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Efficacy of BMY-28142 in Experimental Bacteremia and Meningitis Caused by *Escherichia coli* and Group B Streptococci

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We evaluated the activity of BMY-28142 against a K1 *E. coli* strain and a type III group B streptococcal strain in vitro and in vivo and compared the results with those of cefotaxime and penicillin G, respectively. In vitro, the MICs and MBCs of BMY-28142 were close to those of cefotaxime (≤2-fold difference) for *E. coli* and fourfold less than those of penicillin G for group B streptococci. In vivo studies with an experimental bacteremia and meningitis model in newborn rats revealed that the mean penetration of BMY-28142 into the cerebrospinal fluid was 15% that of concomitant levels in serum and that significantly greater bactericidal titers were achieved in blood and cerebrospinal fluid for both test organisms with BMY-28142 than with cefotaxime and penicillin G. However, the overall efficacy of BMY-28142 was similar to that of cefotaxime for the *E. coli* infection and that of penicillin G for the group B streptococcal infection. This was shown by similar rates of bacterial clearance from blood and cerebrospinal fluid and similar mortality rates. These findings indicate that the activity of BMY-28142 is bactericidal in vitro and in vivo against *E. coli* and group B streptococci, suggesting that this agent may be a suitable alternative for the therapy of *E. coli* and group B streptococcal bacteremia and meningitis.

Because of the significant morbidity and mortality associated with neonatal bacterial infections, particularly due to *E. coli* and group B streptococci (GBS), many new beta-lactam antibiotics with expanded in vitro spectra have generated considerable enthusiasm for studies of their activities in vivo.

BMY-28142 is a novel cephalosporin with potent in vitro activity against most aerobic and facultatively anaerobic gram-positive and gram-negative organisms (1, 4, 5).

This study was performed to evaluate the activity of BMY-28142 in vitro and in vivo against *Escherichia coli* and GBS, the two most common bacterial pathogens in newborn infants (14). The results were compared with those of cefotaxime for *E. coli* and those of penicillin G for GBS.

MATERIALS AND METHODS

Organisms. A serum-resistant *E. coli* K1 strain (C5) and a type III GBS strain (K79) were used in this study. Both organisms were isolated from the cerebrospinal fluid (CSF) of newborn infants with meningitis (6–8).

In vitro studies. MICs and MBCs were measured in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) by the standard macrobroth dilution method (17). The antimicrobial agents tested were BMY-28142 (Bristol Laboratories, Syracuse, N.Y.) for both *E. coli* and GBS, cefotaxime sodium (Hoechst-Roussel Pharmaceuticals, Somerville, N.J.) for *E. coli*, and potassium penicillin G (Bristol) for GBS. Antimicrobial agent solutions were diluted serially twofold from 4 to 0.008 μg/ml in Mueller-Hinton broth. An inoculum of approximately 2×10^5 CFU/ml of the *E. coli* or GBS strain was prepared as described previously from late-logarithmic-phase cultures (6, 7). Equal volumes (0.5 ml) of antibiotic and bacterial dilutions were mixed and incubated at 37°C for 24 h. The MIC was defined as the lowest antibiotic concentration exhibiting 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 concentration of antibiotics resulting in ≥99.9% killing of the original inoculum.

In vivo studies. Outbred, pathogen-free, Sprague-Dawley pregnant rats with timed conception were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass., and gave birth in our vivarium 5 to 7 days after arrival. Bacteremia and meningitis due to *E. coli* and GBS were induced in 5-day-old rats as described previously (7–9; K. S. Kim, Chemotherapy, in press).

