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Pharmacokinetics and Bacteriological Efficacy of Moxalactam (LY127935), Netilmicin, and Ampicillin in Experimental Gram-Negative Enteric Bacillary Meningitis

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Moxalactam (LY127935) is a 1-oxa-β-lactam which was active in vitro against the majority of 128 strains of gram-negative enteric bacilli isolated from meningitis in neonates. Pharmacokinetics and bacteriological efficacy of LY127935 were studied in a lapin meningitis model. The average penetration of this investigational oxa-cephalosporin into cerebrospinal fluid of infected rabbits was 23% compared with 25% for netilmicin and 11% for ampicillin. The cerebrospinal fluid concentrations of LY127935 produced median bactericidal titers of 1:64 to 1:128 against five coliform organisms (two *Escherichia coli* K1 strains, *Klebsiella pneumoniae*, *Salmonella saint-paul*, and *Citrobacter diversus*) used in these experiments compared with median titers of 1:2 to 1:8 for netilmicin and 1:2 to 1:4 for ampicillin. LY127935 was statistically significantly more effective than netilmicin or ampicillin in reducing cerebrospinal fluid bacterial colony counts and in sterilizing cerebrospinal fluid of experimentally infected rabbits. These results suggest that LY127935 has theoretical advantages over netilmicin and ampicillin for therapy of gram-negative bacillary meningitis.

The mortality rate of gram-negative bacillary meningitis in newborn infants is from 15 to 30%, and sequelae are found in approximately one-third of survivors. Results from the two Neonatal Meningitis Cooperative Studies indicate that neither lumbar intrathecal nor intraventricular gentamicin administration combined with systemic therapy has a beneficial effect on outcome from coliform meningitis when compared with results with systemic antimicrobial therapy alone (8) (G. H. McCracken, Jr. et al., manuscript in preparation). Moreover, an increasing percentage of *Escherichia coli* isolated from the cerebrospinal fluid (CSF) of newborns are resistant to ampicillin, which combined with gentamicin represents the currently recommended initial therapeutic regimen for neonatal bacterial meningitis.

Moxalactam (LY127935), a new 1-oxa-β-lactam, is active in vitro against gram-negative organisms including indole-positive *Proteus* species, *Serratia*, and to a limited extent *Pseudomonas aeruginosa* (9). Pharmacokinetic and safety data in adults suggest that this agent is well tolerated and safe (R. Kammer, personal communication). There is presently very little information on clinical efficacy.

The purpose of this study was to compare the pharmacokinetics and bacteriological efficacy of LY127935 to those of netilmicin and ampicillin in the lapin meningitis model (1, 4, 7, 10-12, 16) with five strains of gram-negative bacteria isolated from the CSF of neonates with purulent meningitis.

MATERIALS AND METHODS

Susceptibility studies. In vitro susceptibilities of 128 gram-negative enteric bacilli to LY127935, netilmicin, and ampicillin were determined by an agar dilution method with Mueller-Hinton agar and an inoculum of approximately 5×10^4 colony-forming units (CFU) delivered by the Steers-Foltz replicating device in a volume of 0.001 to 0.002 ml (6, 15). The minimum inhibitory concentration (MIC) was defined as the smallest concentration of antibiotic that inhibited visible growth on agar after 16 to 18 h of incubation at 37°C. The test organisms were isolated from the CSF of newborn infants with bacterial meningitis who had been enrolled in the first and second Neonatal Meningitis Cooperative Studies. They included the following strains: 91 *E. coli* (60 K1), 9 *Enterobacter*, 8 *Salmonella*, 7 *Citrobacter diversus*, 6 *Klebsiella pneumoniae*, and 7 miscellaneous coliform organisms.

Five of these organisms were selected for use in the lapin meningitis model. They were two *E. coli* K1 strains (one ampicillin susceptible and one resistant) and one each of a *K. pneumoniae*, *C. diversus*, and *Salmonella saint-paul*. The MICs and minimum bactericidal concentrations (MBCs) for these five strains were determined in Mueller-Hinton broth by serial twofold dilutions with an inoculum of approximately 5×10^5 CFU/ml. The MBC was taken as the lowest

concentration of drug producing 99.9% bacterial killing determined on eosin-methylene blue agar by quantitative subcultures of each tube containing no visible growth (MIC).

CSF titers. Bacteriostatic and bactericidal titers in CSF against each strain causing meningitis were determined by a microtiter technique with serial twofold dilutions in Mueller-Hinton broth and an inoculum of approximately 5×10^5 CFU/ml.

