Evaluation of ceftriaxone, vancomycin and rifampicin alone and combined in an experimental model of meningitis caused by highly cephalosporin-resistant Streptococcus pneumoniae ATCC 51916

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Received 25 April 2005; returned 16 June 2005; revised 29 June 2005; accepted 15 August 2005

Objectives: The aim of the study was to assess the in vitro and in vivo efficacy of ceftriaxone, vancomycin and rifampicin alone and combined against Streptococcus pneumoniae ATCC 51916 (MIC of ceftriaxone: 32 mg/L).

Methods: In vitro killing curves were performed with clinically achievable CSF antibiotic concentrations. In the rabbit model of pneumococcal meningitis, we studied the efficacy of and effects on inflammation of treatment with ceftriaxone 100 mg/kg/day, vancomycin 30 mg/kg/day and rifampicin 15 mg/kg/day, alone and combined, over a 26 h period.

Results: Time–kill curves showed that vancomycin was bactericidal, and ceftriaxone and rifampicin produced a bacteriostatic effect. An additive effect was observed when combinations of ceftriaxone plus vancomycin were studied at subinhibitory concentrations. Emergence of resistance to rifampicin was detected both when rifampicin was studied alone and when combined with ceftriaxone or vancomycin. In the rabbit meningitis model, ceftriaxone was bacteriostatic, whereas rifampicin and vancomycin were bactericidal at 24 h. Although not synergistic, the combinations of ceftriaxone plus vancomycin or rifampicin, and vancomycin plus rifampicin, improved the efficacy of any antibiotic tested alone—all combinations were bactericidal from 6 h—and significantly decreased inflammatory parameters in CSF compared with control and ceftriaxone groups.

Conclusion: Ceftriaxone plus vancomycin, and vancomycin plus rifampicin appeared to be effective in the therapy of experimental pneumococcal meningitis caused by highly cephalosporin-resistant strains such as ATCC 51916. Our results provide an experimental basis for using these combinations as empirical therapy for pneumococcal meningitis, regardless of the degree of cephalosporin resistance of the causative strain.

Keywords: β-lactams, glycopeptides, empirical therapy, bacterial meningitis

Introduction

The combination of ceftriaxone or cefotaxime with vancomycin is recommended by most experts for pneumococcal meningitis caused by resistant strains.1 This recommendation has proven useful in the clinical setting, where ceftriaxone/cefotaxime MICs of resistant strains rarely exceed 4 mg/L. However, there are no studies that support this approach in meningitis caused by pneumococcal strains with a very high level of cephalosporin resistance.

Strains of this kind have occasionally been identified in the USA and in Spain.2–4 One of them, Streptococcus pneumoniae ATCC 51916 (Tennessee23F–4 clone, CS111 strain) caused otitis media and meningitis in a child who failed to respond to cefotaxime therapy.5 This strain is characterized by a high level of resistance to ceftriaxone/cefotaxime (MICs of 32 mg/L) and intermediate resistance to penicillin.2 Recently, our group reported the capacity of this strain to promote meningitis with a strong inflammatory response in rabbits.5

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The aim of this study was to assess the in vitro and in vivo efficacy of and the effects on inflammation of treatment with ceftriaxone, vancomycin and rifampicin, alone and combined, against *S. pneumoniae* ATCC 51916.

Materials and methods

Susceptibility tests

MIC/MBCs of ceftriaxone, rifampicin and vancomycin for *S. pneumoniae* ATCC 51916 were determined by the macrodilution method according to the NCCLS.

