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Killing Activities of Trovafloxacin Alone and in Combination with β-Lactam Agents, Rifampin, or Vancomycin against *Streptococcus pneumoniae* Isolates with Various Susceptibilities to Extended-Spectrum Cephalosporins at Concentrations Clinically Achievable in Cerebrospinal Fluid

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The killing activities of trovafloxacin alone and in combination with β-lactam agents (extended-spectrum cephalosporins, meropenem), rifampin, or vancomycin were evaluated against 20 genotypically characterized *Streptococcus pneumoniae* isolates for which amoxicillin MICs were ≥4 μg/ml (cefotaxime MICs, ≥4 μg/ml for six strains) at concentrations clinically achievable in cerebrospinal fluid. At 6 h the mean killing activity of trovafloxacin alone (range, 2.6 to 2.9 $\log_{10}$ CFU/ml) did not vary significantly according to the susceptibility of the strains to β-lactam agents. The activities of trovafloxacin or vancomycin added to the β-lactam agents and the combination trovafloxacin-vancomycin were additive or indifferent. Against the ceftriaxone-resistant isolates, the killing activity of the combination of a β-lactam agent and trovafloxacin did not differ significantly from that of a β-lactam agent and vancomycin.

Streptococcus pneumoniae meningitis carries a high rate of morbidity and mortality. Treatment was previously based on penicillin G or aminopenicillins and on extended-spectrum cephalosporins (21, 45), but clinical failures with delayed sterilization of cerebrospinal fluid (CSF) have been reported with amoxicillin and extended-spectrum cephalosporins (4–6, 17, 19, 21, 42, 45). The combination of extended-spectrum cephalosporins and vancomycin is recommended for the treatment of penicillin-resistant pneumococcal meningitis (1, 39). However, isolates with a high level of resistance to cefotaxime have recently been reported (13, 20, 28, 30). Questions as to the appropriateness of this combination have been raised, given the erratic penetration of vancomycin into the CSF and the fact that local concentrations of extended-spectrum cephalosporins are close to the MICs for such isolates (10).

Quinolones have not previously been used for the treatment of community-acquired meningitis because of their limited activity against *S. pneumoniae*. Trovafloxacin, a new fluoroquinolone, is reported to have good activity against *S. pneumoniae* that is independent of the penicillin susceptibility of the organism and to achieve a good concentration in CSF, contrary to older quinolones (25, 44, 46). We tested the efficacy of trovafloxacin against genotypically characterized isolates of *S. pneumoniae* that were recently recovered in France and that had high-level resistance to amoxicillin (amoxicillin MICs, ≥4 μg/ml) but for which cefotaxime MICs were or were not ≥4 μg/ml. We used the time-kill curve method with clinically achievable CSF antibiotic concentrations and a large inoculum to mimic the situation encountered in the clinical setting (3). Trovafloxacin was tested alone and in combination with amoxicillin, cefotaxime, ceftriaxone, cefpirome, meropenem, vancomycin, or rifampin. Combinations of trovafloxacin with a β-lactam agent, rifampin, or vancomycin were compared with the combination of rifampin or vancomycin with a β-lactam agent, used for the treatment of penicillin-resistant pneumococcal meningitis (1, 39).

MATERIALS AND METHODS

Twenty-nine serotyped clinical isolates of *S. pneumoniae* were studied. The serotypes were 6B, 14, 19F, and 23F. The isolates were obtained from blood, middle-ear fluid, conjunctival pus, or the lower respiratory tract between 1996 and 1997. The isolates were genotyped on the basis of the rRNA gene restriction pattern and DNA fingerprinting of the *pbp1a*, *pbp2b*, and *pbp2x* genes as described previously (12). The MICs of penicillin G, amoxicillin, cefotaxime, cefpirome, ceftriaxone, meropenem, trovafloxacin, rifampin, and vancomycin were determined by the dilution method on Mueller-Hinton agar supplemented with 5% sheep blood as described in 1997 by the National Committee for Clinical Laboratory Standards (NCCLS) (33). The replicator prong delivered approximately 10^4 CFU per spot. Killing activity was determined for 20 strains that were genotypically different on the basis of the rRNA gene restriction pattern and/or DNA fingerprinting of the *pbp1a*, *pbp2b*, and *pbp2x* genes. Killing activity was determined in microtiter plates (CML, Nemours, France) with an early-logarithmic-phase culture adjusted to approximately 10^6 to 10^7 CFU/ml in Mueller-Hinton broth supplemented with 5% lysed defibrinated sheep blood as described previously (11, 15). An incubation period of 6 h was chosen because most of the strains underwent spontaneous autolysis in vitro. The antimicrobial agents were used at the mean clinically achievable peak concentration in CSF after administration of doses currently recommended or proposed for the treatment of meningitis, as follows: amoxicillin, 6 μg/ml (35); cefotaxime, 5 μg/ml (10); ceftriaxone, 5 and 8 μg/ml (regimens of 50 and 100 mg/kg of body weight per day, respectively) (24, 26); cefpirome, 4 μg/ml (47); meropenem, 3 μg/ml (8); trovafloxacin, 1 μg/ml (2, 7); rifampin, 1 μg/ml (24, 31); and vancomycin, 2 μg/ml (10). Each β-lactam agent was tested alone and in combination with trovafloxacin, rifampin, or vancomycin. Colonies were counted by the quadrant method after 1:10 sample dilution by plating 50 μl of each dilution onto blood agar plates with a Spiral Plater system and incubating them for 18 h at 37°C with 5% CO_2. The detection limit was 4,000 CFU/ml. With the dilutions and the Spiral Plater system used, antimicrobial carryover does not interfere with bacterial counts (48). The microdilution method was initially compared with the macrodilution method with five strains. The mean difference between the two methods in the bactericidal activities of the antibiotics tested against each strain was 0.2 $\log_{10}$ CFU/ml, with a range of −0.15 to +0.5 $\log_{10}$ CFU/ml (no significant difference by Student's paired *t* test). Additivity and indifference were defined as a less than 10-fold change (increase or decrease) in killing at 6 h with the combination in comparison with that of the most active single agent used alone (14). Since the