Rabbit model. New Zealand White male rabbits (2 to 3 kg) were prepared by the method described by Dacey and Sande (4). Quincke spinal needles (3.5 × 25 gauge) mounted to the frame's geared introducer were advanced without trauma into the cisterna magna of the immobilized rabbits.

Fifteen to eighteen hours after intracisternal inoculation of 2×10^4 to 2×10^6 organisms, CSF was purulent and contained from 3×10^3 to 2.5×10^9 CFU/ml. For each test organism the course of meningitis was observed in four to nine untreated animals until death occurred between 18 to 64 h after inoculation. Blood cultures taken before therapy were sterile except in animals inoculated with *S. saint-paul.*

Antimicrobial therapy. Each antimicrobial agent was dissolved in 54 ml of 0.9% NaCl and administered intravenously over a 9-h period with a constant infusion pump (Holter, model 907) via a femoral venous catheter. During therapy the rabbits were kept lightly anesthetized and then sacrificed by intravenous administration of pentobarbital.

The dosage of each antibiotic was based on attaining serum concentrations that were within the therapeutic range for humans. The following dosages were used: LY127935, 25 mg/kg (loading dose) followed by 25 mg/kg per h; netilmicin, 5 mg/kg (loading dose) followed by 2 mg/kg per h; and ampicillin, 70 mg/kg (loading dose) followed by 70 mg/kg per h. Nine noninfected rabbits received either LY127935 (five animals) or netilmicin (four animals) to determine penetration of drug into CSF. Sixty-one rabbits with experimental gram-negative bacillary meningitis were treated (24 animals with LY127935, 5 animals with LY127935 plus netilmicin, 5 animals with LY127935 plus ampicillin, 17 animals with netilmicin, and 10 animals with ampicillin).

Serial blood (0.5 ml) and CSF samples (0.5 ml) were collected at 0, 3, 6, and 9 h of therapy. CSF was immediately cultured quantitatively on eosin-methylene blue agar. Serum and CSF were kept frozen at −20°C until antibiotic assays and antibacterial titers were performed (within 1 to 4 days of collection).

Antibiotic assay. Concentrations of antibiotic were measured by an agar disk diffusion microbioassay method with *E. coli* ATCC 10536 for LY127935, *Bacillus subtilis* (Difco Laboratories, 0453-36) for netilmicin, and *Sarcina lutea* ATCC 9341 for ampicillin. Serum and CSF from untreated healthy and infected rabbits did not inhibit the assay organisms. Standards and serum samples were diluted in 100% normal rabbit serum for the LY127935 assay, in 25% rabbit serum-phosphate buffer (pH 7.9) for the netilmicin assay, and in phosphate buffer (pH 6.0) for the ampicillin assay. Standards and CSF samples were diluted in phosphate buffer (pH 6.0 for LY127935 and ampicillin, pH 7.9 for netilmicin). Selection of the diluents used in each assay was based on the diffusion characteristics of the antibiotics in agar; in every assay the standards and test specimens for each drug were prepared identically.

Analysis of data. The percentage of penetration of antibiotic into the CSF was defined as CSF concentration/serum concentration × 100.

Statistical analysis on paired observations was done by Student's *t* test. Results of therapy were compared by Dunnett's test (5).

RESULTS

Susceptibility studies. The combination of ampicillin and gentamicin is currently recommended for initial therapy of neonatal meningitis. The concentrations required to inhibit 50 and 90%, respectively, of strains were 0.06 and 0.5 μg of LY127935 per ml, 0.4 and 2.5 μg of gentamicin per ml, and 2.5 and >20 μg of ampicillin per ml. The nine strains that had LY127935 MICs of >0.5 μg/ml were three *E. coli* K1, two *Pseudomonas*, two *Flavobacterium meningosepticum*, one *C. diversus*, and one *Salmonella.*

The MICs and MBCs of LY127935, netilmicin, and ampicillin of the five strains used to produce meningitis in rabbits are presented for each set of experiments (see Fig. 3 to 7).

Drug penetration into CSF. Concentrations of antibiotics in serum and CSF for the different animal experiments are presented in Table 1. The mean values for each drug were similar for all studies, although there was considerable animal-to-animal variation, as indicated by the large standard deviations.

The penetration of drug into CSF of uninfected healthy rabbits ranged from 0.9 to 2.3% (mean, 1.4%) for LY127935 and from 1.7 to 11.8% (mean, 6.1%) for netilmicin.

