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Comparison of Cefotaxime, Imipenem-Cilastatin, Ampicillin-Gentamicin, and Ampicillin-Chloramphenicol in the Treatment of Experimental *Escherichia coli* Bacteremia and Meningitis

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In a search for more effective antimicrobial therapy of neonatal *Escherichia coli* infection, newer beta-lactam antibiotics, cefotaxime and imipenem, were evaluated for their activities against a K1 *E. coli* strain in vitro and in vivo, and the results were compared with those of conventional therapeutic regimens for neonatal *E. coli* infection: ampicillin-gentamicin and ampicillin-chloramphenicol. Measured by MICs and MBCs, cefotaxime and imipenem were 8- to 512-fold more active in vitro than the older agents. For in vivo studies, the following daily doses were used: 50 mg/kg for each of imipenem and cilastatin; 100 mg/kg for each of cefotaxime, ampicillin, and chloramphenicol; and 10 mg/kg for gentamicin. At these doses, the mean bactericidal titers in blood and cerebrospinal fluid were significantly greater with newer agents than with ampicillin-gentamicin and ampicillin-chloramphenicol. However, at the doses used, the newer agents were not more effective in vivo than the older agents. This was shown by the similarities in clearance of bacteria from blood and cerebrospinal fluid, incidences of meningitis in bacteremic animals, and mortality rates. Thus, although these two newer antibiotics are more active in vitro and produce greater bactericidal titers in vivo, they do not appear to be superior to conventional regimens for treatment of neonatal *E. coli* bacteremia and meningitis.

Combinations of ampicillin and an aminoglycoside, or ampicillin and chloramphenicol represent conventional regimens for treatment of gram-negative meningitis in newborns. Because of continuing poor results with these regimens, examinations have been made of the potentials of newer beta-lactam agents with expanded in vitro activity against the gram-negative bacilli and with greater capacity for penetration of the central nervous system (1, 14, 15).

Escherichia coli is the most common gram-negative organism causing meningitis in the neonatal period (16). The present study, therefore, was performed to compare activities of two newer antibiotics (cefotaxime, imipenem) with those of conventional therapeutic regimens (ampicillin-gentamicin, ampicillin-chloramphenicol) in vitro and in vivo against *E. coli*.

MATERIALS AND METHODS

Organism. A serum-resistant *E. coli* K1 strain (C5) isolated from the cerebrospinal fluid (CSF) of a newborn infant with meningitis was used for in vitro and in vivo studies.

In vitro studies. The MICs and MBCs were measured in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) by the standard macrobroth dilution method (17). Antimicrobial agents tested included cefotaxime sodium (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), imipenem (Merck Sharp & Dohme, Rahway, N.J.), ampicillin trihydrate (Bristol Laboratories, Syracuse, N.Y.), chloramphenicol (Parke-Davis, Morris Plain, N.J.), and gentamicin sulfate (Schering Corp., Kenilworth, N.J.). Late logarithmic-phase cultures of the C5 strain containing approximately 5×10^5 CFU/ml were prepared as described previously (5–7). The MIC was defined as the lowest antibiotic concentration in which there was no visual turbidity. From each tube, 10 μl was transferred to a quadrant of sheep blood agar and incubated at 37°C for 24 h to determine the MBC, which was defined as the lowest antibiotic concentration which resulted in ≥99.9% killing of the original inoculum. The MICs and MBCs of ampicillin-chloramphenicol and ampicillin-gentamicin combinations were measured in Mueller-Hinton broth. Twofold dilutions of each antibiotic were used in a checkerboard fashion. MIC and MBC endpoints were read as described above for each antibiotic alone and in various combinations. The results were expressed as the fractional bactericidal concentration (FBC) index, which was calculated by the following equation (2, 12): (MBC of drug A in combination with drug B/MBC of drug A) + (MBC of drug B in combination with drug A/MBC of drug B). When the FBC index is less than 0.5, the combination is considered to be synergistic; when the index is greater than 2, the combination is considered to be antagonistic; when the index is from 0.5 to 2, the combination is considered to be indifferent.

