniae in an experimental meningitis model. However, there are no data on its efficacy against drug-resistant strains causing meningitis.

The aim of the present study was to determine the efficacy of LY333328, alone and in combination with ceftriaxone, in the therapy of cephalosporin-resistant pneumococcal meningitis and the possible influence of dexamethasone in the efficacy of this treatment.

MATERIALS AND METHODS

Bacterial strain. An S. pneumoniae strain recovered from a patient with meningitis was used. The strain, named 2349, belonged to serotype 23F. MICs were determined by the microdilution method in cation-supplemented Mueller-Hinton broth with 5% whole defribinated horse blood and the appropriate concentration of antibiotic. The wells of microdilution plates were inoculated to a volume of 100 μl with an inoculum containing 10^8 CFU/ml. The MIC was defined as the lowest concentration of antibiotic that prevented visible growth, as determined without a microscope after overnight incubation of the plates at 35°C. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of antibiotic able to reduce 99.9% of the initial inoculum. MICs and MBCs were as follows: penicillin, 4 and 4 μg/ml, respectively; ceftriaxone, 2 and 4 μg/ml, respectively; vancomycin, 0.25 and 0.5 μg/ml, respectively; and LY333328, 0.008 and 0.008 μg/ml, respectively.

In vitro killing curves. Killing curve studies were performed with glass tubes containing a final volume of 10 ml. The bacterial strain was grown in cation-adjusted Mueller-Hinton broth with 5% lysed horse blood. The final bacterial inoculum was 5 × 10^5 CFU/ml. Concentrations of one-half the MIC, the MIC, and two times the MIC of ceftriaxone or LY333328 were studied, as were concentrations of one-half the MIC and the MIC of each drug in combination. Bacterial titers were determined at 0, 6, and 24 h of incubation by serial dilution of samples, which were then plated on agar plates containing 5% sheep blood. No carryover effect was observed. The detection limit was 1 log_{10} CFU/ml. S. pneumoniae ATCC 49169, Enterococcus faecalis ATCC 29212, and Escherichia coli were used as control strains.

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coli ATCC 25922 were used as control strains. Synergy was defined as a bactericidal effect of a drug combination with greater than 2-log killing over the level of killing of the most active drug used alone when one of the drugs in the combination was used at a subinhibitory concentration. A bactericidal effect was defined as a decrease in the initial inoculum of ≥3 log CFU/ml.