Figure 1 shows the LY127935 concentrations in 98 concurrent serum and CSF specimens from 34 rabbits with meningitis. The penetration of LY127935 into CSF can be estimated by the position of each point in relation to the diagonal percentage lines. The average penetration of LY127935 was 23%, while that of netilmicin was 25%. By contrast, the average penetration of ampicillin was 11%. The percentage of penetration increased over the 9-h period because of increased concentrations of drug in CSF rather than lower concentrations in serum. The mean percentages at 3 and 9 h, respectively, were 19.9 and 25.3% for LY127935 ($P < 0.001$), 20.5 and 30.6% for netilmicin ($P < 0.001$), and 10.4 and 11.2% for ampicillin ($P < 0.582$).

CSF titers. The median bacteriostatic and bacterial titers in CSF against the five pathogens in the animal experiments are shown in Table 1. The median bactericidal titers for LY127935 were 1:64 or 1:128 compared with 1:2 to 1:8 for netilmicin and 1:2 or 1:4 for ampicillin. There

TABLE 1. *Pharmacological and bacteriological data in rabbits with experimental gram-negative enteric bacterial meningitis*

Pathogen[a]	Antibiotic	No. of animals	Mean ± SD			Median CSF titers		Mean Δ $\log_{10}$ bacteria per ml in CSF ± SD[b] over 9 h
			Serum concn (μg/ml)	CSF Concn (μg/ml)	CSF/serum penetration (%)	Bacterio-static	Bactericidal	
None	LY127935	5	74.3 ± 18.3	1.0 ± 0.4	1.4 ± 0.4			
None	Netilmicin	4	13.9 ± 4.4	0.8 ± 0.3	6.1 ± 2.6			
E. coli K1 (AR)	None (controls)	9						1.48 ± 0.63
	LY127935	5	102.6 ± 22.3	20.4 ± 6.1	20.5 ± 6.5	1:128	1:64	−4.47 ± 0.46
	Netilmicin	5	10.4 ± 1.7	2.7 ± 1.0	26.1 ± 9.1	1:4	1:2	−2.73 ± 0.90
	LY127935 plus Netilmicin	5	92.5 ± 17.7 10.6 ± 1.8	19.7 ± 4.1 2.6 ± 0.6	21.6 ± 3.5 24.8 ± 5.0	1:128	1:64	−4.84 ± 1.14
E. coli K1 (AS)	None (controls)	4						0.31 ± 0.17
	LY127935	6	82.6 ± 26.4	21.6 ± 8.1	26.7 ± 8.5	1:64	1:64	−3.95 ± 0.84
	Ampicillin	6	84.2 ± 23.3	11.1 ± 4.4	12.4 ± 3.1	1:4	1:2	−1.95 ± 1.08
	LY127935 plus Ampicillin	5	113.5 ± 29.0 125.1 ± 32.9	32.1 ± 15.4 12.9 ± 4.5	27.3 ± 8.0 10.4 ± 2.2	1:128	1:128	−4.36 ± 2.09
C. diversus (AR)	None (controls)	4						1.37 ± 0.61
	LY127935	4	96.6 ± 24.0	19.5 ± 8.2	20.2 ± 6.1	1:64	1:64	−3.73 ± 1.04
	Netilmicin	4	10.3 ± 1.8	2.6 ± 0.4	26.3 ± 6.3	1:4	1:4	−2.14 ± 0.84
K. pneumoniae (AR)	None (controls)	6						0.53 ± 0.70
	LY127935	5	88.5 ± 22.0	16.3 ± 5.7	18.5 ± 4.7	1:128	1:128	−4.78 ± 0.87
	Netilmicin	4	12.0 ± 2.8	2.5 ± 0.7	21.4 ± 5.5	1:16	1:8	−2.93 ± 0.83
S. saint-paul (AS)	None (controls)	4						0.74 ± 0.67
	LY127935	4	110.5 ± 19.0	24.3 ± 11.3	21.6 ± 8.4	1:128	1:64	−5.34 ± 0.54
	Netilmicin	4	9.9 ± 1.8	2.3 ± 1.0	22.9 ± 5.8	1:8	1:8	−3.50 ± 0.76
	Ampicillin	4	118.4 ± 36.0	10.3 ± 3.4	8.9 ± 1.5	1:8	1:4	−2.70 ± 0.47

[a] AR, Ampicillin resistant; AS, ampicillin susceptible.
[b] SD, Standard deviation.