In vitro killing curves

Time–kill studies were performed with antibiotic concentrations achievable in CSF. Ceftriaxone (Laboratorios Farmacéuticos Rovi, S.A., Madrid, Spain) was studied at 1/16 and 1/8 × MIC. Rifampicin (Aventis, Madrid, Spain) and vancomycin (Laboratorios Normon S.A., Madrid, Spain) were studied in a range from 1/4–4 × MIC. The antibiotic concentrations used in combinations were 1/16–1/8 × MIC for ceftriaxone and 1/4–1 × MIC for rifampicin and vancomycin. Bactericidal effect was defined as a decrease of ≥3 log cfu/mL. Synergy was defined as a >2 log cfu/mL decrease over the most active agent alone with one of the drugs at subinhibitory concentration. Additive and indifferent effects were, respectively, defined as a reduction of 1–2 log cfu/mL and of ±1 log cfu/mL compared with the most active single antibiotic. Emergence of resistance was studied at 24 h using the Etest method.

Meningitis model

The experimental protocol was approved by the Ethics Committee for Animal Experiments at the University of Barcelona. The animal model originally described by Dacey and Sande was slightly modified. Young female New Zealand White rabbits were anaesthetized intramuscularly with 35 mg/kg of ketamine (Ketolar; Parke-Davis, Prat de Llobregat, Spain) and 5 mg/kg of xylazine (Rompun; Bayer AG, Leverkusen, Germany). Meningitis was induced by intracisternal injection of 250 µL of 10⁸ cfu/mL of the ATCC 51916 strain. Owing to the slow progression of disease with this strain, we modified the usual time between inoculation and start of therapy. Approx. 40 h after inoculation, the rabbits were anaesthetized with urethane (Sigma) at 1.75 g/kg subcutaneously and intraperitoneally (100 mg/kg for control and ceftriaxone groups produced a high inflammatory response characterized by statistically significant increased values of lactate (P = 0.000 compared with all therapies). Ceftriaxone

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Ceftriaxone and rifampicin concentrations were determined by the agar disc diffusion method using as assay organisms *Bacillus subtilis* ATCC 12432 and *Staphylococcus epidermidis* ATCC 27626, respectively. Vancomycin levels were measured by fluorescence polarization immunoassay.

Statistical analysis

ANOVA test (followed by Scheffe’s test) or Kruskal–Wallis test (inflammatory activity) were used. A *P* value of < 0.05 was considered significant.

Results

In vitro studies

MIC/MBCs (mg/L) were: ceftriaxone 32/32, rifampicin 0.06/0.12, and vancomycin 0.25/0.25. In killing curves, vancomycin had a bactericidal effect at 6 h (mean Alog cfu/mL ± SD: −3.27 ± 1.30 at 1 × MIC, and −3.69 ± 1.42 at 4 × MIC). Rifampicin at 4 × MIC reached a bacteriostatic effect with a mean decrease of −0.847 ± 0.257 log cfu/mL. Neither vancomycin and rifampicin at lower concentrations nor ceftriaxone at 1/8 × MIC and 1/16 × MIC showed growth inhibition. An additive effect was found when vancomycin at 1/2 × MIC was combined with ceftriaxone at 1 × MIC or at 1/16 × MIC. Rifampicin combinations showed an indifferent effect. Emergence of resistance was detected when rifampicin was studied alone and combined.

In vivo experiments

Secondary bacteraemia among different groups ranged from 62.5 to 100%. Mortality at 24 h was 58.3% in the untreated group and 0% in all therapies. CSF bacterial counts at 0 h and CSF bacterial decreases for different schedules are summarized in Table 1. Mean CSF peak and trough antibiotic levels of rabbits are shown in Table 2.

Ceftriaxone monotherapy produced a bacteriostatic effect. Therapeutic failure at 24 h occurred in 3/8 animals.

Rifampicin alone was bactericidal at 24 h (*P* = 0.001 compared with ceftriaxone). CSF bacterial counts were below the limit of detection in all rabbits except one that showed a slightly lower bacterial reduction (−2.99 log cfu/mL at 24 h; −3.19 log cfu/mL at 26 h) that was associated with low CSF rifampicin levels (0.227 mg/L).