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TABLE 1. Distribution of MICs of penicillin, amoxicillin, cefotaxime, cefpirome, ceftriaxone, meropenem, vancomycin, rifampin, and trovafloxacin for 29 *S. pneumoniae* isolates

Agent	No. of isolates inhibited at the following concn (μg/ml):									MIC_{90} (μg/ml)
	0.032	0.064	0.125	0.25	0.5	1	2	4	8	
Penicillin G						1	16	10	2	4
Amoxicillin								11	18	8
Cefotaxime					4	17	2	4	2	4
Ceftriaxone					13	12	4			2
Cefpirome				1	15	10	3			1
Meropenem				1	12	14	2			1
Vancomycin				27	2					0.25
Rifampin	29									0.032
Trovafloxacin		1	28							0.125

drugs were tested at a concentration which affected the growth curve of the organism when each drug was tested alone, synergy could not have been detected. Results are expressed as means ± standard deviations. Student's paired *t* test was used to test for statistical significance, and *P* values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The penicillin, amoxicillin, cefotaxime, ceftriaxone, cefpirome, meropenem, rifampin, vancomycin, and trovafloxacin MICs are reported in Table 1. According to NCCLS criteria (32), the strains were classified as resistant to penicillin (MICs, ≥2 μg/ml), resistant to amoxicillin (MICs, ≥2 μg/ml), intermediate (0.5 μg/ml ≤ MIC < 1 μg/ml) or resistant (MICs, ≥1 μg/ml) to meropenem, susceptible, intermediate (0.5 μg/ml < MIC ≤1 μg/ml), or resistant (MICs, ≥2 μg/ml) to cefotaxime and ceftriaxone, susceptible (MICs, ≤1 μg/ml) to vancomycin and rifampin, and susceptible to trovafloxacin (MICs, ≤1 μg/ml). However, the NCCLS breakpoints for amoxicillin and trovafloxacin are for nonmeningeal infections. The penicillin MIC at which 90% of isolates are inhibited (MIC_{90}) was half that of amoxicillin. For seven strains the cefotaxime MICs were equal to or 1 dilution higher than the corresponding penicillin MICs. For 17 strains the ceftriaxone MICs were lower than the cefotaxime MICs (difference of 2 to 4 dilutions for four isolates).

The mean killing activities of the antibiotics alone and in combination are reported in Table 2. The strains were classified into three groups as susceptible, intermediate, or resistant to ceftriaxone according to NCCLS breakpoints. Against strains that were susceptible or intermediate to ceftriaxone, the best killing activity of a β-lactam agent used alone was obtained with meropenem ($P < 0.05$). Against the resistant strains, we observed no significant difference in the killing activities of the β-lactam agents used alone, excluding amoxicillin but including ceftriaxone at 5 and 8 μg/ml. However, with cefotaxime alone, for two resistant strains we observed growth at the concentration clinically achievable in CSF; the cefotaxime MIC for these two strains was 8 μg/ml. The killing activity of trovafloxacin alone did not vary significantly ($P > 0.05$) according to the strain's susceptibility to extended-spectrum cephalosporins. For all the strains (including the two for which the cefotaxime MIC was 8 μg/ml), the effect of the addition of trovafloxacin or vancomycin to the cephalosporins or to meropenem was additive or indifferent, while the effect of the addition of rifampin (except to meropenem) was indifferent only against the strains that were intermediate or resistant to ceftriaxone. The addition of rifampin to the cephalosporins resulted in at least a 10-fold decrease in killing of the strains that were susceptible to ceftriaxone. Similarly, the addition of rifampin to meropenem resulted in a more than 10-fold decrease in the killing of strains that were susceptible or intermediate to ceftriaxone. Similar results were obtained for all