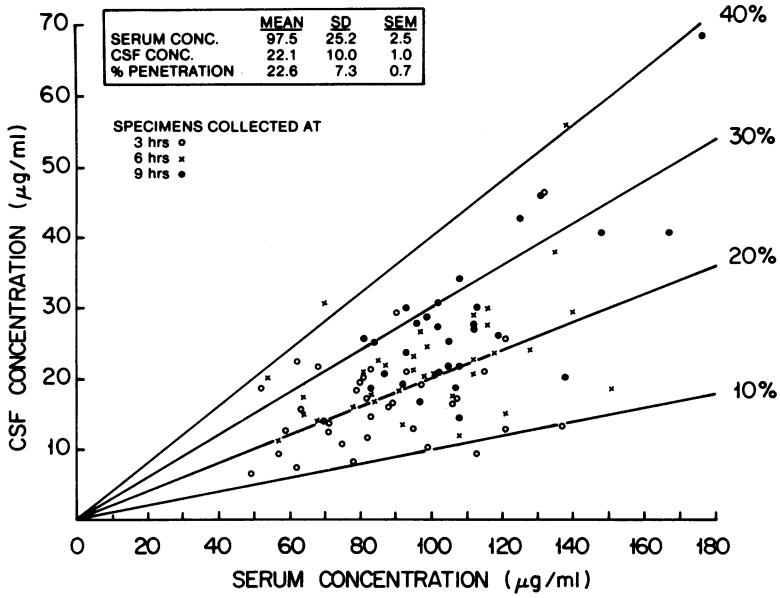

FIG. 1. *Concentrations of LY127935 in paired CSF-serum samples from 34 infected rabbits. The penetration of LY127935 into CSF can be estimated by the position of each point in relation to the diagonal percentage lines.*

was a direct correlation between concentration of LY127935 in CSF, the in vitro MBC for the test strains, and the CSF bactericidal titer (Fig. 2); the average bactericidal titer of LY127935 in CSF was 1:64.

Bacteriological efficacy. The mean changes in the bacterial colony counts in CSF ($\Delta\log_{10}$ bacteria per milliliter) after 9 h of therapy are shown for the different animal studies (Table 1 and Fig. 3 to 7). All 27 untreated, infected rabbits showed stable or increasing CSF bacterial counts ranging from 0 to 2.4 logs. These animals died 18 to 64 h after inoculation. The initial CSF bacterial counts in the 88 infected rabbits ranged from 3.5 to 9.4 $\log_{10}$ CFU/ml. The mean initial CSF counts for each pathogen were the following: 4.8 $\log_{10}$ CFU/ml for *E. coli* K1 (ampicillin resistant), 5.0 $\log_{10}$ CFU/ml for *E. coli* K1 (ampicillin susceptible), 4.5 $\log_{10}$ CFU/ml for *C. diversus*, 5.6 $\log_{10}$ CFU/ml for *K. pneumoniae*, and 7.3 $\log_{10}$ CFU/ml for *S. saint-paul*.

The change in bacterial colony counts in CSF of 61 treated rabbits and the median bactericidal titers at 3, 6, and 9 h of therapy are presented in Fig. 3 to 7. All antibiotic regimens produced bactericidal activity against the infecting pathogens, and the decrease in CSF bacterial concentrations ranged from 1.1 to 5.9 $\log_{10}$. LY127935 therapy produced a significantly ($P < 0.05$) greater decrease in CSF bacterial counts compared with those observed with netilmicin therapy in animals infected with *E. coli* K1 (ampicillin resistant), *C. diversus*, *K. pneumoniae*, and *S. saint-paul* and with those with ampicillin therapy in *E. coli* K1 (ampicillin susceptible) and *S. saint-paul* meningitis. The combination of LY127935 and netilmicin in *E. coli* K1 (ampicillia resistant) and of LY127935 and ampicillin in *E. coli* K1 (ampicillin susceptible) meningitis resulted in only slightly larger reductions in bacterial counts than observed with LY alone.

