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# Treatment of Experimental *Haemophilus influenzae* Type b Meningitis with 1-Oxa-β-Lactam (LY127935)

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1-Oxa-β-lactam (LY127935) (Shionogi 6059-S) is a new type β-lactam antibiotic having a broad spectrum of antibacterial activity. It is highly active against ampicillin-resistant strains of *Haemophilus influenzae* exhibiting minimal inhibitory concentrations as low as 0.06 μg/ml. This compound also has the ability to penetrate into the cerebrospinal fluid of both normal and infected infant rats and attains approximately 10% of the corresponding blood levels. LY127935 was evaluated for its ability to treat ampicillin-resistant *H. influenzae* meningitis in an established experimental model using infant rats. Rats with ampicillin-resistant *H. influenzae* meningitis were treated subcutaneously three times daily for 2 days with various dose levels of LY127935. When given in doses as low as 10 mg/kg, LY127935 sterilized the blood and cerebrospinal fluid in all rats examined at 1 and 5 days posttreatment. In contrast, ampicillin was not effective at this dose in eliminating *H. influenzae* from the blood and cerebrospinal fluid of infected rats. LY127935 was effective against experimental ampicillin-resistant *H. influenzae* meningitis in the dosages employed.

Bacterial meningitis is a life-threatening disease which afflicts approximately 40,000 young individuals each year in the United States (D. H. Smith, *in* International Conference on the Application of Vaccines Against Viral, Rickettsial, and Bacterial Diseases of Man, Pan Am. Health Organ., 1971, 226, p. 353). The majority of reported cases are in children under 5 years of age (6, 21, 22). *Haemophilus influenzae* type b is the causative organism in approximately 50% of these cases (4, 11). Bacterial meningitis occurs less frequently in adults, with *Neisseria meningitidis* and *Streptococcus pneumoniae* being the bacterial species primarily responsible for the disease (3). However, any patient having received a penetrating wound to the head or having undergone a neurosurgical procedure is a potential candidate for meningitis, especially that caused by gram-negative bacteria (7).

In the past, the treatment of choice for *H. influenzae* meningitis has been the parenteral administration of ampicillin. However, the occurrence of increasing numbers of ampicillin-resistant strains of *H. influenzae* cultured from patients with meningitis has produced a serious problem in the management of this disease (5, 8, 23). The present drug of choice for treating meningeal infections involving ampicillin-resistant *H. influenzae* is chloramphenicol (16). Because of the potential toxicological side effects of chloramphenicol therapy, the use of this antibiotic is less than desirable.

An ideal antibiotic to treat such infections would be one with minimal toxicity, which can be easily administered and which penetrates into the cerebrospinal fluid (CSF) at therapeutic concentrations. The purpose of this study was to evaluate the efficacy of such a compound against ampicillin-resistant *H. influenzae* in a previously established infant rat bacterial meningitis model (12, 13, 19). This compound, 1-oxa-β-lactam (LY127935; Shionogi 6059-S), is a new type β-lactam antibiotic with the structure shown in Fig. 1. In addition, it has excellent broad-spectrum activity, is particularly effective against *H. influenzae* (T. Yoshida, M. Narisada, S. Matsuura, W. Nagata, and S. Kuwahara, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 151, 1978), and is relatively nontoxic (S. Matsuura, T. Yoshida, K. Sugeno, Y. Harada, M. Harada, and S. Kuwahara, 18th ICAAC, abstr. no. 152). The present communication describes the penetration of LY127935 into the CSF and its efficacy against experimental ampicillin-resistant *H. influenzae* meningitis in infant rats.

## MATERIALS AND METHODS

**Animals.** Sprague-Dawley [Hap: (SD)BR] lactating rats, having litters with 8 to 12 4-day-old pups, were obtained from Harlan Industries, Indianapolis, Ind. The animals were housed under standard conditions with food and water being available to the dams ad libitum. After inoculation of the pups, both pups and dams were housed in flexible film isolators. Treated and untreated animals were housed separately.

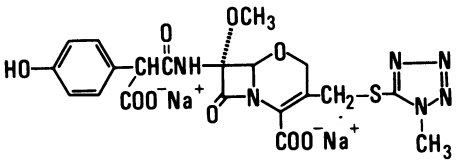

FIG. 1. *Chemical structure of LY127935 (Shionogi 6059-S).*

**Antimicrobial agents.** Ampicillin (sodium salt) lot C7M67 (Bristol Laboratories, Syracuse, N.Y.) and LY127935 (disodium salt) (6059-S, supplied by Shionogi and Company, Ltd., Osaka, Japan) were used.