The activity of vancomycin was similar to that of rifampicin at 6 h, and was bactericidal at 24 h (*P* = 0.033 compared with ceftriaxone). Regrowth occurred in one rabbit at 24 h, which presented CSF levels of 0.03 and 0.53 mg/L at 24 and 26 h, respectively. All combinations achieved a bactericidal effect at 6 h. There was no regrowth and bacterial concentrations at 26 h were below the limit of detection. Ceftriaxone combined with rifampicin or vancomycin improved the activity of both drugs alone and the effects were statistically significant compared with ceftriaxone monotherapy (*P* < 0.03). Rifampicin plus vancomycin did not produce significant variations in the activity of either drug alone, but was more effective than ceftriaxone (*P* < 0.01).

Control and ceftriaxone groups produced a high inflammatory response characterized by statistically significant increased values of lactate (*P* = 0.000 compared with all therapies). Ceftriaxone
Therapy of highly resistant pneumococcal meningitis

Table 1. CSF bacterial counts at 0 h and bacterial killing rates at 6, 24 and 26 h after antimicrobial therapy in experimental pneumococcal meningitis caused by the highly cephalosporin-resistant ATCC 51916 strain

<table>
<thead>
<tr>
<th>Therapy and dosage (n)</th>
<th>Initial titres (log cfu/mL)</th>
<th>Changes in bacterial counts (Δlog cfu/mL) at 6 h</th>
<th>24 h</th>
<th>26 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRO 100 mg/kg/day (8)</td>
<td>5.23 ± 0.88</td>
<td>-0.76 ± 1.70</td>
<td>-0.75 ± 1.72</td>
<td>-1.01 ± 1.78</td>
</tr>
<tr>
<td>RIF 15 mg/kg/day (8)</td>
<td>5.45 ± 0.76</td>
<td>-2.42 ± 0.49</td>
<td>-4.25 ± 0.91*</td>
<td>-5.14 ± 1.09*</td>
</tr>
<tr>
<td>VAN 15 mg/kg/12 h (8)</td>
<td>5.00 ± 0.76</td>
<td>-2.71 ± 1.80</td>
<td>-3.44 ± 1.47*</td>
<td>-4.26 ± 1.48*</td>
</tr>
<tr>
<td>CRO 100 + RIF 15 (10)</td>
<td>5.10 ± 0.80</td>
<td>-3.24 ± 0.75*</td>
<td>-4.31 ± 0.84*</td>
<td>-5.10 ± 0.80*</td>
</tr>
<tr>
<td>CRO 100 + VAN 15 (8)</td>
<td>5.17 ± 1.14</td>
<td>-3.90 ± 1.47*</td>
<td>-4.25 ± 1.17*</td>
<td>-5.05 ± 1.30*</td>
</tr>
<tr>
<td>RIF 15 + VAN 15 (8)</td>
<td>5.18 ± 1.05</td>
<td>-3.56 ± 1.36*</td>
<td>-4.31 ± 1.19*</td>
<td>-5.18 ± 1.05*</td>
</tr>
<tr>
<td>Control (12)</td>
<td>4.78 ± 0.84</td>
<td>1.11 ± 0.87</td>
<td>1.12 ± 2.09</td>
<td>1.07 ± 2.32</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD.

* n, number of animals; CRO, ceftriaxone; RIF, rifampicin; VAN, vancomycin.
* P < 0.04 versus CRO group (ANOVA test).

Table 2. Mean CSF antibiotic levels (mg/L; ±SD) at 2, 24 and 26 h after antimicrobial therapy in experimental pneumococcal meningitis caused by the highly cephalosporin-resistant ATCC 51916 strain

<table>
<thead>
<tr>
<th>Therapya</th>
<th>Peak 2 h</th>
<th>Trough 24 h</th>
<th>Peak 26 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRO 100 mg/kg/day</td>
<td>4.61 ± 2.74</td>
<td>0.30 ± 0.02b</td>
<td>4.84 ± 3.06</td>
</tr>
<tr>
<td>RIF 15 mg/kg/day</td>
<td>0.81 ± 0.32</td>
<td>0.22 ± 0.15b</td>
<td>0.75 ± 0.41</td>
</tr>
<tr>
<td>VAN 15 mg/kg/12 h</td>
<td>1.01 ± 0.59</td>
<td>0.39 ± 0.25</td>
<td>1.44 ± 1.27</td>
</tr>
</tbody>
</table>

a CRO, ceftriaxone; RIF, rifampicin; VAN, vancomycin.
b Measurement of trough concentrations at 24 h was only possible in 2/8 rabbits.

