ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1998, p. 942–944 Vol. 42, No. 4
0066-4804/98/$04.00+0

Comparative In Vitro Killing Activities of Meropenem, Imipenem, Ceftriaxone, and Ceftriaxone plus Vancomycin at Clinically Achievable Cerebrospinal Fluid Concentrations against Penicillin-Resistant *Streptococcus pneumoniae* Isolates from Children with Meningitis

FREDERIC FITOUSSI,[1] CATHERINE DOIT,[1] KARIM BENALI,[2] STEPHANE BONACORSI,[1] PIERRE GESLIN,[3] AND EDOUARD BINGEN[1]*

Service de Microbiologie[1] and Service de Biostatistiques et Informatique Médicale,[2] Hôpital Robert Debré, 75019 Paris, and Centre National de Référence des Pneumocoques, Centre Hospitalier de Créteil, 94010 Créteil,[3] France

Received 8 July 1997/Returned for modification 14 October 1997/Accepted 4 January 1998

The activities of meropenem, imipenem, ceftriaxone, and vancomycin were evaluated against 80 penicillin-susceptible and -resistant *Streptococcus pneumoniae* strains. Meropenem, imipenem, ceftriaxone, and vancomycin MICs at which 90% of the isolates are inhibited were 0.5, 0.25, 1, and 0.25 μg/ml, respectively. Against penicillin-resistant strains, the best killing activity at cerebrospinal fluid concentrations was obtained with imipenem and ceftriaxone-vancomycin. However, while the killing activity of imipenem was significantly greater than that of meropenem, no significant difference was observed between the activities of meropenem and ceftriaxone-vancomycin.

Meningitis caused by *Streptococcus pneumoniae* carries a high rate of morbidity and mortality in both children and adults. Treatment used to be based on penicillin G or aminopenicillins, but the marked increase in pneumococcal resistance to penicillins in the 1980s (20) led to the use of extended-spectrum cephalosporins as empiric therapy for acute bacterial meningitis (15, 28). There have now been reports of *S. pneumoniae* strains resistant to extended-spectrum cephalosporins associated with clinical failure and delayed sterilization of cerebrospinal fluid (CSF) (6, 7, 13, 16, 26). For example, in France, the rate of isolation of *S. pneumoniae* strains for which the cefotaxime or ceftriaxone MICs were greater than >0.5 μg/ml from CSF in patients with meningitis in 1995 was 16.4% (14). This means that antibiotic combinations or new classes of antibacterial agents must be assessed for the treatment of pneumococcal meningitis.

The carbapenem class is highly active against common meningeal pathogens. Imipenem is the first member of this class, and its MICs against penicillin-resistant pneumococci are lower than those of other β-lactam agents (27). However, the use of imipenem in meningitis is limited by its adverse effects (29). Meropenem, a novel carbapenem antibiotic structurally related to imipenem, but with fewer adverse effects, penetrates efficiently into the central nervous system (8). Because meropenem is reported to have good activity against penicillin-intermediate and -resistant *S. pneumoniae* strains (27), we tested its activity against such strains isolated from children with meningitis. We used the time-killing curve method with clinically achievable CSF antibiotic concentrations and a large inoculum to mimic clinical conditions (4). Meropenem was compared to imipenem and, against the most-resistant strains, to ceftriaxone alone and to the ceftriaxone-vancomycin combination, which is recommended for the treatment of penicillin-resistant pneumococcal meningitis (24).

(This work was presented in part at the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 15 to 18 September 1996.)

Eighty serotyped *S. pneumoniae* strains were studied. All were isolated from CSF of children with meningitis between 1987 and 1997. The MICs of penicillin G, meropenem, imipenem, ceftriaxone, and vancomycin were determined by the dilution method on Mueller-Hinton agar supplemented with 5% sheep blood as recommended by the National Committee for Clinical Laboratory Standards (23). The replicator prong delivered approximately 10^4 CFU per spot onto a blood agar plate. The concentration at which 90% of the strains were inhibited was defined as the MIC_{90}. Killing activity against 26 previously described strains (11) for which the penicillin G MICs were increased (>0.125 to 2 μg/ml), was determined with microtiter plates (CML, Nemours, France), with an early-log-phase culture adjusted to approximately 10^6 to 10^7 CFU/ml in Mueller-Hinton broth supplemented with 5% lysed defibrinated sheep blood during 5 h of incubation (10). This incubation period was chosen because most of the strains underwent a spontaneous autolysis in vitro. The antimicrobial agents were used at the mean CSF concentration (CC) and fractions of it (CC/2 and CC/4) after administration (two doses at 4 h) of doses currently recommended for the treatment of meningitis, as follows: meropenem, 3 μg/ml (8); imipenem-cilastatin, 2 μg/ml each (21); ceftriaxone, 8.8 μg/ml (17); vancomycin, 2 μg/ml (9). Ceftriaxone was tested alone and in combination with vancomycin, as recommended for the treatment of penicillin-resistant pneumococcal meningitis (24). Colonies were counted by the quadrant method after 1:10 dilution, by plating of 50 μl of each dilution onto blood agar plates with a Spiral Plater system and incubation for 18 h at 37°C with 5% CO_2. The detection limit was 4,000 CFU/ml. With the dilutions and the Spiral system used, antimicrobial agent carryover does not interfere with bacterial counts (30). The microdilution method used was initially compared with the macromethod with five

