ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1997, p. 2050–2052 Vol. 41, No. 9
0066-4804/97/$04.00+0

Bactericidal Activity against Intermediately Cephalosporin-Resistant *Streptococcus pneumoniae* in Cerebrospinal Fluid of Children with Bacterial Meningitis Treated with High Doses of Cefotaxime and Vancomycin

CATHERINE DOIT,[1] JEROME BARRE,[2] ROBERT COHEN,[3] STEPHANE BONACORSI,[1] ANTOINE BOURRILLON,[4] AND EDOUARD H. BINGEN[1]*

Service de Microbiologie[1] and Service de Pédiatrie Générale,[4] Hôpital Robert Debré, 75019 Paris, and Service Hospitalo-Universitaire de Pharmacologie[2] and Service de Microbiologie,[3] Centre Intercommunal Hospitalier de Créteil, 94010 Créteil, France

Received 3 February 1997/Returned for modification 25 April 1997/Accepted 22 June 1997

Cerebrospinal fluid (CSF) was taken from 19 children with bacterial meningitis treated with cefotaxime (300 mg/kg of body weight/day) and vancomycin (60 mg/kg/day). Median levels of drugs in CSF were smaller than expected, as follows: 4.4 μg/ml for cefotaxime, 3.2 μg/ml for desacetylcefotaxime, and 1.7 μg/ml for vancomycin. The median CSF bactericidal titer against an intermediately cefotaxime-resistant pneumococcus was 1:4. Our data suggest at least an additive interaction between the drugs used in this study.

The widespread increase in penicillin-resistant pneumococci has led to the use of extended-spectrum cephalosporins (cefotaxime [CTX] or ceftriaxone) as an empiric therapy for pneumococcal meningitis in children (5). However, clinical failures with delayed sterilization of cerebrospinal fluid (CSF) have been reported when extended-spectrum cephalosporin MICs are ≥0.5 μg/ml (12). In France in 1995, 44% of the strains isolated from CSF specimens of children had diminished susceptibility to penicillin and for 80% of these strains the CTX MIC was ≥0.5 μg/ml (10). On the basis of in vitro killing curves (9) and experimental data (8), addition of vancomycin (VAN) to extended-spectrum cephalosporin regimens has been recommended for empiric treatment of pneumococcal meningitis in countries with a high incidence of resistant strains (18). Antibiotic efficacy in the treatment of meningitis can be evaluated in vivo on the basis of CSF sterilization in 24 to 36 h (15) and drug penetration into CSF and ex vivo in terms of CSF bactericidal titer (CBT) analysis.

The aim of this study was to assess the efficacy of the combination of high doses of CTX and VAN in the worst-case situation, i.e., against the most resistant strain currently found in France, by studying the activities of CSF specimens from these children against this strain.

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

Patients and CSF samples. As part of a French multicenter study, 19 children with signs of acute pneumococcal meningitis (gram-positive cocci in CSF and/or pneumococcal antigens in CSF, blood, or urine and/or risk factors for invasive pneumococcal infections) were treated intravenously with CTX (300 mg/kg of body weight/day in four doses) and VAN (60 mg/kg/day in four doses). The VAN infusion (1 h) was given after the CTX infusion (20 min). None of the children received dexamethasone. A second lumbar puncture was performed 24 to 36 h after the beginning of treatment, in principle 120 ± 30 min after the end of the CTX infusion (at least the fifth infusion) to control sterilization, to determine antibiotic concentrations, and to assay the bactericidal activity in CSF. CSF samples were stored at −80°C until use.

Antibiotic assays. CTX and desacetylcefotaxime (dCTX) were measured by high-performance liquid chromatography on an octyldecyl silane Hypersil column after liquid extraction with chloroform-isoamyl alcohol; the detection wavelength was 270 nm, ceftizoxime was used as the internal standard, and the limit of quantification was 1 μg/ml (6). VAN was measured by liquid chromatography on an octyldecyl silane Hypersil column; the detection wavelength was 270 nm, trimethoprim was used as the internal standard, and the limit of quantification was 0.5 μg/ml (3).