In vivo studies. Outbred, pathogen-free, pregnant Sprague-Dawley rats with timed conception were purchased from Charles River Breeding Laboratories, Wilmington, Mass., and gave birth in our vivarium 5 to 7 days after arrival. The methodologies to induce *E. coli* bacteremia and meningitis in 5-day-old rats and to monitor the responses of such infections to treatment with antibiotics or saline in terms of bacterial clearance in blood and CSF have been described previously (5–7).

A total of 83 newborn rats from six litters were used. At 5 days of age, all members of each litter were inoculated intraperitoneally with 100 CFU of strain C5. Eighteen hours after inoculation and daily thereafter for 4 days, blood and CSF specimens were obtained for quantitative cultures. Immediately after the first withdrawal of blood and CSF, the pups in each litter were randomly distributed into five treatment groups: group 1, cefotaxime (50 mg/kg), twice

TABLE 1. Bactericidal titers in serum and CSF at 1 to 2 h after subcutaneous administration of antimicrobial agents

Therapeutic regimen (no. of specimens)	Bactericidal titers in:			
	Serum		CSF	
	Mean[a]	Range	Mean[a]	Range
Imipenem-cilastatin (12)	≥181 ± 7.8[b]	1:8->1:512	8 ± 3.7[b]	1:4–1:16
Cefotaxime (10)	≥891 ± 3.0[b]	1:256->1:512	25 ± 3.4[b]	1:16–1:64
Ampicillin-gentamicin (10)	10.5 ± 3.4	1:4–1:32	≤4	<1:4–1:4
Ampicillin-chloramphenicol (12)	13.4 ± 4.0	1:4–1:64	≤4	<1:4–1:4

[a] Geometric mean ± standard error of the mean of reciprocals of bactericidal titers.
[b] Significantly greater ($P < 0.001$) than ampicillin-gentamicin and ampicillin-chloramphenicol.

daily (9 a.m. and 7 p.m.); group 2, imipenem-cilastatin (25 mg/kg each), twice daily; group 3, ampicillin (50 mg/kg)-gentamicin (5 mg/kg), twice daily; group 4, ampicillin (50 mg/kg)-chloramphenicol (50 mg/kg), twice daily; or group 5, saline (0.05 ml), twice daily. Imipenem was administered in combination with cilastatin, an enzyme inhibitor of the renal dipeptidase, because clinical trials will utilize this combination. All drugs were administered subcutaneously. The dose of each antibiotic was chosen based on results of previous studies to produce serum concentrations at 1 to 2 h within the therapeutic range (6, 7; K. S. Kim and T. Aronson, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, abstr. no. 278, 1983; K. S. Kim and B. F. Anthony, Clin. Res. 32:110A, 1984). Therapeutic efficacy was determined by comparing (i) rates of bacterial clearance from blood and CSF, (ii) incidence of meningitis developing during therapy, and (iii) mortality rates among the five groups.

All but one animal had one blood and CSF sample drawn at 1 to 2 h after subcutaneous administration of antibiotics on therapy day 3 for determination of bactericidal titers. Bactericidal titers were determined by a microtiter technique (13) with a serial twofold dilution of serum or CSF in Mueller-Hinton broth and an inoculum of strain C5 of approximately 2×10^5 CFU/ml. The bactericidal titers in serum or CSF were defined as the highest dilution of serum or CSF which resulted in ≥99.9% killing.

Statistical methods. The chi-square test with Yates' correction or Student's t test was used where indicated (3). P values ≤0.05 were considered significant.

RESULTS

In vitro findings. The MIC and MBC of strain C5 were, respectively, 0.06 and 0.12 μg/ml for cefotaxime, 0.5 and 0.5 μg/ml for imipenem, 2 and 4 μg/ml for ampicillin, 2 and 2 μg/ml for gentamicin, and 4 and 64 μg/ml for chloramphenicol. The checkerboard combinations of ampicillin-gentamicin and ampicillin-chloramphenicol revealed that the MBC of ampicillin changed no more than twofold in the presence of gentamicin, whereas the MBC of ampicillin increased two- to eightfold in the presence of 2 to 32 μg of chloramphenicol per ml. The FBC index was 0.86 for a combination of ampicillin and gentamicin, suggesting an additive effect, and 2.1 for a combination of ampicillin and chloramphenicol, suggesting an antagonistic effect.

