Pharmacokinetics and Bacteriological Efficacy of N-Formimidoyl Thienamycin in Experimental Escherichia coli Meningitis

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The pharmacokinetics and bacteriological efficacy of N-formimidoyl thienamycin were determined in rabbits infected with Escherichia coli K1. After a single intravenous dose of 25 mg/kg, a peak N-formimidoyl thienamycin concentration in cerebrospinal fluid (CSF) of 2.5 μg/ml was attained at 45 min. The penetration into CSF was calculated to be 31%. In animals that received continuous intravenous infusions of the drug for 9 h, the mean CSF concentration was 8.3 μg/ml, and the CSF bactericidal titers against the E. coli K1 strain were from 1:16 to 1:32. This infusion produced a reduction in the numbers of E. coli in the CSF of 4 log₁₀ colony-forming units per ml. N-Formimidoyl thienamycin might prove to be useful for therapy of meningitis caused by E. coli and other susceptible bacteria.

N-Formimidoyl thienamycin is a stable derivative of thienamycin, a novel beta-lactam antibiotic with broad antimicrobial activity. The in vitro activity of this compound encompasses both aerobic and anaerobic gram-positive and gram-negative microorganisms, including most strains of coliform bacilli, Pseudomonas aeruginosa, Bacteroides fragilis, Staphylococcus aureus, and S. epidermidis (3, 4, 6, 8). N-Formimidoyl thienamycin is also active in vitro against enterococci and Listeria monocytogenes (2). The in vitro activity against pathogens that frequently cause neonatal meningitis suggests that N-formimidoyl thienamycin might be useful for therapy of this condition. The purpose of this investigation was to determine the penetration of this compound into cerebral spinal fluid (CSF) and its bacteriological efficacy in experimental Escherichia coli K1 meningitis of rabbits.

MATERIALS AND METHODS

Antibiotic. N-Formimidoyl thienamycin (MK0787) was supplied by the Merck Institute. Solutions of the crystalline monohydrate of N-formimidoyl thienamycin in phosphate buffer (pH 7.0) were freshly prepared for each experiment.

Rabbit model. New Zealand white male rabbits (2 to 3 kg) were used for pharmacokinetic and bacteriological efficacy studies. The animals were prepared by the methods of Dacey and Sande (1) and Schaad et al. (3). Rabbits were anesthetized with intravenous pentobarbital sodium. At 15 to 18 h after intracerebral inoculation of 2 × 10⁶ to 2 × 10⁶ colony-forming units (CFU) of an E. coli K1 strain (78-023; minimal inhibitory concentration [MIC] = 0.07 μg/ml; minimal bactericidal concentration [MBC] = 10 μg/ml of Mueller-Hinton broth [BBL Microbiology Systems]) per ml spinal fluid was obtained for a base-line bacterial count before N-formimidoyl thienamycin therapy was given.

Antimicrobial therapy. (I) Single-dose therapy. A 25-mg/kg dose of N-formimidoyl thienamycin was administered intravenously over 5 min. The rabbits were kept lightly anesthetized during therapy and were sacrificed at the termination of the experiment by intravenous administration of pentobarbital. Serial blood and CSF samples were collected periodically for 6 h after the dose. CSF was cultured quantitatively on eosin-methylene blue agar. Serum and CSF were processed on the same day of collection and held at 5°C for determination of concentrations and bactericidal titers.

(ii) Continuous infusion. A loading dose (25 mg/kg) of N-formimidoyl thienamycin was given, followed by a continuous intravenous infusion of 25 mg/kg per h for 9 h with a constant-infusion pump (Holtor model 907). Serial blood and CSF samples were collected at 0, 3, 6, and 9 h of therapy and processed as described above. CSF titers. CSF bactericidal titers against the infecting E. coli K1 strain were determined by a microtiter technique with serial twofold dilutions in Mueller-Hinton broth and an inoculum of approximately 5 × 10⁶ CFU/ml.

Microbiological assay. Concentrations of N-formimidoyl thienamycin were measured by the microbioassay method of Simon and Yin (7). Standard curves were constructed from dilutions of the reference powder and prepared the same way as the serum and CSF specimens. B. subtilis (MB32 SDR, Glaxo, Inc.) was used as the test organism. The lowest concentration of N-formimidoyl thienamycin detected by this bioassay was 0.1 μg/ml, and the error in the assay was ±8%.

Pharmacokinetic determinations. The concentrations of N-formimidoyl thienamycin in CSF and serum measured after single-dose administration were fitted
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FIG. 2. Bacteriological efficacy of N-formimidoyl thienamycin in experimental E. coli K1 meningitis. The bactericidal titers were determined against the infecting E. coli strain (MIC ≤ 0.07 µg/ml; MBC = 10 µg/ml). The bacterial counts are expressed in log_{10} CFU per milliliter.