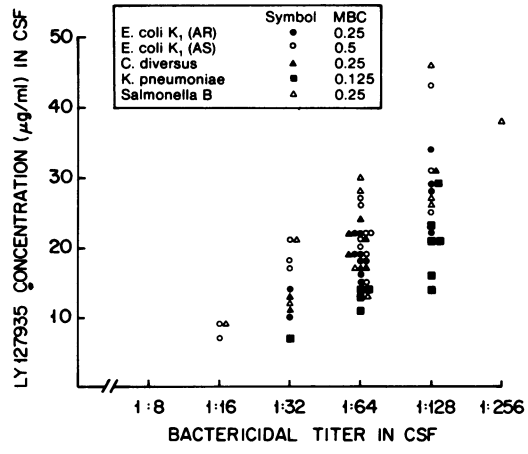

FIG. 2. *Bactericidal titers in relation to concentrations of LY127935 in 61 CSF specimens obtained from rabbits with experimental meningitis. The infecting strains and their in vitro MBCs are shown in the box.*

There was a rough correlation between the

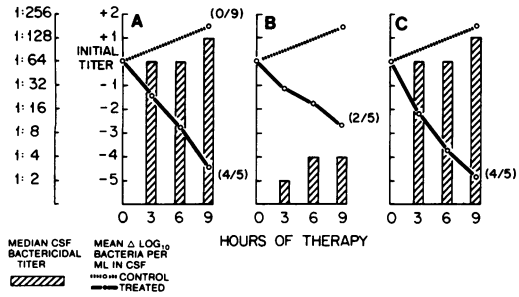

FIG. 3. *Results of antimicrobial therapy in animals with E. coli.K1 (ampicillin resistant) meningitis. The change in concentrations of bacteria in CSF is shown in relation to the CSF bactericidal titers at 3, 6, and 9 h of therapy. Ratios in parentheses represent the number of animals with sterile CSF after therapy to the total treated. (A) LY127935: MIC, 0.125 μg/ml; MBC, 0.25 μg/ml. (B) Netilmicin: MIC, 0.63 μg/ml; MBC, 1.25 μg/ml. (C) LY127935 plus netilmicin.*

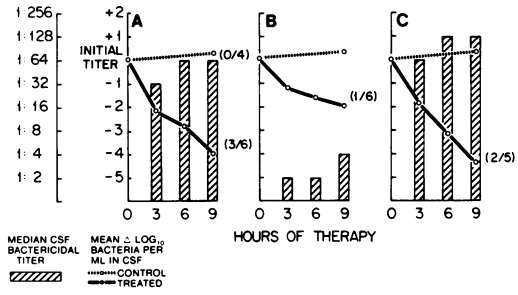

FIG. 4. *Results of antimicrobial therapy in animals with E. coli K1 (ampicillin susceptible) meningitis. See legend to Fig. 3 for further description. (A) LY127935: MIC, 0.5 μg/ml; MBC, 0.5 μg/ml. (B) Ampicillin: MIC, 2.5 μg/ml; MBC, 2.5 μg/ml. (C) LY127935 plus ampicillin.*

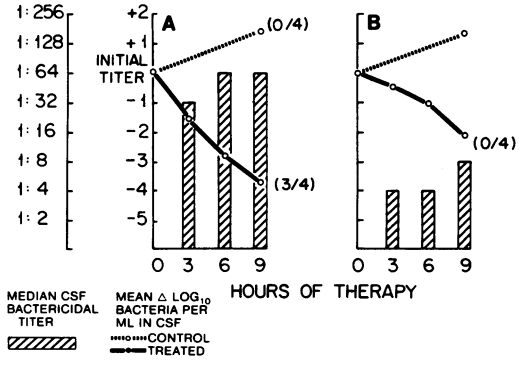

FIG. 5. *Results of antimicrobial therapy in animals with C. diversus meningitis. See legend to Fig. 3 for further description. (A) LY127935: MIC, 0.25 μg/ml; MBC, 0.25 μg/ml. (B) Netilmicin: MIC, 0.63 μg/ml; MBC, 0.63 μg/ml.*

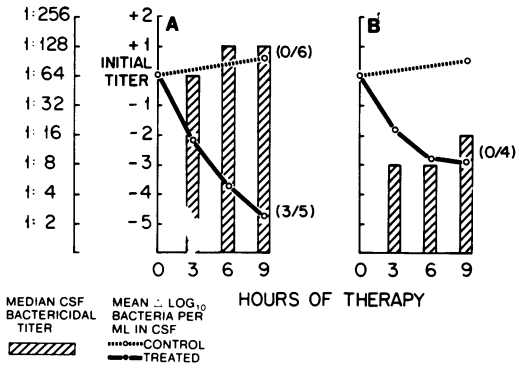

FIG. 6. *Results of antimicrobial therapy in animals with K. pneumoniae meningitis. See legend to Fig. 3 for further description. (A)LY127935: MIC, 0.125 μg/ml; MBC, 0.125 μg/ml. (B) Netilmicin: MIC, 0.32 μg/ml; MBC, 0.32 μg/ml.*

