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Evaluation of a triple-drug combination for treatment of experimental multidrug-resistant pneumococcal meningitis☆

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Abstract

To evaluate the therapeutic efficacy of ceftriaxone+vancomycin+rifampicin (CVR) in the treatment of pneumococcal meningitis caused by a multidrug-resistant strain, single-drug regimens (ceftriaxone 100 mg/kg, rifampicin 15 mg/kg, or vancomycin 20 mg/kg), double-drug regimens (ceftriaxone + vancomycin [CV] and ceftriaxone + rifampicin [CR]) and a triple-drug combination (CVR) with or without dexamethasone were compared in a rabbit meningitis model. Meningitis was induced by a highly penicillin-resistant (MIC 2 mg/l) and ceftriaxone-resistant (MIC 4 mg/l) pneumococcal strain. Final therapeutic efficacy was evaluated by the bacterial concentration at 24 h, and the bacterial killing rate was also evaluated. All combination regimens were superior to ceftriaxone or vancomycin single-drug regimens with regard to sterilisation of CSF and bacterial killing rate. Rifampicin was as effective as combination regimens. Regardless of dexamethasone, therapeutic efficacy of CVR and CR were superior to that of CV. CVR showed comparable therapeutic efficacy to CR. Data suggested that CVR would not have additional therapeutic benefit over CR during the initial 24 h of treatment.

Keywords: *Streptococcus pneumoniae*; Multi-resistance; Meningitis; Animal model; Rifampicin; Ceftriaxone; Vancomycin

1. Introduction

Treatment outcome of pneumococcal meningitis depends on the in vitro susceptibility of the pathogen to antimicrobial agents. During the past decades, the resistance of *Streptococcus pneumoniae* to various antibiotics has been rapidly increasing in many parts of the world [1]. With the spread of pneumococcal resistance, cases of pneumococcal meningitis presenting with therapeutic dilemmas have been increasing. Recent recommendation for empirical treatment of this disease is the combination of third-generation cephalosporins and vancomycin [2]. Some investigators have recommended the addition of rifampicin to this combination if the pathogen proves highly resistant to penicillin and ceftriaxone [3]. However, there have been no experimental or clinical data to support the use of a triple-drug combination for pneumococcal meningitis caused by multidrug-resistant strains. We have evaluated the therapeutic efficacy of combination of ceftriaxone, vancomycin and rifampicin during the initial 24 h of the treatment of experimental pneumococcal meningitis caused by a multidrug-resistant strain.

2. Materials and methods

2.1. Bacterial strain

A highly penicillin- and cephalosporin-resistant strain of *S. pneumoniae* belonging to serotype 23F, originally isolated

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from a patient with pneumococcal meningitis, was used in the experiments. The strain was grown overnight on blood agar plates. The plates were washed with phosphate-buffered saline and aliquots of the resultant suspension were frozen at −70 °C. Aliquots were diluted to a concentration of 5×10^5 colony forming units (CFU)/ml and 0.5 ml of this was injected intracisternally into each rabbit. The minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) were: penicillin 2, 4; ceftriaxone 4, 8; vancomycin 0.5, 0.5; and rifampicin 0.12, 0.25 mg/l, respectively.

2.2. Antimicrobial therapy

Twelve treatment groups (five rabbits each) were evaluated: ceftriaxone (100 mg/kg), vancomycin (20 mg/kg), rifampicin (15 mg/kg), ceftriaxone + vancomycin, ceftriaxone + rifampicin, and ceftriaxone + vancomycin + rifampicin, with or without dexamethasone (1 mg/kg) which was given intravenously just before and 10 h after the initiation of antimicrobial therapy. Antibiotic dosages were chosen to achieve peak and trough concentrations in CSF similar to those observed in humans. The antibiotics were given intravenously every 5 h for two doses except ceftriaxone which was give once at 0 h [4]. Untreated controls (five rabbits) received saline alone.

2.3. Rabbit meningitis model

The rabbit model, originally described by Dacey and Sande, was used [4]. An inoculum that contained approximately 10^5 CFU of multidrug-resistant pneumococci was directly injected into the cisterna magna through a spinal needle. Eighteen to 20 h after the intracisternal injection of the inoculum, CSF was withdrawn and antimicrobial agents were administered through a peripheral ear vein. Untreated and treated animals were euthanised 26 and 40 h after intracisternal inoculation, respectively. Study protocol was approved by the Institutional Animal Care and Use Committee.