**Rabbit model.** Use of the animal model was approved by the Ethical Committee for Animal Experiments at the University of Barcelona (Campus de Bellvitge). Experiments with the animal model were performed by an established protocol (9). Five different therapeutic groups consisting of eight rabbits each were formed, as was a control group which was inoculated but not treated. Female New Zealand White rabbits (weight, 2 kg) were anesthetized intramuscularly with 35 mg of ketamine (Ketolar; Parke-Davis, El Prat de Llobregat, Spain) and 5 mg of xylazine (Rompun; Bayer AG, Leverkusen, Germany) per kg of body weight, and an acrylic dental helmet was affixed to each rabbit’s calvaria. Twenty-four hours later, the animals were anesthetized again and placed in a stereotaxic frame. A spinal needle was introduced into the cisterna magna, 200 μl of cerebrospinal fluid (CSF) was withdrawn, and 200 μl of 10^5 CFU of a strain of S. pneumoniae belonging to serotype 23F per ml of saline was instilled into the subarachnoid space. The rabbits were placed back in their cages, and 18 h later the rabbits were again anesthetized with urethane (Sigma Chemical Company, St. Louis, Mo.) at 1.75 g/kg subcutaneously and phenobarbital (Pentotal Sodico; Abbott Laboratories, Madrid, Spain) 5 mg/kg intravenously (i.v.) and again placed in the stereotaxic frame, and a baseline CSF sample was taken. Then, an i.v. dose of 0.25 mg of dexamethasone (Fortecortin; Merck, Mollet del Vallés, Barcelona, Spain) or saline (Suero fisiológico; Braun S.A. Rubí, Barcelona, Spain) was administered, and 10 min later an i.v. dose of either 10 mg of LY333328 (Lilly S.A., Alcobendas, Madrid, Spain) per kg/day or 10 mg of LY333328 plus 100 mg of ceftriaxone (Rocesfalin; Roche, Madrid, Spain) per kg/day was administered. The total dose of dexamethasone was 0.25 mg/24 h, which was administered every 12 h over a 26-h period (three doses); LY333328 and ceftriaxone were administered every 24 h (two doses). Therapeutic groups were as follows: LY333328 alone, ceftriaxone alone, LY333328 plus ceftriaxone and dexamethasone, and a control group. Even though ceftriaxone alone could not be considered a good therapeutic alternative against this strain (5), a group of rabbits was treated with ceftriaxone alone in order to study whether synergy exists between LY333328 and ceftriaxone. Serial CSF samples were taken at 2 (peak), 6, 24 (trough), and 26 (peak) h of treatment. CSF samples were used to determine white blood cell (WBC) counts and lactic acid concentrations, for direct and quantitative bacterial culture, and to determine bacteriostatic and bactericidal activities CSF and the CSF LY333328 concentrations at the trough and peak time points. WBC counts were determined by optical microscopy with a Neubauer chamber after the red blood cells had been lysed with Turk solution (0.2% acetic acid and methylene blue prepared in-house). CSF lactic acid concentrations were determined with a Lactate PAP kit (Biomérieux, S.A., Marcy l’Etoile, France) and by reading with a spectrophotometer. Serial 10-fold dilution cultures were made to determine the bacterial counts at each time point (the detection limit by this method was 10^5 CFU/ml). A value of 1.9 log CFU/ml was assigned to the first sterile culture, and a value of 0 log CFU/ml was assigned to the subsequent ones. Bactericidal activities in CSF were determined by a microdilution method (18) with cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) with 2 to 5% lysed horse blood. Serial twofold dilutions (range, 1/2 to 1/4,096) of CSF samples were prepared, and a concentration of 5 × 10^6 CFU of the same strain used in the menigitis model per ml was inoculated into each well. After incubation at 35°C for 24 h, the dilution with bacteriostatic activity was the highest dilution without visible turbidity; then, 100 μl of each well without turbidity was subcultured at 35°C for 24 h, and the dilution with bactericidal activity was the highest dilution capable of killing 99.9% of the inoculated bacteria. To avoid interference from antimicrobial agent carryover, the sample was placed onto the plate as a single streak down the center, the sample was allowed to be absorbed into the agar until the plate surface appeared to be dry, and then the inoculum was spread over the plate (7). Aliquots of CSF were carefully frozen and sent to Eli Lilly (Indianapolis, Ind.) for CSF LY333328 level determination by high-performance liquid chromatography (HPLC). LY333328 was assayed by a validated method (15), by which solid-phase extraction followed by HPLC with fluorescence detection was used. The lower limit of quantitation was 0.075 μg/ml, and levels ranged between 0.078 and 2.213 μg/ml. Therapeutic failure was defined as an increase in bacterial concentration of at least 1 log CFU/ml compared with a previous count. A therapy was considered bactericidal when it achieved a reduction of 3 log CFU/ml.

**Statistical analysis.** A Fisher exact test was used to determine categorical variables. Comparisons of means was done by analysis of variance for independent data, and a level of significance of P < 0.05 was assumed. The Mann-Whitney U Wilcoxon rank sum test was used to compare median bactericidal titers.

**RESULTS**

**In vitro killing curves.** In vitro killing curves are shown in Fig. 1 and Table 1. LY333328 alone was bactericidal at 24 h at a concentration of two times the MIC, equivalent to ≤0.01 μg/ml. Ceftriaxone was also bactericidal at 24 h at a concentration of two times the MIC, equivalent to ≤4 μg/ml. Combinations of LY333328 and ceftriaxone at concentrations of one-half the MIC of each drug (subinhibitory concentrations) showed bactericidal activity and synergistic effects at 6 and 24 h (Fig. 2 and Table 1).

**Bacterial concentration from in vivo studies.** CSF bacterial concentration-time curves for the different treatments are shown in Fig. 3 and Table 2. At 2 h, LY333328 promoted a mean reduction of 2 log CFU/ml, and it was bactericidal at 6 h,

![Graph](image-url)  
**FIG. 1.** In vitro killing curves for S. pneumoniae with several antibiotic regimens. CRO, ceftriaxone; LY, LY333328; DEX, dexamethasone.
with a mean reduction of 3.5 log CFU/ml. The combination of LY333328 plus ceftriaxone improved the results and was also bactericidal at 6 h, with a mean reduction of 3.99 log CFU/ml. There were no statistically significant differences or synergistic effects, probably because the decrease in the log CFU per milliliter was very fast in all groups.

The bacterial concentration decreased in most cases with the concomitant use of dexamethasone and LY333328 (Fig. 3 and Table 2). At 2 h, the mean decrease was 3.03 log CFU/ml, which was bactericidal, and at 6 h, the mean reduction was 4.18 log CFU/ml. However, at 24 h, not all samples had counts below the level of detection, because two rabbits presented with regrowth at 24 h and were considered therapeutic failures. The combination of LY333328, ceftriaxone, and dexamethasone was also bactericidal, with mean reductions of 2.86 log CFU/ml at 2 h and 5.12 log CFU/ml at 6 h. At 24 h there were no therapeutic failures. No regrowth or therapeutic failures were found with the combination and dexamethasone.