**Bacteria and preparation of inoculum.** An ampicillin-susceptible strain of *H. influenzae* type b (no. M112J6) was obtained from Indiana University Hospital, Indianapolis. This organism had been isolated from the CSF of a child with meningitis and was originally cultured on chocolate agar. The ampicillin-resistant strain of *H. influenzae* type b (no. R272), also a clinical isolate, was obtained from The Childrens' Hospital Medical Center, Boston, Mass. Passages were made through infant Sprague-Dawley rats six times for the ampicillin-susceptible strain and three times for the ampicillin-resistant strain. After the last passage, the organisms were reisolated from the CSF, grown to log phase, centrifuged, suspended in skim milk broth, and stored in liquid nitrogen for subsequent use in the meningitis model.

The inoculum was prepared by adding 0.5 ml of the frozen stock culture into 50 ml of brain heart infusion broth containing 1% Difco Supplement C. The culture was incubated at 37°C with 5% $CO_2$ being bubbled continuously through the broth. The culture was harvested during log phase by centrifugation at 12,000 × *g* for 20 min at 4°C. At this point the culture contained $10^9$ colony-forming units per ml, which corresponded to a Nephalos reading of 90 on a Coleman model 9 nepho-colorimeter. The cell pellets were suspended in a total volume of 5 ml of cold 0.01 M phosphate buffered saline (pH 7.2), containing 0.1% gelatin. This cell suspension contained $10^{10}$ colony-forming units per ml and was used immediately to inoculate the infant rats. Plate counts of the inoculum were performed on chocolate agar plates containing 1% Difco Supplement C. The plates were incubated at 37°C for 18 h in an 8% $CO_2$ atmosphere.

**Experimental bacterial meningitis.** Experimental *H. influenzae* meningitis was established in infant rats according to the method of Moxon et al. (12, 13). Briefly, the pups were inoculated intranasally with 0.01 ml of a freshly harvested *H. influenzae* culture which contained $10^{10}$ colony-forming units per ml. This was accomplished by using a Hamilton microsyringe fitted with a blunt-ended 27-gauge needle. A heart puncture was performed on each animal 24 h postinoculation. Approximately 0.1 ml of blood was obtained and streaked on supplemented chocolate agar plates and incubated as previously described. Animals with positive blood cultures for *H. influenzae* were always found to have a concomitant meningitis. These animals were retained for therapeutic studies. Treated animals and untreated infected control animals were tested at 1 and 5 days after termination of antibiotic therapy for the presence of *H. influenzae* in the blood and CSF. Several 10-fold dilutions of each blood and CSF sample were plated on supplemented chocolate agar plates for quantitation of *H. influenzae* organisms. These samples were also tested for the presence of antibiotic.

**MIC.** To determine minimal inhibitory concentration (MIC), supplemented brain heart infusion agar containing serial twofold dilutions of antibiotic was prepared. Inocula obtained from a 1:100 dilution of an early-log-phase culture were spotted onto the surface of the agar plates with a 5-μl micropipette (applied sample contained $10^6$ colony-forming units per ml). The inoculated plates were incubated for 18 h at 37°C in a $CO_2$ atmosphere.

**Antibiotic assay method.** Standard curves for each antibiotic were obtained using an agar microwell technique. Seeded agar plates were poured, and small plugs of agar (2 mm in diameter) were removed by aspiration. The resulting microwells were of sufficient size to contain 2 μl of the appropriately diluted antibiotic. Since no significant differences were found between standards prepared in normal pooled rat serum or CSF and normal saline, both blood and CSF zone sizes were read from standard curves of antibiotics diluted in normal saline. The assay organisms for ampicillin and LY127935 were *Micrococcus luteus* (ATCC 9341) and *Escherichia coli* (ATCC 10536), respectively.

**Antibiotic concentration in blood and CSF.** Normal or infected infant rats were injected subcutaneously with antibiotic. At various time intervals after administration of the drug, blood and CSF samples were obtained from 5 to 10 animals, and 2 μl of each sample was placed in microwells of the seeded assay plates. Blood was drawn by cardiac puncture. CSF was obtained by inserting a 27-gauge needle through the posterior alantooccipital membrane into the cisterna magna. By using a slight amount of suction with a 0.5-ml syringe and carefully withdrawing the needle to minimize capillary damage, 5 to 10 μl of CSF was obtained. Blood in the CSF sample of less than 1% can be assessed visually (1). Therefore, a CSF sample demonstrating visible blood contamination was discarded. As additional controls, solutions containing known antibiotic concentrations were run simultaneously with each experiment to assess assay plate variation.