Discussion

The virulence of the ATCC 51916 strain in the rabbit meningitis model has been demonstrated previously and was confirmed in the present experiment by the findings in control animals, in which a high inflammatory response combined with a high bacterial concentration in CSF led to a mortality rate of 58.3% at 24 h.

In view of the high cephalosporin resistance of the ATCC 51916 strain, we expected ceftriaxone monotherapy to be totally ineffective. However, although ceftriaxone peak levels were negligible compared with the MBC, therapeutic failures occurred in only 37.5% of the rabbits and a bacteriostatic effect was seen in the rest. If we consider that animals were sacrificed at 26 h, the low rate of ceftriaxone-associated therapeutic failures should be attributed to the short duration of the experiment.

Vancomycin was an effective therapy against this pneumococcal strain. However, its erratic penetration across the blood–brain barrier was confirmed in our model, where therapeutic failure occurred once CSF levels declined.

Friedland et al. demonstrated that ceftriaxone plus vancomycin was synergistic against both cephalosporin-intermediately susceptible and -resistant pneumococci. However, we did not observe synergy either in vitro or in the animal model as reported by other investigators.

In our in vitro studies, vancomycin plus ceftriaxone at concentrations achievable in CSF had an additive effect. These results had a good correlation with in vivo experiments, where this combination was effective against the ATCC 51916 strain (P < 0.002 compared with ceftriaxone, no differences compared with vancomycin).

It is well known that dexamethasone may decrease the penetration of vancomycin into the subarachnoid space. The effect of adjunctive dexamethasone on the efficacy of ceftriaxone plus vancomycin against this highly resistant strain should be evaluated in further studies.

Our in vitro data confirmed previous findings of an indifferent effect when rifampicin was added to ceftriaxone or vancomycin. However, in the animal model, although not statistically superior, rifampicin combinations improved the activity of rifampicin alone; and rifampicin plus ceftriaxone showed better in vivo activity than ceftriaxone alone. This discordance between in vitro and in vivo results has been previously reported.

Emergence of resistance did not occur in the animal model when rifampicin was tested either in monotherapy or in combination, although it was detected in time–kill curves. Single use of rifampicin may not be warranted in clinical practice owing to its low activity of the associated drug, as occurs in the combination of ceftriaxone plus rifampicin against the ATCC 51916 strain. This fact may limit the use of this combination in the clinical setting.

In the initial phase of treatment with a bactericidal antibiotic such as ceftriaxone, we would expect a burst of meningeal inflammation as a consequence of rapid bacterial lysis. However, possibly due to its low activity against this highly resistant strain, the inflammatory response induced by ceftriaxone was similar to that observed in control animals. Inflammatory data obtained in our experiments with rifampicin confirm its low potential for inducing a CSF inflammatory response, and that the addition of rifampicin or vancomycin to ceftriaxone led to a reduction in inflammatory parameters compared with ceftriaxone monotherapy.

In conclusion, ceftriaxone plus vancomycin, and vancomycin plus rifampicin appeared to be effective and appropriate...
combinations in treating experimental pneumococcal meningitis caused by highly cephalosporin-resistant strains. Our results provide an experimental basis for using these antibiotic combinations for empirical therapy of pneumococcal meningitis, regardless of the degree of cephalosporin resistance of the causative strain.

Acknowledgements

This study was supported by a grant FIS 01/1235. S. Ribes, F. Taberner and A. Domenech were supported by grants from IDIBELL and Universitat de Barcelona.

References