TABLE 2. Killing activities of amoxicillin, cefotaxime, ceftriaxone, cefpirome, and meropenem alone or in combination with vancomycin, rifampin, or trovafloxacin after 6 h of incubation, according to ceftriaxone susceptibility[a]

Agent used in combination and susceptibility	Mean ± SD change in log_{10} CFU/ml after 6 h of incubation							
	Alone	Trovafloxacin	Amoxicillin	Cefotaxime	Ceftriaxone (5 μg/ml)	Ceftriaxone (8 μg/ml)	Cefpirome	Meropenem
None (drug alone)								
S[b]		−2.9 ± 0.3	−1.2 ± 1.2	−2.7 ± 0.6	−2.7 ± 0.6	−2.7 ± 0.6	−2.6 ± 0.5	−3.2 ± 0.5
I[c]		−2.6 ± 0.4	−0.5 ± 0.9	−2.0 ± 0.6	−2.4 ± 0.5	−2.5 ± 0.5	−2.4 ± 0.5	−2.8 ± 0.5
R[d]		−2.8 ± 0.2	−1 ± 1.2	−0.8 ± 1.8	−2.0 ± 0.6	−2.4 ± 0.4	−2.3 ± 0.5	−2.7 ± 0.3
With vancomycin								
S	−1.8 ± 0.4	−2.2 ± 0.5	−2.2 ± 0.3	−2.9 ± 0.3	−3.0 ± 0.3	−3.0 ± 0.3	−3.0 ± 0.4	−3.0 ± 0.4
I	−1.6 ± 0.2	−2.0 ± 0.3	−2.0 ± 0.5	−2.3 ± 0.6	−2.3 ± 0.6	−2.3 ± 0.6	−2.3 ± 0.6	−2.5 ± 0.6
R	−1.9 ± 0.2	−2.2 ± 0.5	−2.5 ± 0.6	−2.8 ± 0.3	−2.8 ± 0.4	−2.7 ± 0.2	−2.8 ± 0.3	−2.8 ± 0.4
With rifampin								
S	−1.1 ± 0.4	−1.4 ± 0.3	−1.3 ± 0.2	−1.4 ± 0.2	−1.5 ± 0.4	−1.5 ± 0.4	−1.5 ± 0.4	−1.4 ± 0.2
I	−1.2 ± 0.5	−1.4 ± 0.5	−1.4 ± 0.4	−1.5 ± 0.5	−1.5 ± 0.5	−1.6 ± 0.5	−1.5 ± 0.5	−1.6 ± 0.5
R	−1.6 ± 0.2	−1.9 ± 0.4	−2.1 ± 0.7	−1.7 ± 0.4	−1.7 ± 0.4	−1.7 ± 0.5	−1.7 ± 0.4	−1.9 ± 0.8
With trovafloxacin								
S			−3.3 ± 0.2	−3.4 ± 0.4	−3.3 ± 0.3	−3.4 ± 0.4	−3.3 ± 0.3	−3.4 ± 0.4
I			−3.0 ± 0.4	−2.9 ± 0.5	−3.0 ± 0.3	−3.0 ± 0.4	−2.9 ± 0.5	−3.0 ± 0.4
R			−2.8 ± 0.3	−2.8 ± 0.1	−2.8 ± 0.15	−2.8 ± 0.1	−2.8 ± 0.2	−2.8 ± 0.1

[a] Antibiotics were tested at the mean concentration achieved in CSF after administration of doses currently recommended or proposed for the treatment of meningitis, as follows: amoxicillin, 6 μg/ml; cefotaxime, 5 μg/ml; ceftriaxone, 5 and 8 μg/ml; cefpirome, 4 μg/ml; meropenem, 3 μg/ml; trovafloxacin, 1 μg/ml; rifampin, 1 μg/ml, vancomycin, 2 μg/ml.

[b] Strains susceptible to ceftriaxone; $n = 5$.

[c] Strains intermediate to ceftriaxone; $n = 11$.

[d] Strains resistant to ceftriaxone; $n = 4$.

strains when rifampin was added to trovafloxacin. The trovafloxacin-vancomycin combination was indifferent. Against the ceftriaxone-resistant isolates, the killing activity of the combinations meropenem-trovafloxacin or extended-spectrum cephalosporin–trovafloxacin was not significantly different from that of the combination meropenem-vancomycin or extended-spectrum cephalosporin–vancomycin ($P > 0.05$).