**Antibiotic therapy.** Compounds were administered subcutaneously to infant rats with meningitis at various dose levels three times daily (at 4-h intervals) for 1 or 2 days as shown in Table 2 of the Results.

## RESULTS

Antibiotic concentrations in blood and CSF were determined at 15, 30, and 60 min postadministration (Table 1). When given at a dose of 100 mg/kg, LY127935 was shown to penetrate into the CSF of both normal animals and animals with inflamed meninges. In each case, the concentration of drug in the CSF was approximately 10% of the concentration in blood of the same animal. Ampicillin also penetrated into the CSF of infected animals, attaining 15 to 20% of

the corresponding blood concentration at the time periods depicted. In contrast, the ampicillin CSF levels in normal rats were ≤4% of the concentration in blood. Although the observed CSF concentrations of ampicillin in infected animals were sufficiently above the MIC for the susceptible strain of *H. influenzae* studied (0.1 μg/ml), these CSF concentrations did not exceed the MIC for the ampicillin-resistant *H. influenzae* strain studied (>64 μg/ml).

LY127935 had an MIC of 0.06 μg/ml for the ampicillin-resistant strain as determined by the agar dilution technique described above. The peak concentration of LY127935 attained in the CSF of the infected animals at this dose was approximately 150 times the MIC. From these data it appeared that LY127935 might have shown therapeutic efficacy in the treatment of this ampicillin-resistant *H. influenzae* meningitis even when administered at a dose lower than 100 mg/kg.

Initially, infected animals were treated subcutaneously with 50 mg/kg three times daily for 2 days. When blood and CSF samples were tested for the presence of *H. influenzae* organisms 1 day after the termination of therapy, all samples from seven infant rats were found to be free of *H. influenzae* (Table 2). At 5 days post-treatment, blood and CSF samples from an additional 10 animals were cultured. No *H. influenzae* organisms were detected in any of these samples. Since a 100% cure rate was achieved with this treatment regimen, the treatment time was decreased to 1 day, thus reducing the total

TABLE 1. *Antibiotic concentrations in blood and CSF from normal and infected infant rats*

| Antibiotic tested[a] | Animal | Sample | Concn (μg/ml)[b] | | |
|---|---|---|---|---|---|
| | | | 15 min[c] | 30 min | 60 min |
| LY127935 | Normal | Blood | 57.0 ± 3.0 | 67.0 ± 3.0 | 43.0 ± 3.0 |
| | | CSF | 4.4 ± 2.0 | 3.8 ± 0.7 | 3.9 ± 0.6 |
| | Infected | Blood | 80.0 ± 6.0 | 103.0 ± 16.0 | 95.0 ± 18.0 |
| | | CSF | 5.6 ± 2.0 | 9.0 ± 3.0 | 3.3 ± 0.7 |
| Ampicillin | Normal | Blood | 71.0 ± 6.0 | 58.0 ± 8.0 | 48.0 ± 3.0 |
| | | CSF | 1.0 ± 0.4 | 2.2 ± 0.2 | 1.7 ± 0.2 |
| | Infected | Blood | 130.0 ± 21.0 | 109.0 ± 11.0 | 100.0 ± 14.0 |
| | | CSF | 22.0 ± 6.0 | 18.0 ± 3.2 | 15.0 ± 7.0 |

[a] Antibiotic administered subcutaneously at 100 mg/kg.
[b] Mean ± standard error of 5 to 10 animals per time period.
[c] Time post-administration of antibiotic.

TABLE 2. *Treatment of ampicillin-resistant H. influenzae meningitis in infant rats*