For the control group, the mean log CFU per milliliter was stable, with high counts detected in the control group.

**Bacteriostatic and bactericidal activities in CSF.** The median bacteriostatic and bactericidal activities in the CSF of the various therapeutic groups are shown in Table 2. The bacteriostatic and bactericidal activities were similar in all groups at 2 h (peak; activity range, 1:4 to 1:8) and 24 h (trough; activity, 1:2). The best activity was achieved with the combination of LY333328 and ceftriaxone, with or without dexamethasone, at 26 h, after administration of the second antibiotic dose (activities, 1:32 and 1:16, respectively).

**CSF LY333328 concentration.** It was not possible to determine LY333328 levels in CSF by the microbiological method because of the large molecular size of LY333328, which makes its diffusion in agar difficult. CSF LY333328 levels were low by HPLC, ranging from 0.09 to 0.33 μmol/ml; they were lower in the groups treated with dexamethasone (Table 2), but the difference was not statistically significant. The volumes of the CSF samples used for drug level determinations were small in most cases.

**Inflammatory activity.** WBC counts in CSF and lactate and protein concentrations are shown in Table 3. There were no statistically significant differences between groups. The decrease in inflammatory parameters of the greatest magnitude (without statistical significance) was achieved with the combination of LY333328, ceftriaxone, and dexamethasone. The WBC counts were 6,511/mm³ at 0 h and 1,586/mm³ at 26 h, the lactate concentrations were 6.8 mmol/liter at 0 h and 3.0 mmol/liter at 26 h, and the protein concentrations were 1.98 mg/ml at 0 h and 0.8 mg/ml at 26 h.

**DISCUSSION**

LY333328 was effective in the rabbit model of cephalosporin-resistant pneumococcal meningitis, suggesting that this glycopeptide antibiotic might be a good option in the treatment of such infections. The decrease in the log CFU per milliliter produced by LY333328 was impressive and was apparently faster than that produced by vancomycin in previous studies with the rabbit model (4, 19). The very low MIC of LY333328
for this strain of *S. pneumoniae* (0.008 µg/ml) was similar to those for other *S. pneumoniae* strains (13) and might explain this increased efficacy of LY333328.

Doses of LY333328 (oritavancin) ranging from 20 to 25 mg/kg have been used in several studies of experimental staphylococcal and enterococcal endocarditis (17, 21). In this study, we used a LY333328 dose of 10 mg/kg, which was found to be the most adequate in the experience of Gerber et al. (14), and we obtained efficacy results similar to those achieved by Gerber et al. Consequently, as would be anticipated, the antibi-

cacy of LY333328.

The addition of ceftriaxone improved the decrease in the bacterial concentration produced by LY333328 prevented the manifestation of a synergistic effect of the two drugs. Combinations of beta-lactams and glycopeptides have been suggested to be potentially efficacious against infections caused by *S. aureus* strains with different antibiotic susceptibilities (8). Moreover, a synergistic effect of ceftriaxone and vancomycin against resistant strains has been found in an experimental model of pneumococcal meningitis. In fact, these combinations are recommended as empirical therapy for pneumococcal meningitis (11, 12). According to our data, LY333328 plus ceftriaxone would be an even better combination in this setting. Given the present levels of cephalosporin resistance, synergism will not be relevant in most cases; however, hypothetically, for very highly cephalosporin-resistant strains, such synergy might be useful, as it would increase the activity of LY333328. CSF LY333328 levels were slightly higher in the two groups in which ceftriaxone was included as part of the treatment. One might speculate that the lytic activity of ceftriaxone might induce a higher level of inflammatory activity and perhaps the passage of larger amounts of LY333328 through the blood-brain barrier. Another explanation for the higher LY333328 levels in ceftriaxone-treated animals could be that ceftriaxone might impair the clearance of LY333328 from the CSF. Unlike the combination of vancomycin plus dexamethasone (4), the combination of LY333328 plus dexamethasone performed well in terms of reducing the bacterial concentration (there were no differences in the bacterial concentration after treatment with LY333328 plus dexamethasone compared with those obtained after treatment with LY333328 alone) and bactericidal activities. Although the combination was bactericidal at 2 h, the two therapeutic failures are intriguing. This fact might be related to the low levels

### Table 2. Bacteriostatic and bactericidal activities in CSF (BD range) and LY333328 levels in CSF of rabbits with pneumococcal meningitisa

