Experimental study of the efficacy of daptomycin for the treatment of cephalosporin-resistant pneumococcal meningitis

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Objectives: To determine the efficacy of daptomycin for the treatment of penicillin- and cephalosporin-resistant pneumococcal meningitis using in vitro and in vivo methods.

Methods: In vitro killing curves were determined with clinically achievable CSF antibiotic concentrations. In a rabbit model of pneumococcal meningitis, we studied the efficacy (Δ cfu/mL) of daptomycin used at 15 and 25 mg/kg, comparing it with ceftriaxone 100 mg/kg/24 h and ceftriaxone plus vancomycin 30 mg/kg/24 h over a 26 h period against two different strains: HUB 2349 and ATCC 51916, with MICs of 2 and 32 mg/L of cefotaxime/ceftriaxone, respectively.

Results: The penetration of daptomycin into CSF ranged between 9% and 11%. Daptomycin therapy achieved an excellent response, being bactericidal within 2 h of antibiotic administration. Against strain HUB 2349, daptomycin at both doses was as effective as ceftriaxone plus vancomycin. Against the highly resistant strain, daptomycin 25 mg/kg was significantly better than ceftriaxone plus vancomycin at 2 and 6 h.

Conclusions: Daptomycin at standard doses, and especially at high doses, may be a useful alternative for the treatment of penicillin- and cephalosporin-resistant pneumococcal meningitis.

Keywords: lipopeptides, CNS infections, Streptococcus pneumoniae

Introduction

Bacterial meningitis is still associated with high morbidity and mortality. The annual incidence is around three cases per 100000 people and mortality may be as high as 50%, depending on the aetiology. This incidence has fallen since the introduction of conjugate vaccines. Streptococcus pneumoniae is one of the most common causative pathogens of bacterial meningitis worldwide. In adults, 61% of cases in the USA and 40% in Spain are due to this microorganism. Mortality ranges from 16% to 37%, and between 30% and 52% of surviving adults have neurological sequelae. The worldwide increase in penicillin- and cephalosporin-resistant isolates of S. pneumoniae in the 1980s and 1990s necessitated a reassessment of the empirical treatment of pneumococcal meningitis. In Spain, the incidence of penicillin-resistant strains rose during the 1980s, before stabilizing and then falling during the last decade. The emergence of this resistance and the occasional need for alternatives to β-lactams, due to allergy or adverse effects, have triggered an active search for alternative antibiotic regimens for pneumococcal meningitis. Alternatives such as high doses of third-generation cephalosporins have been successfully used for treatment of meningitis caused by intermediate cephalosporin-resistant pneumococci (MICs 1–2 mg/L), but therapeutic failures have been described with highly resistant strains (MICs ≥ 2 mg/L). Therapy failures with cefotaxime and ceftriaxone in partially penicillin-resistant strains have been reported. Other treatments using β-lactam antibiotics have been tested. A guinea pig model used to test meropenem showed great efficacy against two strains with different susceptibilities to β-lactams. Another experimental study using a highly cephalosporin-resistant strain of S. pneumoniae confirmed better activity with combined therapy using either ceftriaxone plus vancomycin or vancomycin plus rifampicin. Studies of fosfomycin in combination with ceftriaxone and vancomycin with two strains with different resistance patterns showed that these combined regimens performed better than the drugs used alone and were as effective as the combination of ceftriaxone plus vancomycin. Teicoplanin alone was effective in the same rabbit model, but the addition of dexamethasone reduced penetration into the CSF. Several new quinolones were also tested as alternatives,
but some of them presented severe problems of toxicity and are no longer available.13,14

Daptomycin is a new lipopeptide antibiotic with excellent activity against a variety of Gram-positive microorganisms,15–18 including pneumococci. Daptomycin’s rapid bactericidal activity, with no lysis and a limited inflammatory response, makes it an attractive alternative for the treatment of multidrug-resistant pneumococcal meningitis. In addition, its non-bacteriolytic activity may represent an advantage even in cases of full β-lactam susceptibility.