A total of 86 animals from eight litters were used for infection with *E. coli* and 66 animals from six litters for infection with GBS. At 5 days of age, all members of each litter were inoculated subcutaneously with 10^2 CFU of the *E. coli* strain C5 or with 10^5 CFU of the GBS strain K79 in a volume of 0.05 ml. As shown in our previous experiments (7–9; Kim, in press), this inoculum of *E. coli* or GBS produces nonlethal bacteremia (with or without meningitis) in 100% of the animals within 18 h of inoculation. At 18 h after inoculation and daily thereafter for 4 days, 0.1 ml of blood and 0.01 ml of CSF were obtained as previously described for quantitative cultures (7–9; Kim, in press). Briefly, blood was diluted 10-fold and CSF 100-fold in brain heart infusion broth and further diluted 10-fold (up to 10^{-4}) in sterile normal saline; 0.025 ml of each dilution was spread on sheep blood agar. The lowest dilution was also incubated overnight at 37°C, and a loopful was streaked on blood agar to detect bacteremia of $<4 \times 10^2$ CFU/ml and CSF counts of $<4 \times 10^3$ CFU/ml. Since blood and CSF samples were obtained at 8 a.m., more than 12 h after the last dose of antibiotics, no attempt was made to inactivate any residual antibiotics in blood and CSF except for serial dilutions. Immediately after the first blood and CSF specimens were obtained, each litter was randomly divided for *E. coli* studies into three treatment groups to receive (i) BMY-28142, 50

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TABLE 1. Comparison of bactericidal titers in blood and CSF after subcutaneous administration of antibiotics

Organism	Antibiotic (no. of specimens)	Dose (mg/kg)	Bactericidal titer[a] (mean ± SEM) in:		
			Serum		CSF
			1 to 2 h	7 to 8 h	1 to 2 h
E. coli	BMY-28142 (17)	50	>512	11.7 ± 2.8[b]	≥643 ± 13.4[c]
	Cefotaxime (17)	50	>512	≤2.5 ± 1.3	34 ± 21.7
GBS	BMY-28142 (15)	50	>512	20.1 ± 1.6[d]	>512[e]
	Penicillin G (20)	50	≥245 ± 9.3	≤3.4 ± 1.6	≤8.4 ± 4.8
	Penicillin G (8)	100	>512	4.5 ± 2.0	24 ± 6.9

[a] Expressed as geometric mean ± SEM of reciprocals of bactericidal titers.
[b] Significantly greater ($P < 0.01$) than cefotaxime.
[c] Significantly greater ($P < 0.001$) than cefotaxime.
[d] Significantly greater ($P < 0.01$) than penicillin G (100 mg/kg).
[e] Significantly greater ($P < 0.001$) than penicillin G (100 mg/kg).

mg/kg twice daily (at 9 a.m. and 7 p.m.), (ii) cefotaxime, 50 mg/kg twice daily, or (iii) saline, 0.05 ml twice daily for 4 days. Similarly, for GBS studies, infected animals were randomly divided into three groups to receive (i) BMY-28142, 50 mg/kg twice daily, (ii) penicillin G, 50 or 100 mg/kg twice daily, or (iii) saline, 0.05 ml twice daily for 4 days. All drugs were administered subcutaneously. Animals that died during therapy were removed, and postmortem blood specimens were obtained by cardiac puncture. Blood cultures obtained from animals dying more than 1 h before daily cultures were excluded from quantitative bacteriologic analysis. CSF specimens could not be obtained from dead animals. Therapeutic efficacy was determined by comparing the rates of bacterial clearance from blood and CSF, the incidence of meningitis developing during therapy, and the mortality rates among the treatment groups.

Both blood (0.1 ml) and CSF (0.02 ml) samples were collected from most animals at 1 to 2 h after subcutaneous administration of antibiotics on therapy day 3, and blood alone was collected at 7 to 8 h for the determination of bactericidal titers by a microtiter technique (10). Serial twofold dilutions of serum or CSF in Mueller-Hinton broth and an inoculum of approximately 10^5 CFU/ml of infecting organisms were used. The serum or CSF bactericidal titers were defined as the highest dilution which resulted in ≥99.9% killing.