* Corresponding author. Mailing address: Service de Microbiologie, Hôpital R. Debré, 48 Bd. Sérurier, 75019 Paris, France. Phone: 33 (1) 40 03 23 40. Fax: 33 (1) 40 03 24 50. E-mail: edouard.bingen@rdb.ap-hop-paris.fr.

TABLE 1. Susceptibility of pneumococcal CSF isolates to antimicrobial agents by penicillin susceptibility

Antimicrobial agent and penicillin susceptibility of isolates (*n*)[a]	MIC (μg/ml)[b]		
	Range	50%	90%
Penicillin			
S (29)	0.016–0.06	0.032	0.064
I (29)	0.125–1.0	0.5	1.0
R (22)	2.0	2.0	2.0
Meropenem			
S	<0.015–0.06	0.015	0.06
I	<0.015–0.5	0.12	0.5
R	0.12–0.25	0.5	0.5
Imipenem			
S	<0.015–0.03	<0.015	0.015
I	<0.015–0.25	0.06	0.25
R	0.12–0.25	0.25	0.25
Ceftriaxone			
S	0.015–0.06	0.015	0.015
I	0.015–0.5	0.12	0.5
R	0.5–1.0	1.0	1.0
Vancomycin			
S	0.125–0.25	0.25	0.25
I	0.25	0.25	0.25
R	0.25	0.25	0.25

[a] S, susceptible (≤0.06 μg/ml); I, intermediate (0.125 to 1.0 μg/ml); R, resistant (≥2.0 μg/ml). *n*, number of strains.
[b] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

strains. The mean difference in bactericidal activity of the antibiotics tested for each strain between the two methods was 0.2 $\log_{10}$ CFU/ml, with a range of −0.15 to +0.5 $\log_{10}$ CFU/ml. Student's paired *t* test shows that there was no significant difference between the two methods. Bactericidal activity was defined as a reduction of more than 3 $\log_{10}$ CFU/ml after 5 h of incubation. Results were expressed as means ± standard deviations. One-way analysis of variance was used to compare the killing activities of the antibiotics. Post hoc tests with Bonferroni correction were used to test the killing activities of all possible pairs of antibiotics (*P* values were two tailed and were considered significant when probabilities were less than 0.012).

The penicillin, meropenem, imipenem, ceftriaxone, and vancomycin MICs are reported in Table 1. The serotypes of strains for which penicillin MICs were increased were 6A, 6B, 9V, 14, 15C, 19A, 19F, 23A, and 23F. According to National Committee for Clinical Laboratory Standards criteria, strains were classified as intermediate (0.1 μg/ml ≤ MIC ≤ 1 μg/ml) or resistant (MIC of ≥2 μg/ml) to penicillin, susceptible (MIC of ≤0.12 μg/ml) or intermediate (0.25 μg/ml ≤ MIC ≤ 0.5 μg/ml) to meropenem (data not yet published) and imipenem, susceptible (MIC of ≤ 0.05 μg/ml) or intermediate (MIC of 1 μg/ml) to ceftriaxone, and susceptible (MIC of ≤ 1 μg/ml) to vancomycin. The meropenem, imipenem, ceftriaxone, and vancomycin MIC_{90}s were 0.5, 0.25, 1, and 0.25 μg/ml, respectively.