The aim of this study was to determine the efficacy of daptomycin against two penicillin- and cephalosporin-resistant strains of S. pneumoniae in an experimental rabbit meningitis model.

Materials and methods

Bacterial strains

Two strains of S. pneumoniae belonging to serotype 23F, originally isolated from patients with meningitis, were used. The HUB 2349 strain is penicillin and cephalosporin resistant, and the ATCC 51916 strain (Tennessee 23F-4 clone) is intermediately penicillin resistant and highly cephalosporin resistant.13,14

In vitro studies

In all in vitro experiments with daptomycin, the Mueller–Hinton broth medium was supplemented with 50 mg/L of calcium (Sigma–Aldrich, Madrid, Spain).

Determination of MICs and MBCs and 24 h killing curves

The MIC was defined as the minimum concentration of antibiotic that was able to inhibit visible bacterial growth, and the MBC as the lowest concentration that killed 99.9% of the original inoculum. MICs and MBCs were determined using the macrodilution/microdilution method following the CLSI (formerly NCCLS) guidelines.20

The methodology used for the 24 h killing curves followed previously standardized recommendations:19 Time–kill curves were derived using glass tubes containing a final volume of 10 mL of Mueller–Hinton broth supplemented with 5% lyzed horse blood and a final inoculum of 105 cfu/mL. Antibiotic concentrations achievable in CSF were studied, ranging from 1/4 to 8 × the MIC of antibiotics alone, as were concentrations of 1/4, 1/2, 1 and 2 × the MIC of each drug in combination. Samples were removed at 0, 6 and 24 h of incubation. The detection limit was 10 cfu/mL. Bactericidal effect was defined as a decrease in the initial inoculum of 3 log cfu/mL. Synergy of a combination was defined as a >2 log cfu/mL reduction over the most active agent alone, with one of the drugs at subinhibitory concentration. Additive and indifferent effects were defined respectively as reductions of between 1 and 2 log cfu/mL and of −1 log cfu/mL compared with the most active single antibiotic.22

All 24 h killing curves were carried out in triplicate. To avoid carryover antimicrobial agent interference, the sample was placed on the plate in a single streak down the centre and allowed to be absorbed into the agar until the plate surface appeared dry; the inoculum was then spread over the plate. As described previously,23 this methodology was checked by comparing the results obtained with the centrifugation and resuspension of the fluid from tubes of killing curves.

In vivo studies

Meningitis model

The experimental protocol complied with Spanish legislation on animal experimentation and was approved by the University of Barcelona’s Ethics Committee for Animal Experiments. The rabbit model of meningitis described originally by Dacey and Sande24 was modified slightly. Young female New Zealand White rabbits were anaesthetized intramuscularly with 35 mg/kg of ketamine (Ketolar; Parke-Davis, Prat de LL, Spain) and 5 mg/kg of xylazine (Rompum; Bayer AG, Leverkusen, Germany). Meningitis was induced using an intracisternal injection of 250 µL of a saline suspension containing 106 cfu/mL of inoculum. In rabbits infected with the HUB 2349 strain, therapy was started 18 h post-inoculation. In animals infected with the ATCC 51916 strain, therapy was initiated 40 h after inoculation owing to the slow progression of meningitis.25

Rabbits were anaesthetized using urethane (Sigma Chemical Company, St Louis, MO, USA) at 1.75 g/kg subcutaneously and thiopental sodium (Thiopenal; B. Braun Medical S.A., Rubí, Spain) at 5 mg/kg intravenously (iv). A blood sample was collected to assess secondary bacteraemia. Animals were placed in the stereotactic frame, and a baseline CSF sample was taken (0 h). Further CSF samples were taken after 2, 6, 24 and 26 h of therapy. Hydration was ensured throughout the experiment. Mortality was assessed at 26 h. Surviving animals were euthanased using a lethal dose of thiopental sodium at the end of each experiment.