CSF bactericidal assay. The test strain was a clinical CSF isolate from a patient included in the study (patient 1401). It was representative of the prevalent resistant clone (serotype 23F) currently found in France, which has intermediate susceptibility to CTX (7). MICs for the strain were determined by microdilution in Mueller-Hinton broth supplemented with 5% lysed and defibrinated sheep blood by using a standard inoculum (10^5 CFU/ml) and a heavy inoculum (10^7 CFU/ml). MBCs (99.9% killing) were determined by plating 50-μl aliquots from clear microtiter wells onto Columbia agar and incubating the wells in 5% CO_2 for 24 h. The CSF bactericidal assay was a microtiter broth dilution method. Serial twofold dilutions of CSF in normal saline were made in microtiter plates (CML, Nemours, France) to provide a range of 1:1 to 1:64 in a volume of 100 μl. One hundred microliters of an early-log-phase inoculum was added to provide a final bacterial density of 10^6 to 10^7 CFU/ml and a final CSF dilution of 1:2 to 1:128. This heavy inoculum was chosen to simulate that frequently found in CSF of children with pneumococcal meningitis (4). The early-log-phase inoculum was prepared by suspending bacteria from an overnight agar culture in broth and incubating the culture for 1 h. Survivors were counted after 6 h of incubation by plating 50 μl of culture onto Columbia agar with a spiral plater (Spiral Systems Inc., Cincinnati, Ohio) (11). Colonies were counted after 18 h of incubation at 37°C in room atmosphere with 5% CO_2 by the quadrant counting

* 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.

TABLE 1. Patient data and individual CSF antibiotic concentrations and CBTs against an intermediately cephalosporin-resistant pneumococcal strain

Patient	Age	Time (min) between CSF sampling and end of infusion of:		CSF antibiotic concn (μg/ml)			CBT
		CTX	VAN	CTX	dCTX	VAN	
1401	11 yr	130	70	4.4	1.9	1.5	1:4
2701	12 yr	100	45	5.6	3.5	2.0	<1:2
3002[a]	6 mo	345	45	4.4	3.2	1.5	1:8
4201	8 yr	190	120	2.7	2.3	1.7	1:2
2401	17 yr	100	40	4.8	5.5	1.8	1:8
3801	6 mo	100	40	4.4	3.5	2.0	1:4
3803	5.5 yr	130	70	4.4	3.5	1.8	1:4
1601	6.5 mo	165	95	6.1	5.1	2.6	1:8
3001	9 mo	190	130	7.3	4.4	2.2	1:4
3601	5 mo	120	60	15.1	7.0	2.9	1:4
4101	10 yr	130	40	2.9	2.0	1.1	1:2
4001	9 mo	130	60	5.8	3.2	1.3	1:2
3401	9 mo	160	90	5.1	3.2	1.5	1:8
1602	5 mo	80	20	3.7	1.9	0.9	1:2
1603	1.5 yr	180	120	7.9	5.0	1.7	1:4
1604	7 mo	130	60	15.5	10.7	3.3	1:16
1402	10 mo	110	300	1.7	2.8	0.4	<1:2
1605	12 mo	130	60	2.6	1.4	0.6	1:4
2801	5 yr	150	90	1.0	1.2	0.3	1:2

[a] This patient received CTX in rapid (2-min) intravenous injections.

method. The CFU detection limit was 400/ml. Preliminary experiments excluded a significant antibiotic carryover effect. They were performed as follows. Inocula containing low numbers of bacteria (approximately 1 to 3 $\log_{10}$ CFU/ml) were plated in the presence or absence of the combination of the two antibiotics at the highest concentration observed in the CSF of our patients. The viable counts were compared with those of the original inoculum, and no significant difference was observed. The CBT was defined as the highest dilution that killed more than 99.9% of the initial inoculum. Reproducibility was checked by repeating the assay on six CSF samples (two assays for each sample). The same CBTs were found in the duplicate assays, and the maximum difference in the change of the initial inoculum was 0.6 $\log_{10}$ CFU/ml. We used the mean of the two decreases in CFU for these six samples.