The killing activities of the antibiotics at the mean CSF antibiotic concentration and fractions of it are reported in Table 2. With all antibiotics tested, alone or in combination, bactericidal activity was observed at 5 h against all strains with penicillin G MICs of <0.5 μg/ml. Against strains with penicillin G MICs of ≥1 μg/ml, killing was significantly lower for the three CSF concentrations for each agent ($P = 0.008$). The strains for which penicillin G MICs were 2 μg/ml were intermediate to ceftriaxone. The best killing activity against these strains was obtained at 5 h with imipenem and ceftriaxone-vancomycin at the mean concentrations of antibiotic in the CSF (no significant difference between the two [$P = 0.04$]). However, while the killing activity of imipenem was significantly greater than that of meropenem against the strains for which the penicillin G MICs were highest ($P = 10^{-4}$), no significant difference was observed between meropenem and ceftriaxone-vancomycin ($P = 0.05$). Ceftriaxone alone was the least active antibiotic ($P > 0.01$). For all antibiotics, whether with the mean concentrations in the CSF or fractions of it, we did not observe a significant difference in the killing ($P = 0.7$).

Changes in β-lactam susceptibility among *S. pneumoniae* isolates have led to recommendations that high-dose cefotaxime or ceftriaxone, combined with vancomycin, be used to treat meningitis in children (24). Alternatively, new therapeutic compounds must be evaluated. In our study, the MIC_{90}s of meropenem, imipenem, ceftriaxone, and vancomycin of penicillin-sensitive, -intermediate, and -resistant pneumococci were consistent with those reported elsewhere (3, 27). On the basis

TABLE 2. Killing activity of meropenem, imipenem, ceftriaxone, and ceftriaxone-vancomycin against the 26 *S. pneumoniae* isolates according to the penicillin G MIC

Antibiotic and concn	Change in $\log_{10}$ CFU/ml after incubation with isolates (*n*) at penicillin G MIC (μg/ml) of [a]:				
	0.12 (*n* = 4)	0.25 (*n* = 3)	0.5 (*n* = 2)	1 (*n* = 4)	2 (*n* = 13)
Meropenem					
CC (3 μg/ml)	≥3.4	≥3.4	3.2 ± 0.2	2.62 ± 0.25	2.46 ± 0.25
CC/2 (1.5 μg/ml)	≥3.4	≥3.4	3.2 ± 0.2	2.50 ± 0.23	2.38 ± 0.33
CC/4 (0.75 μg/ml)	≥3.4	≥3.4	3.2 ± 0.2	2.45 ± 0.20	2.36 ± 0.34
Imipenem					
CC (2 μg/ml)	≥3.4	≥3.4	≥3.4	2.90 ± 0.52	2.95 ± 0.42
CC/2 (1 μg/ml)	≥3.4	≥3.4	3.3 ± 0.1	2.95 ± 0.63	2.98 ± 0.52
CC/4 (0.5 μg/ml)	≥3.4	≥3.4	3.3 ± 0.1	2.80 ± 0.67	2.91 ± 0.54
Ceftriaxone					
CC (8.8 μg/ml)	≥3.4	≥3.4	3.0 ± 0.3	2.45 ± 0.24	2.08 ± 0.22
CC/2 (4.4 μg/ml)	≥3.4	≥3.4	2.9 ± 0.2	2.4 ± 0.2	1.98 ± 0.25
CC/4 (2.2 μg/ml)	≥3.4	≥3.4	2.8 ± 0.2	2.3 ± 0.2	1.90 ± 0.27
Ceftriaxone-vancomycin[b]					
CC (8.8 and 2 μg/ml)	≥3.4	≥3.4	3.2 ± 0.1	2.75 ± 0.3	2.75 ± 0.32
CC/2 (4.4 and 1 μg/ml)	≥3.4	≥3.4	3.2 ± 0.1	2.65 ± 0.3	2.7 ± 0.4
CC/4 (2.2 and 0.5 μg/ml)	≥3.4	≥3.4	3.2 ± 0.1	2.60 ± 0.2	2.7 ± 0.4

[a] Values represent mean (± standard deviation) change after 5 h of incubation with isolates at various penicillin MICs.
[b] Concentrations for the ceftriaxone-vancomycin combination are given respectively.

of the MICs, imipenem was the most active β-lactam agent. The MIC_{90} of meropenem for penicillin-resistant pneumococci was 0.5 μg/ml; it is half that of ceftriaxone. Although small, such a difference may influence the therapeutic response in meningitis, because the concentration of β-lactams in CSF is close to the relevant MICs (12). This is even more important in dexamethasone administration, which by reducing meningeal inflammation, reduces antibiotic diffusion into the CSF (25).