Changes in β-lactam susceptibility among *S. pneumoniae* isolates have led to recommendations that high-dose cefotaxime or ceftriaxone in combination with vancomycin be used to treat meningitis in children (1, 39). However, the recent emergence and spread of strains with high-level resistance to extended-spectrum cephalosporins may compromise the efficacy of this treatment in patients with meningitis (13, 20, 28, 30). So far, *S. pneumoniae* isolates with high-level resistance to extended-spectrum cephalosporins have been reported in Spain, the United States, and the United Kingdom (20, 28, 30). Recently, pneumococci with high-level resistance to amoxicillin (MICs, ≥4 μg/ml) and to cefotaxime (MICs, ≥4 μg/ml) have also been identified in France (13). Interestingly, the penicillin MIC_{90} for those isolates was half that of amoxicillin, suggesting the emergence of high-level resistance to amoxicillin within preexisting penicillin-resistant clones (13). Although none of the strains were isolated from CSF, their serotypes were those usually recovered from patients with meningitis. Given the reported spread of clonal epidemic strains (12, 28–30, 41), the killing activities of the antibiotics and their combinations were tested only against the 20 genotypically different strains among the 29 isolates. In our study the MIC_{90}s of cefotaxime and ceftriaxone were 4 and 2 μg/ml, respectively. Mean clinically achievable peak concentrations in CSF are 5 μg of cefotaxime per ml after the administration of 300 mg/kg/day and 5 and 8 μg of ceftriaxone per ml after the administration of 50 and 100 mg/kg/day, respectively (10, 24, 26). Even though the difference is minimal, such a difference in antibiotic susceptibility may lead to delayed sterilization of CSF, as studies of experimental pneumococcal meningitis have shown a correlation between peak CSF antibiotic concentrations and maximal bactericidal efficacy (27, 43). In agreement with the MICs, amoxicillin alone showed a nonefficient killing activity. We observed no significant difference in the killing activities of ceftriaxone when it was tested at 5 and 8 μg/ml. This may be explained by the short incubation period and the time-dependent actions of β-lactam drugs. The addition of vancomycin to cephalosporins or meropenem was additive or indifferent. However, against the two strains for which the cefotaxime MIC was 8 μg/ml, the vancomycin-cefotaxime combination prevented growth at concentrations clinically achievable in CSF. This is consistent with at least additive activity of extended-spectrum cephalosporin–vancomycin combinations in an experimental model of pneumococcal meningitis (16). The use of rifampin has been proposed against such strains for which the expected clinical or bacteriologic response may be delayed (1). In our study the addition of rifampin to cephalosporins resulted in at least a 10-fold reduction in the killing of ceftriaxone-susceptible strains, while this combination was indifferent against resistant or intermediate strains. Note, however, that the rifampin-cephalosporin combination is effective in the rabbit model of pneumococcal meningitis (38).

All the isolates were susceptible to trovafloxacin, regardless of their β-lactam susceptibilities, as reported previously (24, 36, 39). The killing activity of trovafloxacin ranged from 2.6 to 2.9 $\log_{10}$ CFU/ml. Studies based on experimental models of meningitis have indicated that trovafloxacin has effective bactericidal activity in CSF against penicillin- or cephalosporin-resistant pneumococci (22, 37, 40). However, selection of antibiotic-resistant mutants may be a cause for concern when using quinolone antibiotics alone, and use of a drug combination may be required to prevent the emergence of resistance. This is why we tested trovafloxacin in combination. Very few data on the combination of fluoroquinolones with other drugs against *S. pneumoniae* are available. In vitro, the rifampin-fluoroquinolone combination was found to be indifferent in a study by Klugman et al. (23), while Giron et al. (18) found that rifampin reduced the killing activities of quinolones, as supported by our results. However, in an experimental model of pneumococcal meningitis, Kim et al. (22) found that the trovafloxacin-rifampin combination was indifferent. Nicolau et al. (34) reported that the trovafloxacin-vancomycin and trovafloxacin-ceftriaxone combinations were generally indifferent or synergistic against ceftriaxone-resistant *S. pneumoniae* strains. In our study both combinations were indifferent against all the ceftriaxone-resistant strains. This discrepancy between our data and those from the study of Nicolau et al. (34) may be explained by methodology considerations (use of the time-kill curve in our study and the checkerboard method in the work of Nicolau et al. [34]), although it has been suggested that the time-kill method is more predictive of the outcome of antibiotic treatment (9, 40). Against the ceftriaxone-resistant pneumococcal isolates, no significant difference in killing activity was observed between the meropenem-vancomycin or extended-spectrum cephalosporin–vancomycin combination (the recommended treatment) on the one hand and the meropenem-trovafloxacin or extended-spectrum cephalosporin–trovafloxacin combination on the other hand. The last two combinations may thus prove to be satisfactory alternatives for patients who do not tolerate vancomycin. Experimental studies and clinical trials are required to corroborate our in vitro data.

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