Results. The subjects' ages ranged from 5 months to 17 years. Pneumococcal meningitis was confirmed for 18 patients by isolation of pneumococcal strains in their CSF. The 19th patient had meningococcal meningitis. The pneumococcal isolates were susceptible to penicillin and cephalosporin in eight cases, intermediately resistant to penicillin and susceptible to cephalosporin in seven cases, intermediately resistant to penicillin and cephalosporin in one case, and resistant to penicillin and intermediately resistant to cephalosporin in two cases, according to criteria of the National Committee for Clinical Laboratory Standards (17). No cephalosporin-resistant strains were isolated. All control CSF samples were sterile. At this time, the clinical outcome is not available for all patients. The follow-up evaluations at 6 to 12 months have been done for 9 of the 19 patients. No relapse or recurrence of meningitis has been observed. The complete clinical study will be presented in a subsequent report.

The same MICs and MBCs were obtained for the test strain whatever the inoculum (10^5 and 10^7 CFU/ml) and were as follows (MIC listed first): 2 and 4 μg/ml for penicillin G, 1 and 2 μg/ml for CTX, and 0.5 and 0.5 μg/ml for VAN. Individual results of the CSF sampling times, antibiotic concentrations in CSF, and CBT for each patient are shown in Table 1. Median times (range) between CSF sampling and the end of the infusion were 130 min (80 to 345 min) for CTX and 60 min (20 to 300 min) for VAN. Mean antibiotic concentrations in CSF ± the standard deviation (median) were 5.5 ± 3.9 (4.4), 3.7 ± 2.3 (3.2), and 1.6 ± 0.8 (1.7) μg/ml for CTX, dCTX, and VAN, respectively. The CBTs were 1:16, 1:8, 1:4, 1:2, and <1:2 for one, four, seven, five, and two patients, respectively. The median CBT was 1:4. Individual results for the decreases in the initial inoculum at dilutions 1:2, 1:4, 1:8, 1:16, and 1:32 are shown in Table 2. The mean decrease (± standard deviation) was 3.4 ± 0.5 $\log_{10}$ CFU/ml with the 1:2 dilution. With dilutions 1:4, 1:8, 1:16, and 1:32, bacterial killing of 1 $\log_{10}$ CFU/ml or more was observed with 17 CSF samples, 12 CSF samples, 6 CSF samples, and 1 CSF sample, respectively.

The need for accurate methods to predict bacteriologic and clinical outcome in pneumococcal meningitis has become increasingly acute. High doses of CTX have been proposed for the treatment of meningitis due to pneumococcal strains with decreased susceptibilities to penicillin (21). However, clinical failures have been reported despite high doses, e.g., when CTX or ceftriaxone MICs were ≥0.5 μg/ml (12). We have developed an ex vivo method for clinical CSF specimens to measure the activities of the combination of high doses of CTX and VAN against the most common penicillin-resistant pneumococcal strain found in French children. With high doses of CTX, the median level of CTX in CSF was 4.4 μg/ml. By comparison to results of previous studies using classical doses of CTX (160 to 200 mg/kg/day) (1, 20), increased doses do not appear to increase concentrations of CTX in CSF. In experimental meningitis, successful outcome correlates with a peak concentration of antibiotic in CSF 10 times greater than the MBC for the infecting organism (19). CSF CTX levels obtained in our study were not sufficient to obtain consistently in all patients such a concentration against an intermediately cephalosporin-resis-

TABLE 2. Bactericidal activity in diluted CSF from 19 children treated with the CTX-plus-VAN combination against an intermediately cephalosporin-resistant pneumococcal strain