Optimal treatment requires rapid bactericidal activity within the CSF, because delayed CSF sterilization is associated with a poor prognosis and a high risk of sequelae in children (19). We therefore used the time-killing curve method after 5 h of incubation with clinically achievable concentrations in CSF (CC, CC/2, and CC/4) to predict antimicrobial activity in CSF during the first few hours of treatment. The best killing activity of imipenem is in agreement with those of previous reports (1, 10). However, its use for treatment of meningitis is limited because of its seizure activity (29). In contrast, with meropenem, no greater incidence of neurological toxicity in patients with meningitis was reported (5, 18). Meropenem at the mean CSF concentration and fractions of it gave a mean killing activity superior to 2.3 $\log_{10}$ CFU/ml against penicillin-resistant pneumococcal strains after 5 h of incubation. Barakett et al. reported comparable results, despite the use of a different methodology (1, 2). Meropenem had significantly superior killing activity to that of ceftriaxone used alone. Nairn et al. reported similar results in experimental pneumococcal meningitis with resistant strains (22). The bactericidal activity of meropenem was similar to that of ceftriaxone-vancomycin. However, there are concerns over the moderate penetration of the glycopeptide with significant interpatient variability (5). Meropenem has already been tested for the treatment of meningitis in both adults and children (5, 18), but only a very few data for patients infected by cephalosporin-resistant pneumococci are available. Our in vitro data suggest that meropenem may be effective in the treatment of cephalosporin-resistant pneumococcal meningitis; more clinical data are required in this setting.