<table>
<thead>
<tr>
<th>Therapy</th>
<th>BC/BDa</th>
<th>LY level (µg/ml)b</th>
<th>BC/BDa</th>
<th>LY level (µg/ml)b</th>
<th>BC/BD</th>
<th>LY level (µg/ml)b</th>
<th>Change in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
<td>24 h</td>
<td>26 h</td>
<td></td>
<td></td>
<td></td>
<td>∆Log CFU/ml</td>
</tr>
<tr>
<td>LY</td>
<td>1:4–1:8/1:4–1:8 BQL</td>
<td>1:4/1:2</td>
<td>BOL</td>
<td>1:16/1:4–1:8 BOL</td>
<td>0.18 ± 0.09</td>
<td>−3.5</td>
<td>−4.48</td>
</tr>
<tr>
<td>CRO</td>
<td>1:2/1:2</td>
<td>NA</td>
<td>1:2–1:4/1:2 NA</td>
<td>NA</td>
<td>−3.96</td>
<td>−3a</td>
<td>0.66 ± 0.10</td>
</tr>
<tr>
<td>LY + DEX</td>
<td>1:8/1–4:1:4 BQL</td>
<td>1:2/1:2</td>
<td>BOL</td>
<td>1:16/1:4–1:8 BOL</td>
<td>0.18 ± 0.09</td>
<td>−4.18</td>
<td>−4.32</td>
</tr>
<tr>
<td>LY + CRO</td>
<td>1:16/1:4–1:4</td>
<td>0.39 ± 0.31</td>
<td>1:4/1:2</td>
<td>0.09 ± 0.00</td>
<td>1:32/1:32</td>
<td>0.26 ± 0.09</td>
<td>−3.99</td>
</tr>
<tr>
<td>LY + CRO + DEX</td>
<td>1:4–1:8/1:8</td>
<td>0.33 ± 0.16</td>
<td>1:4/1:2</td>
<td>BOL</td>
<td>1:16/1:16</td>
<td>0.20 ± 0.17</td>
<td>−4.24</td>
</tr>
</tbody>
</table>

a Abbreviations: BC, bacteriostatic activity; BD, bactericidal activity; LY, LY333328; DEX, dexamethasone; CRO, ceftriaxone; BQL, below quantitation limit (the quantitation limit was 0.075 µg/ml); NA, not available.

b Values are medians or ranges.

c Values are means ± standard deviations.

d P < 0.05 versus LY333328-ceftriaxone-dexamethasone.

### Table 3. Inflammatory parameters in CSF of rabbits with pneumococcal meningitis

<table>
<thead>
<tr>
<th>Therapya</th>
<th>No. of WBCs µl</th>
<th>Change in WBC count from 26 to 0 h</th>
<th>Lactate concn (mmol/liter)</th>
<th>Change in lactate concn from 26 to 0 h</th>
<th>Protein concn (mg/ml)</th>
<th>Change in protein concn from 26 to 0 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>LY</td>
<td>5,998 ± 4,279</td>
<td>1,929 ± 1,748</td>
<td>−4.069</td>
<td>6.44 ± 2.22</td>
<td>3.12 ± 1.64</td>
<td>−3.32</td>
</tr>
<tr>
<td>CRO</td>
<td>7,607 ± 7,683</td>
<td>3,391 ± 4,502</td>
<td>−4.216</td>
<td>6.23 ± 3.34</td>
<td>3.49 ± 0.49</td>
<td>−2.74</td>
</tr>
<tr>
<td>LY + CRO</td>
<td>4,470 ± 4,516</td>
<td>1,569 ± 1,317</td>
<td>−2.901</td>
<td>6.68 ± 2.88</td>
<td>3.97 ± 1.33</td>
<td>−2.71</td>
</tr>
<tr>
<td>LY + DEX</td>
<td>5,120 ± 4,327</td>
<td>1,079 ± 1,453</td>
<td>−4.041</td>
<td>6.20 ± 1.53</td>
<td>3.06 ± 0.78</td>
<td>−3.14</td>
</tr>
<tr>
<td>LY + CRO + DEX</td>
<td>6,511 ± 4,796</td>
<td>1,586 ± 715</td>
<td>−4.925</td>
<td>6.81 ± 1.21</td>
<td>3.09 ± 0.73</td>
<td>−3.72</td>
</tr>
</tbody>
</table>

a LY, LY333328; DEX, dexamethasone; CRO, ceftriaxone.
achieved in CSF at the time of the trough concentration. In addition, bacteriostatic activities at the time of regrowth (24 h, which was the time of the trough concentration) were very low (<1:2), and the LY333328 concentrations in all CSF samples were below the level of detection. This trend suggests that the anti-inflammatory activity of dexamethasone may reduce the amount of LY333328 that passes through the blood-brain barrier, resulting in lower levels in CSF. For this reason, dexamethasone and LY333328 should not be used concomitantly.

In conclusion, LY333328 might be a good alternative in the therapy of penicillin- and cephalosporin-resistant pneumococcal meningitis. The combination of LY333328 and dexamethasone led to some therapeutic failures, whereas the combination of LY333328 plus ceftriaxone and dexamethasone was effective in all cases. According to this experience, clinical studies may be scheduled.

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