Patient	Change in the initial inoculum ($\log_{10}$ CFU/ml) after 6 h of incubation at dilution:				
	1:2	1:4	1:8	1:16	1:32
1401	−3.9	−3.7	+0.1	Regrowth[a]	Regrowth
2701	−2.5	−2.3	−1.2	+0.5	Regrowth
3002	−3.4	−3.1	−3.0	+0.9	Regrowth
4201	−3.1	−2.8	−2.7	+0.5	Regrowth
2401	−4.0	−3.7	−3.0	−2.4	Regrowth
3801	−4.0	−3.2	−2.5	−1.7	Regrowth
3803	−4.0	−3.2	−2.7	−0.5	Regrowth
1601	−3.4	−3.4	−3.0	−2.0	Regrowth
3001	−3.1	−3.1	−2.2	−1.0	Regrowth
3601	−3.2	−3.1	−2.2	−1.0	Regrowth
4101	−4.0	−2.0	+1.0	Regrowth	Regrowth
4001	−3.1	−2.8	+0.2	Regrowth	Regrowth
3401	−4.1	−3.5	−3.4	Regrowth	Regrowth
1602	−3.0	Regrowth	Regrowth	Regrowth	Regrowth
1603	−3.1	−3.0	−1.5	Regrowth	Regrowth
1604	−3.9	−3.9	−3.8	−3.3	−2.4
1402	−2.6	−1.7	Regrowth	Regrowth	Regrowth
1605	−4.0	−4.0	Regrowth	Regrowth	Regrowth
2801	−3.5	Regrowth	Regrowth	Regrowth	Regrowth

[a] Regrowth is ≥2 $\log_{10}$ CFU/ml.

tant strain. Therefore, use of a synergistic antibiotic appears justified (8, 9). CSF VAN concentrations were expected to be higher than those observed in our study. In a recent report, using the same VAN doses to treat nine children with bacterial meningitis, Klugman et al. (14) found levels in CSF of 3.3 ± 1.1 μg/ml (mean ± standard deviation), i.e., significantly higher than in our study ($P < 0.001$, Student's t test). These differences may be due to the CSF sampling time after the end of the VAN infusion (mean of 80 min in our study and 170 min in Klugman et al.'s study), the assay method (high-performance liquid chromatography versus polarization of fluorescence in Klugman et al.'s study), and the pathogens responsible for meningitis (only one *Streptococcus pneumoniae* isolate among the 10 patients in the study by Klugman et al. and 18 isolates among the 19 patients in our study).

Data from the rabbit meningitis model have shown that a peak CBT of ≥1:8 is necessary to achieve maximal bacterial killing (16). Sixty-three percent of our patients had a CBT of ≥1:4 or more, and only one-quarter had the optimal titer (≥1:8). No clear correlation between CSF antibiotic concentrations and CBT values was observed with our patients. Bacterial killing of 0.5 to 3.3 $\log_{10}$ CFU/ml was observed with the 1:16 dilution in samples from seven patients, and killing of 2.4 $\log_{10}$ CFU/ml was observed with the 1:32 dilution in a sample from one patient. At these dilutions, antibiotic concentrations were below the MIC for the test strain. Our data therefore suggest at least an additive interaction between the drugs used. In vitro synergistic interactions between CTX and its major active metabolite dCTX have been reported from experiments using numerous species, including *S. pneumoniae* (13). Moreover, a synergistic effect on penicillin-resistant pneumococcal strains of the combination of extended-spectrum β-lactams (ceftriaxone and cefpirome) and VAN has been reported from in vitro experiments (2, 9) and from experimental treatment of meningitis (8). In the same way, Klugman et al. (14) reported that CSF of patients treated with ceftriaxone plus VAN showed greater bactericidal activity against two cephalosporin-resistant pneumococcal strains than CSF of patients treated with ceftriaxone alone. Three of our 19 patients had pneumococcal strains for which the CTX MIC was 1 μg/ml. In all three cases, CSF CTX levels were below 10 times the MBC for the infective strain; the CBT of these patients was 1:4, but no delayed sterilization of CSF was observed.

We thank M. Rosembaum and M. Bouvier d'Yvoire for helpful discussions and the pediatricians and microbiologists participating in the multicenter study.