Serum and CSF samples obtained from animals receiving BMY-28142 were also assayed for drug levels. Serum and CSF levels of BMY-28142 were measured by an agar-disk diffusion method using *E. coli* ATCC 10536 (12). The serum and CSF pharmacokinetics of penicillin G and cefotaxime in this model have been previously reported from our laboratory (9; Kim, in press). At the doses used in the present study, the mean concentrations in serum (± standard deviation [SD]) at 1 to 2 and 6 to 7 h after subcutaneous administration were, respectively, 21.5 ± 13.9 and 2.2 ± 1.2 μg/ml for cefotaxime, 14.0 ± 8.6 and <0.1 μg/ml for penicillin G (50 mg/kg), and 20.2 ± 10.6 and 0.14 ± 0.03 μg/ml for pencillin G (100 mg/kg). The penetration into CSF was 16.2% for cefotaxime and 5.3% for penicillin G.

Statistical methods. The chi-square test with Yates' correction or Student's *t* test was used where indicated (2).

RESULTS

In vitro findings. The MIC and MBC of BMY-28142 for the *E. coli* strain (C5) were both 0.03 μg/ml, and those of cefotaxime were both 0.06 μg/ml. The MIC and MBC of BMY-28142 for the GBS strain (K79) were both 0.015 μg/ml, and those of penicillin G were both 0.06 μg/ml.

Pharmacology and bactericidal titers in infant rats. The bactericidal titers (geometric mean ± standard error of the mean) in the serum and CSF of infected animals are summarized in Table 1. Serum and CSF from untreated healthy and infected animals did not kill the strain C5 or K79. The mean reciprocals of the serum bactericidal titers in animals receiving BMY-28142 were >10 against both *E. coli* and GBS for a 7- to 8-h period after administration. These levels were significantly greater ($P < 0.01$) than those achieved with cefotaxime for *E. coli* or with penicillin G (50 or 100 mg/kg per dose) for GBS. Similarly, the mean CSF bactericidal titers achieved with BMY-28142 at 1 to 2 h after administration were significantly greater ($P < 0.001$) than those achieved with cefotaxime (for *E. coli*) and with penicillin G (for GBS).

The mean concentrations in serum (±SD) of BMY-28142 were 38.0 ± 14.2 and 5.5 ± 3.3 μg/ml at 1 to 2 and 7 to 8 h, respectively, after subcutaneous administration of 50 mg/kg, whereas the mean CSF level (±SD) at 1 to 2 h was 5.4 ± 1.3 μg/ml. The penetration of BMY-28142 into CSF was 15% that of concomitant levels into serum.

In vivo efficacy. *E. coli* bacteremia was present in 86 (100%) animals, and meningitis (positive CSF culture) was present in 24 (23%) animals 18 h after inoculation and before therapy (Table 2). At this time, the prevalence of meningitis and the bacterial counts in blood and CSF were not significantly different among the three treatment groups (Table 2).

The overall mortality and bacterial clearance from blood and CSF among the three treatment groups are compared in Table 2. All control animals receiving saline died within 2 days of therapy, whereas 11 (17%) of 64 animals receiving BMY-28142 or cefotaxime died. Among the latter groups, the mortality with BMY-28142 therapy was not significantly different from that with cefotaxime.

The bacterial clearance from blood of the three treatment groups was compared by determining bacterial counts in animals with positive blood cultures at 1, 2, and 3 days of completed therapy (Table 2). The number of animals available for these observations decreased with the deaths in every group. Both BMY-28142 and cefotaxime completely eradicated the *E. coli* from blood afer 2 days of therapy, whereas bacterial counts in blood increased in animals receiving saline.