| Antibiotic | Treatment[a] | Days post-treatment | Sample | Animals cured/total | Avg CFU/ml[b] |
|---|---|---|---|---|---|
| LY127935 | 50 mg/kg t.i.d. (2 days) | 1 | Blood | 7/7 | 0 |
| | | | CSF | 7/7 | 0 |
| | | 5 | Blood | 10/10 | 0 |
| | | | CSF | 10/10 | 0 |
| LY127935 | 50 mg/kg t.i.d. (1 day) | 1 | Blood | 7/8 | $5.0 \times 10^3$ |
| | | | CSF | 7/7 | 0 |
| | | 5 | Blood | 6/8 | $1.5 \times 10^5$ |
| | | | CSF | 6/8 | $2.5 \times 10^6$ |
| LY127935 | 10 mg/kg t.i.d. (2 days) | 1 | Blood | 9/9 | 0 |
| | | | CSF | 9/9 | 0 |
| | | 5 | Blood | 9/9 | 0 |
| | | | CSF | 9/9 | 0 |
| Ampicillin | 10 mg/kg t.i.d. (2 days) | 1 | Blood | 1/5 | $2.0 \times 10^4$ |
| | | | CSF | 1/5 | $1.0 \times 10^6$ |
| | | 5 | Blood | 0/3 | $4.8 \times 10^5$ |
| | | | CSF | 0/3 | $1.1 \times 10^6$ |
| Untreated | | 1 | Blood | 0/10 | $2.0 \times 10^7$ |
| | | | CSF | 0/10 | $1.0 \times 10^8$ |
| | | 5 | Blood | 0/10 | $2.0 \times 10^4$ |
| | | | CSF | 0/10 | $2.0 \times 10^5$ |

[a] Administered subcutaneously. t.i.d., Three times daily.
[b] CFU, Colony-forming units.

dose administered by one half. This treatment regimen initially demonstrated clearance of the organisms from the CSF. However, one animal did display a positive, but significantly reduced number of organisms on blood culture. By 5 days posttreatment, positive blood and CSF cultures were observed in two of eight additional animals tested. Therefore, the initial 2-day treatment schedule was reinstituted, but the individual doses were decreased to 10 mg/kg three times daily in the subsequent experiment. As shown in Table 2, no *H. influenzae* organisms were cultured from either blood or CSF at 1 or 5 days posttreatment. In contrast, all untreated control animals displayed positive blood and CSF *H. influenzae* cultures on the corresponding days.

When ampicillin was given subcutaneously at 10 mg/kg three times daily for 2 days, only one of five rats showed negative blood and CSF cultures at 1 day posttreatment. However, at 5 days posttreatment, all the blood and CSF samples from an additional three rats tested contained *H. influenzae* organisms equal to or greater than the number of organisms exhibited in the untreated control animals. It was noted that neither antibiotic could be detected in any of the 1- or 5-day posttreatment samples.

## DISCUSSION

An increasing incidence in the numbers of ampicillin-resistant *H. influenzae* isolated from patients with bacterial meningitis (5, 8) suggests that ampicillin may no longer be appropriate as the drug of choice in this infection. Whereas chloramphenicol is often selected for treatment of meningitis due to ampicillin-resistant *H. influenzae*, its potential toxicity presents a major drawback. Currently there is no single antibiotic or combination of antibiotics available to treat ampicillin-resistant *H. influenzae* meningitis that offers the combined characteristics of uncomplicated administration, ability to enter the CSF in therapeutic concentrations, and minimal toxicity. The present communication describes a new type β-lactam broad-spectrum antibiotic, LY127935, which has met the above criteria in an experimental animal model and was therapeutically effective against *H. influenzae* meningitis in infant rats.

The effectiveness of LY127935 in treating experimental meningitis undoubtedly is due, in part, to its ability to penetrate into the CSF. Results from this study indicate that LY127935 is able to achieve therapeutic concentrations in the CSF of infant rats in the absence of inflammation. Furthermore, when given to infected infant rats at 10 mg/kg three times daily for only 2 days, a dose which we estimate to be equivalent to 2 g per day for a 70-kg person, the blood and CSF samples cultured 5 days after the termination of treatment showed no *H. influenzae* organisms.

Another factor influencing the treatment success of LY127935 is its activity against *H. influenzae*. The MIC of this antibiotic for the ampicillin-resistant *H. influenzae* strain used in this study is 0.06 μg/ml. Results from the present investigation indicate that LY127935 attained concentrations of sufficient magnitude above the MIC to completely eradicate the organisms from the infected animals. In contrast, an equivalent dose of ampicillin was not able to attain therapeutic concentrations or effectively treat the experimental meningitis caused by the same strain of ampicillin-resistant *H. influenzae* (MIC > 64).

## ADDENDUM

Compound LY127935 is described in this issue as an oxa-β-lactam. In past tissues it was called 1-oxacephalosporin. The name cephem was proposed in 1962 (J. Am. Chem. Soc. 84:3401) as a generic designation for the fused β-lactam dihydrothiazine ring system found in the cephalosporins. This system has been generally accepted. The oxa-β-lactam compound LY127935 has a β-lactam dihydrooxazine ring system and thus cannot qualify as a cephem or cephalosporin by definition. To avoid confusion, the nonspecific term oxa-β-lactam is used, pending approval of a generic name.

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