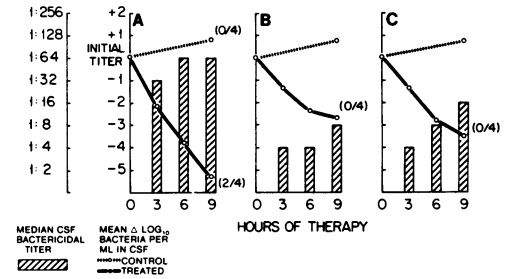

FIG. 7. *Results of antimicrobial therapy in animals with S. saint-paul meningitis. See legend to Fig. 3 for further description. (A) LY127935: MIC, 0.25 μg/ml; MBC, 0.25 μg/ml. (B) Ampicillin: MIC, 1.25 μg/ml; MBC, 2.5 μg/ml. (C) Netilmicin: MIC, 0.63 μg/ml; MBC, 1.25 μg/ml.*

change in CSF bacterial counts and the CSF bactericidal activity against the pathogen.

CSF cultures were sterile after 9 h of therapy in 15 of 24 LY127935-treated (63%), 2 of 17 netilmicin-treated (12%), and 1 of 10 ampicillin-treated (10%) animals. The rate of CSF sterilization produced by LY127935 was significantly ($P < 0.01$) greater than that by netilmicin or ampicillin.

DISCUSSION

Because of the excellent in vitro activity of LY127935 against gram-negative enteric bacilli isolated from CSF of neonates, we initiated this study of the pharmacokinetics and bacteriological efficacy of LY127935 in experimentally produced meningitis in rabbits. Netilmicin, a synthetic derivative of sisomicin, was chosen for study because, compared with gentamicin, it has greater in vivo CSF bactericidal activity in rabbits (12) and less nephrotoxicity in rats (2).

The average concentrations of LY127935 in CSF were approximately 23% of those in concurrent serum specimens. This penetration is substantially greater than has been demonstrated for the other β-lactam antibiotics (1, 11), including ampicillin, in the present study. The greatly enhanced penetration of LY127935 into CSF of infected rabbits compared with that in control animals (1.4%) may be explained in part by increased permeability through inflamed meninges and by reversible depression of the saturable choroid plexus efflux transport system (14). It is also possible that the significantly increased penetration of LY127935 and netilmicin during constant intravenous infusion over 9 h resulted from achieving concentrations in CSF that exceeded the transport maximum of the choroid plexus "pump" (13, 14).

The CSF bactericidal activity of the three drugs tested was a product of both the in vitro MBCs for the pathogens and the concentrations of drug achieved in CSF. In general, the larger bactericidal titers were associated with greater reductions in bacterial colony counts and with sterilization of CSF after 9 h of therapy. Infected animals treated with LY127935 demonstrated a significantly greater reduction in CSF bacterial concentrations during therapy compared with animals treated with netilmicin or ampicillin. Additionally, CSF cultures from 63% of LY127935-treated animals were sterile after 9 h of therapy compared to 12 and 10%, respectively, for netilmicin-treated and ampicillin-treated animals. Although we were able to show synergism by an in vitro "kill-curve" technique for LY127935 plus netilmicin and for LY127935 plus ampicillin against ampicillin-resistant and -susceptible *E. coli* strains, respectively, this effect could not be demonstrated by comparing bacterial concentrations and bactericidal activity in CSF of infected rabbits treated with combination therapy versus LY127935 alone.

These experiments in rabbits suggest that LY127935 has theoretical advantages over netilmicin and ampicillin for therapy of coliform meningitis in newborn infants. However, extrapolation of the data to meningitis in neonates must be done with care because formation and circulation of CSF differ in humans and rabbits (3). Furthermore, meningitis in humans usually occurs from hematogenous infection, and perivascular involvement is prominent. Experimental meningitis in rabbits was produced by direct instillation of bacteria into the cisterna magna, and infection usually localized to the meninges and CSF space. We are presently investigating the pharmacokinetics of LY127935 in newborn infants and, depending on the results, may undertake evaluation of the efficacy and safety of LY127935 in a third Neonatal Meningitis Cooperative Study.

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