2.4. Measurement of bacterial and antibiotic concentrations

Bacterial concentrations in CSF were measured at 0, 5, 10, and 24 h after therapy was started, by plating undiluted and serial 10-fold dilutions of CSF (100 μl) on sheep blood agar and incubating in 5% CO_2 at 35 °C for 24 h (12). Bacterial killing rate in CSF was assessed by a reduction of bacterial concentrations in CSF during the time period. Final therapeutic efficacy of each treatment group was assessed by the final bacterial concentrations in CSF and the numbers of animals surviving at 24 h. In vivo synergism was defined when combination therapy reduced the bacterial concentration from the start of therapy by more than 1 $\log_{10}$ CFU/ml compared with the sum of the reduction with each agent alone. Antibiotic concentrations were measured in CSF sampled from treated rabbits at 60 min (peak) and 5 h (trough) after each antibiotic dose, and in serum sampled 30 min and 5 h after an intravenous dose. All specimens were frozen at −70 °C until they could be analysed. Ceftriaxone and rifampicin concentrations were determined by disk diffusion microbioassay using *Escherichia coli* (ATCC 10536), *Bacillus subtilis* (ATCC 6633) and *Micrococcus lutea* (ATCC 9341), respectively. Vancomycin values were measured by reverse-phase high-performance liquid chromatography. The lower limits of detection of the antibiotic were as follows: ceftriaxone 0.06, rifampicin 0.06, and vancomycin 0.01 mg/l.

2.5. Statistical analysis

Mean changes in bacterial concentrations in each treatment group were compared using the Mann–Whitney test. A P-value of <0.05 was considered significant.

3. Results and discussion

Peak and trough antibiotic concentrations in the CSF and serum for each drug with or without dexamethasone are presented in Table 1. Among the single-drug regimens, rifampicin was the most effective therapy evaluated, whereas ceftriaxone was the least effective (Table 2). Therapeutic failures of third-generation cephalosporins have been well documented in pneumococcal meningitis caused by ceftriaxone-resistant strains (MIC ≥ 2 mg/l) [5]. Since the strain used in the experiment was highly resistant to ceftriaxone, peak CSF concentrations of ceftriaxone were lower than the MBC of the strain. By pharmacodynamic analysis, sterilization of CSF by ceftriaxone could be achieved only when the time above MBC ($T >$ MBC) was 95–100% of the dosing interval [6]. This was the basic reason for poor therapeutic efficacy of ceftriaxone alone in our study. Given the increasing prevalence and level of ceftriaxone resistance among clinical isolates of pneumococci, single use of ceftriaxone should not be an initial empirical choice. With the widespread emergence of β-lactam resistance among pneumococci, vancomycin has become the mainstay of treatment regimens for pneumococcal meningitis. However, vancomycin alone may also fail due to inadequate concentrations of vancomycin in CSF, particularly with concomitant use of dexamethasone [7]. In our model, vancomycin with dexamethasone also showed poor sterilization effect at 24 h. Regardless of dexamethasone, rifampicin was shown to be as effective as ceftriaxone + rifampicin or ceftriaxone + vancomycin + rifampicin and more effective than ceftriaxone + vancomycin with regard to sterilization of CSF at 24 h and the bacterial killing rate during 0–24 h (Table 2). However, single use of rifampicin may not be warranted in clinical practice due to rapid emergence of resistance during the treatment. Therefore, any single-drug

Table 1
Mean antibiotic concentrations in CSF and serum in rabbits with pneumococcal meningitis (mg/l)

Antibiotics	CSF concentrations		Serum concentrations	
	Peak	Trough	Peak	Trough
Ceftriaxone	5.7 ± 1.5[a]	2.2 ± 0.8	193.1 ± 77.9	13.0 ± 1.7
Ceftriaxone (+dexa)	5.3 ± 2.6	1.7 ± 1.0	205.7 ± 17.4	16.2 ± 7.1
Vancomycin	0.9 ± 0.5	0.8 ± 0.3	34.7 ± 5.3	4.9 ± 2.5
Vancomycin (+dexa)	0.8 ± 0.3	0.5 ± 0.2	36.8 ± 8.0	4.2 ± 1.0
Rifampicin	0.4 ± 0.1	0.3 ± 0.1	21.0 ± 4.0	6.1 ± 2.9
Rifampicin (+dexa)	0.6 ± 0.1	0.5 ± 0.1	15.8 ± 3.5	9.7 ± 1.0

[a] All values are means with standard deviation.

regimens cannot be used in the treatment of pneumococcal meningitis caused by multidrug-resistant strains.

For these reasons, combination of vancomycin and ceftriaxone (or cefotaxime) has been recommended as a standard choice for initial treatment of presumed pneumococcal meningitis since the mid-1990s. Synergistic interaction of this combination was documented in the rabbit meningitis model [4] and in the CSF of children with meningitis [8]. Our data also documented synergistic bacterial killing of this combination without dexamethasone at both 10 and 24 h, although this regimen failed to sterilise the CSF at 24 h in some animals. However, dexamethasone significantly decreased the sterilization efficacy and bacterial killing rate ($P = 0.03$) of this combination, which was consistent with other observations [7]. The effect of steroids on vancomycin may be circumvented by the use of larger doses of vancomycin, which produce higher peak CSF concentrations.