Therapeutic groups

Antibiotic iv therapy (n = 8 rabbits/group) was then administered for 26 h using one of the following therapy schedules: ceftriaxone at 100 mg/kg once daily, vancomycin at 15 mg/kg every 12 h plus ceftriaxone, daptomycin at 15 mg/kg once daily and daptomycin at 25 mg/kg once daily. Doses of ceftriaxone and vancomycin were the same as those used in previous experiments.26,27 Untreated controls (n = 10) received saline.

Sample processing

CSF samples were used to determine CSF white blood cells (WBCs), bacterial counts and antibiotic concentrations at peak and trough timepoints. For leucocyte counts, 10 µL of each sample was diluted 1:1 with Turk Solution and read with a Neubauer chamber. Animals presenting at least 300 cells/mm3 were included in the therapeutic groups of the study. Serial 10-fold dilutions were made to determine bacterial counts at each timepoint. The lowest bacterial concentration detectable was 10 cfu/mL. For purposes of analysis, a value of 0.99 log cfu/mL was assigned to the first sterile culture, and a value of 0 log cfu/mL was assigned to subsequent ones. Changes in bacterial counts (Δ log cfu/mL) were calculated as the differences between bacterial concentrations at the start of therapy and those at 2, 6, 24 and 26 h. 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 a reduction of 3 log cfu/mL was achieved. Samples were centrifuged at 5000 g for 10 min, and the supernatants were stored at −70°C.

Pharmacokinetics

Pharmacokinetic data were compiled from a study of eight infected animals after a single iv dose of 15 or 25 mg/kg of daptomycin. Blood and CSF samples were taken at different timepoints depending on the therapy. A computer-assisted method (pharmacokinetic functions for Microsoft Excel; J. I. Usansky, A. Desai and D. Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA 92606, USA) was used to determine the following parameters in serum and CSF: maximum concentration (Cmax), AUC over 24 h (AUC0–24) and CSF penetration as the comparison of areas under the curve (AUCCSF/AUCserum).

Antibiotic assays

Daptomycin concentrations were measured using the agar disc diffusion method,28 using Micrococcus luteus ATCC 9341 [linearity of assay (r2), 0.99; lower detection limit, 2 mg/L] as the assay organism.
Statistical analysis

All bacterial counts are presented as log numbers of cfu/mL (mean ± SD). Differences in bacterial counts between treated and untreated animals were evaluated for statistical significance using analysis of variance. An unpaired Student t-test with Bonferroni’s correction was used to determine statistical significance. WBCs were compared using the non-parametric Mann–Whitney and Wilcoxon tests. For all tests, differences were considered to be statistically significant when P values were <0.05.

Results

In vitro studies

MICs and MBCs

The MICs and MBCs (mg/L) of daptomycin, vancomycin and ceftriaxone were 0.09/0.18, 0.25/0.5 and 2/4, respectively, for the HUB 2349 strain, and 0.19/0.38, 0.25/0.25 and 32/32, respectively, for the ATCC 51916 strain.

Figure 1. Time–kill curves for log phase with a bacterial inoculum of 10^5 cfu/mL with the antibiotics alone and in combination. Values shown in the key are multiples/fractions of the MIC. DAP, daptomycin; VAN, vancomycin; CRO, ceftriaxone. Control = no antibiotic.
24 h killing curves
For HUB 2349 strain, a bactericidal effect was observed at daptomycin concentrations of $8 \times$ and $4 \times$ MIC at 6 and 24 h and was almost achieved at concentrations of $2 \times$ MIC and $1 \times$ MIC at 6 h. Ceftriaxone showed a bactericidal effect at 24 h at 2 and $1 \times$ MIC; the same result was observed with vancomycin. The combination ceftriaxone 1/4 plus vancomycin 1/2 at 6 and 24 h was synergistic, as was ceftriaxone 1/4 plus vancomycin 1/4 at 6 h.