In vivo findings. (i) Bactericidal titers. Table 1 summarizes bactericidal titers (geometric mean ± standard error of the mean) in serum and CSF obtained 1 to 2 h after subcutaneous injection of the various antimicrobial agents. Serum and CSF specimens from untreated healthy and infected animals did not kill strain C5. The mean bactericidal titers of cefotaxime and imipenem in serum and CSF were significantly greater than those of ampicillin-gentamicin and ampicillin-chloramphenicol ($P < 0.001$). Of note, 5 of 10 animals (50%) receiving ampicillin-gentamicin and 5 of 12 animals (42%) receiving ampicillin-chloramphenicol had bactericidal titers in CSF of <1:4.

(ii) In vivo efficacy. Just before therapy (18 h after inoculation), all 83 rats were bacteremic and 31 (37%) had positive CSF cultures. The incidence of meningitis and numbers of bacteria in blood and CSF were not significantly different in the animals assigned to the different treatment groups (Table 2).

Table 2 compares the overall mortality and bacterial clearance from blood and CSF among the five treatment groups. All control animals receiving saline died within 2 days of therapy. Otherwise, the mortality rates among the four antibiotic therapy groups were not significantly different.

The bacterial clearance from blood of the five treatment groups was compared by determining bacterial counts of animals with positive blood cultures at 1, 2, and 3 days of completed therapy (Table 2). In every group, the number of animals available for these observations decreased with time. Bacterial counts in blood increased in animals receiving saline, while treatment with one of the four antibiotic regimens enhanced bacterial clearance from the blood of surviving animals. Only one animal each in the imipenem-cilastatin (1 of 12 or 8%) and ampicillin-chloramphenicol (1 of 13 or 8%) groups had positive blood cultures ($<4 \times 10^2$ CFU/ml) after 3 days of therapy.

Few animals with positive CSF cultures before therapy were alive at the end of the first treatment day and available for repeated cisternal puncture. Among the survivors, the four antibiotic regimens were equally effective in eradication of the *E. coli* from the CSF (Table 2).

Among the 52 bacteremic rats without meningitis before therapy, 37 survived beyond 1 day of therapy and were available for studying the development of meningitis. All survivors (2 of 2) in the control group and 2 of 11 (18%) animals treated with ampicillin-chloramphenicol developed meningitis during 4 days of therapy. In contrast, none of the 24 animals developed meningitis in the cefotaxime, imipenem-cilastatin, and ampicillin-gentamicin groups.

DISCUSSION

Cefotaxime and imipenem (*N*-formimidoyl thienamycin) are two of the newer beta-lactam antibiotics reported to have broad antimicrobial activity in vitro against both aerobic and anaerobic gram-positive and gram-negative microorganisms (4, 8, 9, 11) and may deserve further evaluation of their efficacies against infections caused by a variety of gram-positive and gram-negative organisms.

Results of this study showed that cefotaxime and

TABLE 2. Comparison of mortality and bacterial clearance in blood and CSF among the five groups of animals treated with imipenem-cilastatin, cefotaxime, ampicillin-gentamicin, ampicillin-chloramphenicol, or saline