REFERENCES

1. **Barakett, V., D. Lesage, F. Delisle, G. Richard, and J. C. Petit.** 1994. Killing kinetics of imipenem against penicillin-resistant *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **33:**1025–1028.
2. **Barakett, V., D. Lesage, F. Delisle, G. Richard, and J. C. Petit.** 1996. Killing kinetics of meropenem against penicillin-resistant pneumococci. J. Antimicrob. Chemother. **38:**1085–1089.
3. **Barry, A. L., S. D. Brown, and W. J. Novick.** 1995. In vitro activities of cefotaxime, ceftriaxone, ceftazidime, cefpirome, and penicillin against *Streptococcus pneumoniae* isolates. Antimicrob. Agents Chemother. **39:**2193–2196.
4. **Bingen, E., N. Lambert-Zechovsky, P. Mariani-Kurdjian, C. Doit, Y. Aujard, F. Fournerie, and H. Mathieu.** 1990. Bacterial count in cerebrospinal fluid of children with meningitis. Eur. J. Clin. Microbiol. Infect. Dis. **9:**278–281.
5. **Bradley, J. S., and W. M. Scheld.** 1997. The challenge of penicillin-resistant *Streptococcus pneumoniae* meningitis: current antibiotic therapy in the 1990s. Clin. Infect. Dis. **24:**(Suppl. 2)**:**213–221.
6. **Bradley, J. S., and J. D. Connor.** 1991. Ceftriaxone failure in meningitis caused by *Streptococcus pneumoniae* with reduced susceptibility to beta-lactams antibiotics. Pediatr. Infect. Dis. J. **10:**871–873.
7. **Canton, E.** 1993. Cefotaxime breakpoint for *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **37:**616–617.
8. **Dagan, R., L. Velghe, J. L. Rodda, and K. P. Klugman.** 1994. Penetration of meropenem into the cerebrospinal fluid in humans with inflamed meninges. J. Antimicrob. Chemother. **34:**175–179.
9. **Doit, C., J. Barre, R. Cohen, A. Bourrillon, E. Bingen, and the Study Group.** 1996. Bactericidal activity in cerebrospinal fluid (CSF) of children treated with cefotaxime (CTX) + vancomycin (VAN) against an intermediately cephalosporin-resistant *Streptococcus pneumoniae* (Sp) strain, abstr. A105, p. 21. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
10. **Doit, C. P., S. P. Bonacorsi, A. J. Fremaux, G. Sissia, R. Cohen, P. L. Geslin, and E. H. Bingen.** 1994. In vitro killing activities of antibiotics at clinically achievable concentrations in cerebrospinal fluid against penicillin-resistant *Streptococcus pneumoniae* isolated from children with meningitis. Antimicrob. Agents Chemother. **38:**2655–2659.
11. **Fitoussi, F., C. Doit, A. Sandin, A. Pechinot, A. Kazmierczack, P. Geslin, and E. Bingen.** 1995. Killing activity of cefpirome against penicillin-resistant *Streptococcus pneumoniae* isolates from patients with meningitis in a pharmacodynamic model simulating the cerebrospinal fluid concentration profile. Antimicrob. Agents Chemother. **39:**2560–2563.
12. **Friedland, I. R., M. Paris, S. Ehrett, S. Hickey, K. Olsen, and G. H. McCracken, Jr.** 1993. Evaluation of antimicrobial regimens for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob. Agents Chemother. **37:**1630–1636.
13. **Friedland, I. R., S. Shelton, M. Paris, S. Rinderknecht, S. Ehrett, K. Krisher, and G. H. McCracken, Jr.** 1993. Dilemmas in diagnosis and management of cephalosporin-resistant *Streptococcus pneumoniae* meningitis. Pediatr. Infect. Dis. J. **12:**196–200.
14. **Geslin, P.** 1996. Centre National de Référence des Pneumocoques. Rapport d'activité année 1994–1995. Centre National des Pneumocoques.
15. **Jackson, M. A., Y. Mithal, R. M. Robins-Browne, M. N. Gaspar, and H. J. Koornhof.** 1984. Relatively penicillin-resistant pneumococcal infections in pediatric patients. Pediatr. Infect. Dis. J. **3:**129–132.
16. **John, C. C.** 1994. Treatment failure with use of third-generation cephalosporin for penicillin-resistant pneumococcal meningitis: case report and review. Clin. Infect. Dis. **18:**188–193.
17. **Klugman, K. P., I. R. Friedland, and J. S. Bradley.** 1995. Bactericidal activity against cephalosporin-resistant *Streptococcus pneumoniae* in cerebrospinal fluid of children with acute bacterial meningitis. Antimicrob. Agents Chemother. **39:**1988–1992.
18. **Klugman, K. P., R. Dagan, and the Meropenem Meningitis Study Group.** 1995. Randomized comparison of meropenem with cefotaxime for treatment of bacterial meningitis. Antimicrob. Agents Chemother. **39:**1140–1146.
19. **Lebel, M. H., and G. H. McCracken, Jr.** 1989. Delayed cerebro-spinal fluid sterilization and adverse outcome of bacterial meningitis in infants and children. Pediatrics **83:**161–167.
20. **Mason, E. O., Jr., S. L. Kaplan, L. B. Lamberth, and J. Tillman.** 1992. Increased rate of isolation of penicillin-resistant *Streptococcus pneumoniae* in a children's hospital and in vitro susceptibilities to antibiotics of potential therapeutic use. Antimicrob. Agents Chemother. **36:**1703–1707.
21. **Modai, J., D. Vittecocq, J. M. Decazes, and A. Meulemans.** 1985. Penetration of imipenem and cilastatin into cerebrospinal fluid of patients with bacterial meningitis. J. Antimicrob. Chemother. **16:**751–755.
22. **Nairn, K., G. L. Shepherd, and J. R. Edwards.** 1995. Efficacy of meropenem in experimental meningitis. J. Antimicrob. Chemother. **36**(Suppl. A)**:**73–84.
23. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
24. **Paris, M. M., O. Ramilo, and G. H. McCracken, Jr.** 1995. Management of meningitis caused by penicillin-resistant *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **39:**2171–2175.
25. **Paris, M. M., S. M. Hickey, M. I. Uscher, S. Shelton, K. D. Olsen, and G. H. McCracken, Jr.** 1994. Effect of dexamethasone on therapy of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob. Agents Chemother. **38:**1320–1324.
26. **Sloas, M. M., F. F. Barrett, P. J. Chesney, B. K. English, B. C. Hill, F. C. Tenover, and R. J. Legiadro.** 1992. Cephalosporin treatment failure in penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* meningitis. Pediatr. Infect. Dis. J. **11:**662–666.
27. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1994. Susceptibilities of 177 penicillin-susceptible and -resistant pneumococci to FK 037, cefpirome, cefepime, ceftriaxone, cefotaxime, ceftazidime, imipenem, biapenem, meropenem, and vancomycin. Antimicrob. Agents Chemother. **38:**898–900.
28. **Weingarten, R. D., Z. Markiewicz, and D. N. Gilbert.** 1990. Meningitis due to penicillin-resistant *Streptococcus pneumoniae* in adults. Rev. Infect. Dis. **12:**118–124.
29. **Wong, V. K., H. T. Wright, Jr., L. A. Ross, W. H. Mason, C. B. Inderlied, and K. S. Kim.** 1991. Imipenem/cilastatin treatment of bacterial meningitis in children. Pediatr. Infect. Dis. J. **10:**122–125.
30. **Yourassowsky, E., M. P. Van der Linden, F. Crokaert, and Y. Glupezynski.** 1988. Effect of antibiotics carry-over on bacterial counting by "spiral plating." J. Antimicrob. Chemother. **21:**138–140.