For ATCC 51916 strain, a bactericidal effect was observed at 6 and 24 h only at concentrations of $8 \times$ MIC. Ceftriaxone and vancomycin alone produced a bactericidal effect at 6 and 24 h at $2 \times$ MIC.

The following combinations were synergistic at 6 h: ceftriaxone 1/2 plus vancomycin 1 and vancomycin 1/2, and ceftriaxone 1/4 plus vancomycin 1. The following combinations were synergistic at 24 h: ceftriaxone 1 plus vancomycin 1, vancomycin 1/2, and vancomycin 1/4, ceftriaxone 1/2 plus vancomycin 1 and vancomycin 1/2, and ceftriaxone 1/4 plus vancomycin 1.

The most representative results of the killing curves with antibiotics in combination with ceftriaxone and vancomycin are shown in Figure 1.

In vivo studies
Pharmacokinetics
Daptomycin at 15 mg/kg/24 h presented a peak concentration in serum of 73.08 mg/L at 1 h, and a trough concentration of 3.83 mg/L at 24 h. The peak CSF concentration was 8.66 mg/L at 4 h. The AUC was 416.51 mg·h/L in serum and 110.75 mg·h/L in CSF. The AUC/MIC was 1230.55 for the S. pneumoniae 2349 strain and 582.89 for the ATCC 51916 strain. The $C_{\text{max}}$/MIC was 96.22 for the 2349 strain and 45.6 for the ATCC 51916 strain.

Using daptomycin 25 mg/kg/24 h, peak serum concentration was 110.81 mg/L at 0.5 h, and the trough concentration was 3.48 at 24 h. The peak CSF concentration was 9.5 mg/L at 2 h. Serum AUC was 773.77 mg·h/L, and the CSF AUC was 138.53 mg·h/L. The AUC/MIC was 1539.22 for the S. pneumoniae 2349 strain, and 729.10 for the ATCC 51916 strain. The $C_{\text{max}}$/MIC was 105.56 for the 2349 strain and 50 for the ATCC 51916 strain.

The penetration of daptomycin into the CSF ranged between 9% and 11%. Other pharmacokinetic parameters and the pharmacodynamic parameters related to MIC for each strain of S. pneumoniae are included in Figures 2 and 3.

Experimental meningitis: HUB 2349 strain
The CSF of infected rabbits showed inflammation in terms of high WBCs throughout the experiment. CSF WBCs at 24 h were statistically lower: median (IQR) was 1050 (867–2420) in the daptomycin 25 group versus 5340 (2307–7830) in the ceftriaxone plus vancomycin group.

Secondary bacteraemia at 0 h was 100%. Mortality at 26 h was 50% in the control group, 12.5% in the ceftriaxone group, 11.1% for ceftriaxone plus vancomycin, 12.5% for daptomycin 15 mg/kg and 0% for daptomycin 25 mg/kg.
CSF bacterial counts at 0 h and reduction in CSF bacterial counts after the different therapies are summarized in Table 1. All therapy groups were significantly better than the control group ($P<0.05$). The bactericidal activity of daptomycin 15 and 25 mg/kg doses was as effective as that of ceftriaxone plus vancomycin.

Daptomycin therapy obtained an excellent response, being bactericidal 2 h after administration. At 6 h, CSF samples from all antibiotic groups were below 3 log from the initial inocula and at 26 h all CSF samples were below the level of detection.

**Experimental meningitis: ATCC 51916 strain**

The CSF of infected rabbits showed inflammation in terms of high WBCs throughout the experiment. CSF WBCs at 0 and 24 h were not statistically different among the therapy groups.

Secondary bacteraemia at 0 h in the different groups ranged from 75% to 100%. Mortality at 26 h was 50% in the control group, 25% in the ceftriaxone group, 12.5% for ceftriaxone plus vancomycin, 11.1% for daptomycin 15 mg/kg and 0% for daptomycin 25 mg/kg. CSF bacterial counts at 0 h and reduction in CSF bacterial counts after the different therapies are summarized in Table 1. Daptomycin 15 mg/kg and daptomycin 25 mg/kg therapies were statistically better than the control and ceftriaxone groups throughout the treatment ($P<0.05$). Daptomycin 25 mg/kg was statistically better compared with ceftriaxone plus vancomycin at 2 and 6 h, and at 2 h also versus the daptomycin 15 mg/kg group ($P<0.05$). The combination of ceftriaxone plus vancomycin was significantly more effective, reducing bacterial counts versus control and ceftriaxone groups at 6, 24 and 26 h ($P<0.05$).