REFERENCES

1. **Asmar, B. I., M. C. Thirumoorthi, J. A. Buckley, D. M. Kobos, and A. S. Dajani.** 1985. Cefotaxime diffusion into cerebrospinal fluid of children with meningitis. Antimicrob. Agents Chemother. **28:**138–140.
2. **Bajaksouzian, S., M. A. Visalli, M. R. Jacobs, and P. C. Appelbaum.** 1996. Antipneumococcal activities of cefpirome and cefotaxime, alone and in combination with vancomycin and teicoplanin, determined by checkerboard and time-kill methods. Antimicrob. Agents Chemother. **40:**1973–1976.
3. **Bauchet, J., E. Pussard, and J. J. Garaud.** 1987. Determination of vancomycin in serum and tissues by column liquid chromatography using solid-phase chromatography. J. Chromatogr. **414:**472–476.
4. **Bingen, E., N. Lambert-Zechovsky, P. Mariani-Kurkdjian, 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. **Committee on Infectious Diseases.** 1988. Treatment of bacterial meningitis. Pediatrics **81:**904–907.
6. **Demotes-Mainard, F. M., G. A. Vincon, C. H. Jarry, and H. C. Albin.** 1984. Micromethod for the determination of cefotaxime and desacetylcefotaxime in plasma and urine by high-performance liquid chromatography. J. Chromatogr. **336:**438–445.
7. **Doit, C., E. Denamur, B. Picard, P. Geslin, J. Elion, and E. Bingen.** 1996. Mechanisms of spread of penicillin resistance in *Streptococcus pneumoniae* strains causing meningitis in children in France. J. Infect. Dis. **174:**520–528.
8. **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.
9. **Friedland, I. R., M. Paris, S. Shelton, and G. H. McCracken, Jr.** 1993. Time-kill studies of antibiotic combinations against penicillin-resistant and -susceptible *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **34:**231–237.
10. **Geslin, P.** 1996. Centre National de Référence des Pneumocoques: rapport d'activité année 1994–1995. Centre National de Référence de Pneumocoques.
11. **Gilchrist, J. E., J. E. Campbell, C. B. Donnely, J. T. Peller, and J. M. Delaney.** 1973. Spiral plate method for bacterial determination. Appl. Environ. Microbiol. **25:**244–252.
12. **John, C. C.** 1994. Treatment failure with the use of a third-generation cephalosporin for penicillin-resistant pneumococcal meningitis: case report and review. Clin. Infect. Dis. **18:**188–193.
13. **Jones, R. N.** 1995. Cefotaxime and desacetylcefotaxime antimicrobial interactions. The clinical relevance of enhanced activity: a review. Diagn. Microbiol. Infect. Dis. **22:**19–33.
14. **Klugman, K. P., I. 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.
15. **Lebel, M. H., and G. H. McCracken, Jr.** 1989. Delayed cerebrospinal fluid sterilization and adverse outcome of bacterial meningitis in infants and children. Pediatrics **83:**161–167.
16. **McCracken, G. H., Jr., and Y. Sakata.** 1985. Antimicrobial therapy of experimental meningitis caused by *Streptococcus pneumoniae* strains with different susceptibilities to penicillin. Antimicrob. Agents Chemother. **27:**141–145.
17. **National Committee for Clinical Laboratory Standards.** 1994. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3, vol. 14, no. 16. National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. **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.
19. **Schaad, U. B., G. H. McCracken, Jr., C. A. Loock, and C. L. Thomas.** 1981. Pharmakcokinetics and bacteriologic efficacy of moxalactam, cefotaxime, cefoperazone, and rocephin in experimental bacterial meningitis. J. Infect. Dis. **143:**156–163.
20. **Trang, J. M., R. F. Jacobs, G. L. Kearns, A. L. Brown, T. G. Wells, F. L. Underwood, and R. B. Kluza.** 1985. Cefotaxime and desacetylcefotaxime pharmacokinetics in infants and children with meningitis. Antimicrob. Agents Chemother. **28:**791–795.
21. **Viladrich, P. F., C. Cabellos, R. Pallares, F. Tubau, J. Martínez-Lacasa, J. Liñares, J., and F. Gudiol.** 1996. High doses of cefotaxime in treatment of adult meningitis due to *Streptococcus pneumoniae* with decreased susceptibilities to broad-spectrum cephalosporins. Antimicrob. Agents Chemother. **40:**218–220.