After 1 day of treatment, 10 of the 24 animals with *E. coli* meningitis (positive CSF culture) before therapy were alive for repeated cisternal puncture. Again, both BMY-28142 and

TABLE 2. Comparison of mortality and bacterial clearance in blood and CSF

Organism	Antibiotics (dose, mg/kg b.i.d.)[a]	No. of animals	Treatment day	Deaths	Overall mortality (%)	Bacterial counts (log 10 CFU/ml)[b] in:	
						Blood	CSF
E. coli	BMY-28142 (50)	32	0			5.21 ± 1.15 (32/32)	3.06 ± 0.18 (7/7)
			1	5		1.30 ± 0.00 (3/27)	0 (0/5)
			2	0		0 (0/27)	0 (0/5)
			3	0	5 (16)	0 (0/27)	0 (0/5)
	Cefotaxime (50)	32	0			5.43 ± 0.89 (32/32)	3.85 ± 1.53 (10/10)
			1	6		1.30 ± 0.00 (3/26)	0 (0/5)
			2	0		0 (0/26)	0 (0/5)
			3	0	6 (25)	0 (0/26)	0 (0/5)
	Saline	22	0			4.82 ± 1.28 (22/22)	4.02 ± 1.82 (7/7)
			1	17		6.99 ± 1.91 (5/5)	NA[c]
			2	5		NA	NA
			3	0	22 (100)	NA	NA
GBS	BMY-28142 (50)	24	0			5.42 ± 1.87 (24/24)	5.59 ± 1.45 (13/13)
			1	5		1.97 ± 0.88 (2/19)[d]	4.44 (1/8)
			2	0		1.30 ± 0.00 (1/19)	0 (0/8)
			3	0	5 (21)	0 (0/19)	0 (0/8)
	Penicillin G (50)	24	0			5.55 ± 1.48 (24/24)	4.10 ± 2.50 (14/14)
			1	4		2.51 ± 1.30 (9/20)[d]	7.39 (1/10)
			2	0		0 (0/20)	4 (1/10)
			3	0	4 (17)	0 (0/20)	0 (0/10)
	Penicillin G (100)	10	0			5.33 ± 1.63 (10/10)	5.39 ± 1.98 (3/3)
			1	1		1.70 ± 0.80 (4/9)	1.80 (1/2)
			2	0		0 (0/9)	4.95 (1/2)
			3	1	2 (20)	0 (0/8)	0 (0/1)
	Saline	8	0			4.00 ± 0.98 (8/8)	4.48 ± 2.90 (3/3)
			1	4		5.92 ± 2.41 (4/4)	NA
			2	3		5.74 ± 0.00 (1/1)	NA
			3	1	8 (100)	NA	NA

[a] b.i.d., Twice daily.

[b] Values are expressed as mean ± SD. Numbers in parentheses indicate the number of animals positive for culture after completion of treatment day versus the number of animals positive for culture before therapy and available for subsequent determination of bacterial counts.

[c] NA, Not applicable (no survivors available).

[d] The incidence of bacteremia after 1 day of therapy was significantly less ($\chi^2 = 4.14$, $P < 0.05$) with BMY-28142 than with penicillin G (100 mg/kg per day).

cefotaxime were very effective in a complete eradication of the *E. coli* from the CSF of surviving animals (Table 2).

Among the 62 bacteremic animals without meningitis before therapy, 48 animals survived beyond 1 day of therapy and were available for comparisons of therapeutic efficacy to prevent the development of meningitis in bacteremic animals. All five survivors in the control group developed meningitis, whereas none of other 43 animals developed meningitis during 4 days of BMY-28142 or cefotaxime therapy.

GBS. GBS bacteremia was present in 66 (100%) animals, and meningitis was present (positive CSF culture) in 33 (50%) animals at 18 h after infection and before therapy. At this time the prevalence of meningitis and the bacterial counts in blood and CSF were not significantly different among the four treatment groups (Table 2). Overall, antimicrobial therapy significantly decreased the mortality rates (19% of 58 animals receiving antibiotics versus 100% of 8 animals receiving saline: $\chi^2 = 18.7$, $P < 0.001$). The mortality rate for animals receiving BMY-28142 was not significantly different from that for animals receiving penicillin G (50 or 100 mg/kg twice daily).