Since rifampicin showed high CSF concentrations and excellent CSF activity against penicillin-resistant, rifampicin-susceptible pneumococci in the rabbit meningitis model, combination of ceftriaxone and rifampicin has also been recommended [4]. Previous data have shown that the addition of rifampicin to ceftriaxone was as effective as vancomycin + ceftriaxone in treating ceftriaxone-resistant pneumococcal meningitis [4]. In our model using a rifampicin-susceptible strain, however, rifampicin-containing combination regimens showed better bactericidal activity than ceftriaxone + vancomycin, especially when used with dexamethasone. Therapeutic benefit of rifampicin-containing combinations may be partly due to decreased release of proinflammatory cell wall products (lipoteichoic and teichoic acids) from pneumococci in rifampicin-treated animals [9]. Therapeutic efficacy of the combination of ceftriaxone and rifampicin was not affected by dexamethasone. In contrast to our data, however, rifampicin was reported to decrease the bactericidal activity of ceftriaxone, imipenem, or vancomycin when tested in vitro in combination with these agents [10]. Given the increasing prevalence of rifampicin resistance among pneumococci in some parts of the world, ceftriaxone + rifampicin would be used if the organism is susceptible to rifampicin, or if there is a delay in the treatment response to standard regimen [2].

Table 2
Numbers of rabbits with sterile CSF and bacterial killing rate in the CSF with each antibiotic regimen in the rabbit meningitis model

Treatment group	Dose[a] (mg/kg)	Rabbits treated	Rabbits with sterile CSF[b] at		$\Delta \log_{10}$ CFU/ml in the CSF during each time period	
			10 h	24 h	0–10 h	0–24 h
Untreated		5	0	Not done[c]	1.0	
Ceftriaxone	100	5	1	0	−2.7	−0.2
Ceftriaxone + dexa[d]	100	5	0	1	−2.1	−0.6
Vancomycin	20	5	4	3	−4.6	−1.2
Vancomycin + dexa	20	5	3	1	−3.5	−1.6
Rifampicin	15	5	1	5	−3.3	−4.4
Rifampicin + dexa	15	5	0	5	−3.3	−5.9
Ceftriaxone + vancomycin	100/20	5	5	2	−5.9	−3.9
Ceftriaxone + vancomycin + dexa	100/20	5	3	0	−5.4	−1.4[e]
Ceftriaxone + rifampicin	100/15	5	4	5	−4.5	−5.5
Ceftriaxone + rifampicin + dexa	100/15	5	3	5	−4.2	−5.6
Ceftriaxone + vancomycin + rifampicin	100/20/15	5	5	5	−5.8	−5.8
Ceftriaxone + vancomycin + rifampicin + dexa	100/20/15	5	5	5	−3.6	−5.4

[a] Two doses of each antibiotic except ceftriaxone (once) were given at 0 and 5 h.
[b] Specimens with ≤10 CFU/ml were considered sterile.
[c] Animals were agonal before this time and were euthanised.
[d] Dexamethasone.
[e] At 24 h, CSF bacterial killing rate in cetriaxone+vancomycin+dexa group was significantly lower than in cetriaxone+vancomycin group ($P = 0.032$).

Although there have been no supporting data, triple combination of ceftriaxone, vancomycin, and rifampicin has been recommended if an infecting pathogen is resistant to penicillin (≥0.1 mg/l) and to cefotaxime (or ceftriaxone) (≥2 mg/l) [3]. In our study, with regard to sterilization of CSF at 24 h, the triple combination was as effective as ceftriaxone + rifampicin and more effective than ceftriaxone + vancomycin regardless of dexamethasone. Bacterial killing of triple combination was more rapid than ceftriaxone + vancomycin when dexamethasone was given, while it was comparable with that of ceftriaxone + rifampicin. Triple combination was not affected by dexamethasone with regard to sterilization effect and bacterial killing rate.

Our study had some limitations. First, since vancomycin and other agents were given only once or twice, we could only assess initial responses to various regimens for 24 h. Second, since only a rifampicin-susceptible strain was used in the experiment, we could not evaluate the therapeutic efficacy of rifampicin-containing regimens in the treatment of pneumococcal meningitis caused by rifampicin-resistant strains.

Data from this study suggested that triple combination may not have additional therapeutic benefits over combination of ceftriaxone and rifampicin, although it may be more effective than the combination of ceftriaxone and vancomycin, particularly when dexamethasone is given.

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