Therapy regimen (daily dose)	No. of animals	Treatment day	No. of deaths	Overall mortality (%)	Blood			CSF		
					Bacterial counts[a]	No. of cultures $<4 \times 10^2$ CFU/ml	Incidence of bacteremia (%)[b]	Bacterial counts[a]	No. of cultures $<4 \times 10^3$ CFU/ml	Incidence of meningitis (%)[b]
Imipenem-cilastatin (50 mg/kg each)	19	0			5.43 ± 0.85 (19)		19/19 (100)	5.93 ± 1.39 (5)	3	8/8 (100)
		1	6		2.60 (1)	3	4/13 (31)	0	1	1/3 (33)
		2	1		0	1	1/12 (8)	0	1	1/3 (33)
		3	0	7 (37)	0	1	1/12 (8)	0	0	0/3
Cefotaxime (100 mg/kg)	19	0			5.10 ± 0.98 (19)		19/19 (100)	6.36 ± 1.66 (3)	2	5/5 (100)
		1	9		2.60 (1)	1	1/10 (10)	0	1	1/2 (50)
		2	0		0	0	0/10	0	0	0/2
		3	0	9 (47)	0	0	0/10	0	0	0/2
Ampicillin (100 mg/kg)-gentamicin (10 mg/kg)	15	0			5.23 ± 0.86 (15)		15/15 (100)	5.43 ± 1.21 (6)	1	7/7 (100)
		1	3		0	3	3/12 (25)	0	1	1/5 (20)
		2	1		0	1	1/11 (9)	0	0	0/3
		3	1	5 (33)	0	0	0/10	0	0	0/3
Ampicillin-chloramphenicol (100 mg/kg each)	18	0			5.03 ± 1.74 (18)		18/18 (100)	6.00 ± 1.60 (4)	2	6/6 (100)
		1	4		3.07 (1)	3	4/14 (28)	0	1	1/3 (33)
		2	1		0	3	3/13 (23)	0	0	0/2
		3	0	5 (28)	0	1	1/13 (8)	0	0	0/2
Saline	12	0			5.00 ± 1.58 (12)		12/12 (100)	5.03 ± 1.24 (4)	1	5/5 (100)
		1	4		8.16 ± 1.98 (8)		8/8 (100)	NA[c]		
		2	8		ND[d]			NA		
		3	0	12 (100)	ND			NA		

[a] Expressed as mean ± standard deviation (log 10 CFU/ml). Numbers in parentheses indicate the number of animals that had blood cultures that contained $>4 \times 10^2$ CFU/ml or CSF cultures that contained $>4 \times 10^3$ CFU/ml. These animals were used in determining bacterial counts.

[b] Numbers indicate the number of animals positive for culture after completion of treatment day/the number of animals positive for culture before therapy and available for subsequent examination of blood or CSF.

[c] NA, No animals with positive CSF cultures before therapy surviving for subsequent examination of CSF.

[d] ND, No animals with positive blood cultures before therapy surviving for subsequent examination of blood.

imipenem are very active in vitro against a K1 *E. coli* strain. Their MICs and MBCs were 8- to 512-fold lower than those of ampicillin, gentamicin, and chloramphenicol. Furthermore, the mean bactericidal titers in blood and CSF were significantly greater with cefotaxime and imipenem-cilastatin than with ampicillin-gentamicin and ampicillin-chloramphenicol. However, at the doses used, the outcome of *E. coli* infections treated with the newer agents was not significantly different from that attained with more conventional regimens. This was shown by similar rates of bacterial clearance from the blood and CSF, similar incidence of meningitis developing in bacteremic animals, and similar mortality among the four antibiotic therapy groups. As has been shown previously (6), the combination of ampicillin and chloramphenicol is clearly beneficial in vivo against a K1 *E. coli* strain despite its antagonistic effect in vitro.

These in vivo findings of relatively limited efficacy with newer agents were somewhat unexpected because of their excellent in vitro activities. The reasons for this in vitro and in vivo discrepancy are not completely understood. One possibility may be that some neonatal gram-negative infections run a rapid and fulminant course, and antimicrobial chemotherapy alone may not be able to reverse the profound alterations noted in these fulminant infections. In the present study, all but one death in animals treated with newer antimicrobial agents occurred within 24 h of therapy, suggesting the fulminant nature of *E. coli* infection in this model. A recent multicenter cooperative study of neonatal gram-negative meningitis also showed that the outcome was comparable between neonates treated with moxalactam, a potent new beta-lactam antibiotic, or a combination of ampicillin and aminoglycoside (10), suggesting that there may be a limit to the efficacy of antibiotic therapy alone. It is therefore prudent to suggest that future studies include other forms of therapy (e.g., immunotherapy; K. S. Kim, D. Green, A. Cross, B. Kaufman, W. Zollinger, J. Sadoff, and M. Apicella. Pediatr. Res. **18**:279A, 1984) for better management of neonatal gram-negative infections.

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