As with *E. coli*, the bacterial clearance from blood was compared by determining bacterial counts of animals with positive blood cultures at 1, 2, and 3 days of completed therapy (Table 2). As noted, the incidence of bacteremia was significantly less ($P < 0.05$) with BMY-28142 therapy than with penicillin G therapy (50 mg/kg twice daily) at the end of 1 day of treatment. However, the bacterial clearance with BMY-28142 was not significantly different from that with penicillin G (50 or 100 mg/kg twice daily). Similarly, both BMY-28142 and penicillin G were equally effective in a complete eradication of the GBS from the blood of surviving animals after 3 days of therapy (Table 2).

After 1 day of treatment, 20 of the 33 animals with GBS meningitis before therapy were available for repeated cisternal puncture. Both BMY-28142 and penicillin G completely eradicated the GBS from the CSF of surviving animals after 2 and 3 days of therapy, respectively (Table 2).

Among the 33 bacteremic animals without meningitis before therapy, 32 animals survived beyond 1 day of treatment and were available for comparisons of drug efficacy to prevent the development of meningitis in bacteremic animals. All such animals (4 of 4) in the control group developed meningitis, whereas none of the 28 bacteremic animals developed meningitis during 4 days of BMY-28142 or penicillin G therapy.

DISCUSSION

There has been remarkable progress in recent years in the development of new beta-lactam antibiotics by chemical modifications of existing compounds. BMY-28142 is such a compound, with a quaternary substitution with *N*-methylpyrrolidine of the 3-side chain of aminothiazole cephalosporin (J. Okumura, S. Aburaki, H. Kamachi, Y. Narita, T. Naito, and H. Kawaguchi, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 576, 1983). Recently, Khan et al. (5) showed that BMY-28142 was the

most active cephalosporin in vitro against the majority of aerobic and facultatively anaerobic microorganisms studied. However, the in vivo evaluation of BMY-28142 has been limited (4, 16).

The present study was therefore performed to examine the in vivo activity of BMY-28142 against *E. coli* and GBS using the newborn rat model. As shown previously (7–9; Kim, in press), *E. coli* and GBS bacteremia and meningitis induced in newborn rats have several important similarities to those infections in human infants: age dependency, hematogenous infection of the meninges without the need for adjuvants or mechanical disruption of the blood-brain barrier, and high mortality. In addition, using the techniques for serial collection of blood and CSF specimens without sacrificing animals, we were able to examine the in vivo efficacy in terms of the mortality rate and the rates of bacterial clearance from blood and CSF.

BMY-28142 was extremely active in vitro and its MIC/MBC values were close to those of cefotaxime for *E. coli* and fourfold less than those of penicillin G for GBS. In vivo, BMY-28142 produced significantly greater bactericidal titers in blood and CSF than did cefotaxime and penicillin G. It should be noted that the mean concentration of BMY-28142 in serum at 7 to 8 h after administration was still at approximately 14% of the 1- to 2-h level, suggesting a slow decay in vivo. This finding of presumed longer half-life is similar to half-lives observed with ceftriaxone and cefoperazone (3, 11, 13).

However, the overall efficacy of BMY-28142 was similar to that of cefotaxime for *E. coli* and of penicillin G for GBS. This was indicated by similar rates of mortality and of bacterial clearance from blood and CSF. These findings of similar efficacy for BMY-28142 versus cefotaxime and penicillin G are consistent with those of Tauber et al. (15), who demonstrated that drugs with peak concentrations in CSF of >100 times the MBC cannot be expected to provide more benefits than drugs with peak CSF concentrations of >10 times the MBC. In our study, the mean concentrations of BMY-28142 in CSF at 1 to 2 h after administration were 180 and 360 times the MBCs of *E. coli* and GBS, respectively, whereas the mean concentrations of cefotaxime and penicillin G (100 mg/kg per dose) in CSF were 37 and 18 times the respective MBCs of *E. coli* and GBS. Therefore, this study also confirms our previous findings (7) that peak concentrations of antibiotics in CSF ≥10 to 20 times the MBC provide a consistent bactericidal effect in CSF.

Based on our findings, BMY-28142 is a suitable alternative agent for therapy of *E. coli* and GBS bacteremia and meningitis in this model and may deserve further evaluation in human infections caused by these organisms.

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