### Discussion

The availability of an antibiotic with very fast and effective bactericidal activity would represent a step forward in the treatment of pneumococcal meningitis, since slow-acting antibiotic therapy has been associated with negative sequelae. The results obtained with our animal model suggest that daptomycin is useful for the treatment of cephalosporin-resistant pneumococcal meningitis. Its efficacy is similar to that of the empirically used combination of ceftriaxone plus vancomycin. Daptomycin was as effective as this combination but bactericidal activity was more rapid with daptomycin and it was statistically more effective than ceftriaxone alone against the highly resistant strain.

At high doses (25 mg/kg), daptomycin was also more rapid in reducing bacterial counts than the lower dose (15 mg/kg) in meningitis due to both strains. Against the highly cephalosporin-resistant strain it was also significantly more effective, reducing bacterial counts more than standard ceftriaxone plus vancomycin ($P<0.05$ at 2 and 6 h; Table 1).

An experimental study with infant rat pneumococcal meningitis found that daptomycin treatment resulted in more rapid bacterial killing and lower CSF inflammation than ceftriaxone treatment. Another study in the experimental rat pneumococcal meningitis model also showed that daptomycin in combination with ceftriaxone may be more beneficial for the treatment of meningitis than the combination of ceftriaxone plus rifampicin.

Our findings corroborate these results and those reported by Cottagnoud et al. in two studies with a rabbit model. In both studies, daptomycin bacterial killing was achieved earlier than with ceftriaxone plus vancomycin.
Table 1. Mean (±SD) antibiotic concentrations (mg/L) in CSF of rabbits with pneumococcal meningitis caused by two tested strains of S. pneumoniae

<table>
<thead>
<tr>
<th>Therapy groupa</th>
<th>initial titres (log cfu/mL)</th>
<th>changes in bacterial counts (Δ cfu/mL)</th>
<th>initial titres (log cfu/mL)</th>
<th>changes in bacterial counts (Δ cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2h</td>
<td>6h</td>
<td>2</td>
<td>4h</td>
</tr>
<tr>
<td>CRO 100 mg/kg/24 h</td>
<td>4.91±0.75</td>
<td>-2.22±0.85</td>
<td>-3.71±0.82</td>
<td>-3.26±0.81</td>
</tr>
<tr>
<td>CRO 100 mg/kg/24 h plus VAN 15 mg/kg/12 h</td>
<td>4.56±0.43</td>
<td>-2.63±1.42</td>
<td>-3.66±0.44</td>
<td>-3.76±0.49</td>
</tr>
<tr>
<td>DAP 15 mg/kg/24 h</td>
<td>4.83±0.45</td>
<td>-3.33±1.17</td>
<td>-3.93±0.66</td>
<td>-3.95±0.48</td>
</tr>
<tr>
<td>DAP 25 mg/kg/24 h</td>
<td>4.44±0.53</td>
<td>-3.54±0.52</td>
<td>-3.53±0.53</td>
<td>-3.54±0.53</td>
</tr>
<tr>
<td>Control</td>
<td>4.93±0.38</td>
<td>0.12±0.44a</td>
<td>0.05±0.34a</td>
<td>0.72±0.82a</td>
</tr>
</tbody>
</table>

CRO, ceftriaxone; VAN, vancomycin; DAP, daptomycin.

aCeftriaxone and daptomycin were administered twice (at 0 and 24 h) and vancomycin three times (at 0, 12 and 24 h).
bP≤0.05 versus daptomycin 25 mg/kg/24 h.