FIG. 1. Serum and CSF concentrations of N-formimidoyl thienamycin after a 25-mg/kg dose given intravenously to rabbits with (or without) experimental E. coli K1 meningitis. Half-life (T1/2) values are in hours and AUC values are in microgram hours per milliliter.

to a regression line by the method of least mean squares. The half-life of this agent in CSF or serum was calculated by dividing ln 2 by the slope of the line. The area-under-the-concentration versus time curve (AUC) for CSF and serum was obtained by successive trapezoidal approximation from time = 0 to time = ∞.

RESULTS

Pharmacokinetics. A mean peak N-formimidoyl thienamycin concentration of 77.2 µg/ml was observed at the completion of the 5-min 25-mg/kg infusion (Fig. 1). The serum half-life was 1.5 h, and the AUC value was 18.2 µg.h/ml. The CSF concentrations of N-formimidoyl thienamycin in animals without meningeval inflammation were 0.1 µg/ml or less at 15, 30, and 45 min after the infusion. By contrast, the CSF levels in animals with E. coli meningitis were approximately 2.0 µg/ml during the first 45 min after the dose and declined thereafter in a curve similar to that in serum. The half-life in CSF was 1.4 h, and the AUC value was 5.7 µg.h/ml. The ratio of CSF AUC to serum AUC was 0.31.

In the 9-h continuous-infusion studies, serum concentrations at 3, 6, and 9 h were comparable and ranged from 19.0 to 66 µg/ml (mean ± 1 standard error of the mean, 40.9 ± 3.5 µg/ml). CSF concentrations were from 4.7 to 14.8 µg/ml (mean ± 1 standard error of the mean, 8.3 ± 0.5 µg/ml) in these experimentally infected animals. The CSF penetration, defined as the mean CSF concentration/serum concentration × 100, was 20.3%.

Bacteriological efficacy. We determined the effect of a single dose and a 9-h continuous infusion of N-formimidoyl thienamycin on the CSF bacterial counts of animals with experimental E. coli K1 meningitis (Fig. 2). In untreated animals, the bacterial count in CSF increased 2 log_{10} CFU/ml, and no cultures were sterile in 9 h. A single 25-mg/kg dose reduced the E. coli
but there was no further change in the CSF count at 4 and 6 h. The largest concentration of N-formimidoyl thienamycin measured was 2.5 \( \mu g/ml \) at 45 min, and the median CSF bactericidal titers against the infecting organism were 1:4 and 1:2 at 1 and 2 h after the dose, respectively. There was no measurable bactericidal activity at 4 and 6 h. CSF cultures in two of five animals were sterile after a single dose of N-formimidoyl thienamycin.

In the 9-h continuous-infusion studies, the CSF E. coli counts declined 4 \( \log_{10} \) CFU/ml, and the CSF cultures of four of six rabbits were sterile at 9 h. The concentrations of N-formimidoyl thienamycin in CSF were from 4.7 to 14.8 \( \mu g/ml \) (mean 8.3 \( \mu g/ml \)), and the bactericidal titers against the E. coli were 1:16 at 3 h and 1:32 at 6 and 9 h.

**DISCUSSION**

The concentrations of N-formimidoyl thienamycin in the CSF of uninfected rabbits were 0.1 \( \mu g/ml \) or less, whereas those in E. coli K1-infected animals were approximately 2.0 \( \mu g/ml \) for 45 min after a single 25-mg/kg dose and remained greater than 0.1 \( \mu g/ml \) for the 6-h study period. Based on the ratio of CSF AUC to serum AUC \((x100)\), the penetration of N-formimidoyl thienamycin into CSF was estimated at 31%. In the 9-h continuous-infusion studies of infected animals, the CSF penetration, determined from the ratio of CSF to serum concentration \((x100)\), was 20%. These results indicate that this compound diffuses well into the CSF of animals with purulent meningitis. In addition, the concentrations present after continuous infusion produced a bactericidal titer against the infecting E. coli K1 strain of from 1:16 to 1:32 and reduced the bacterial count by 4 \( \log_{10} \) CFU/ml in 9 h. Cultures of CSF at the completion of the studies were sterile in four of six animals, whereas those in six untreated animals had a median count of 10^6 CFU/ml.

The MBC for E. coli K1 was large compared with the MIC when Mueller-Hinton broth \((\text{MIC} \leq 0.07 \mu g/ml; \text{MBC} = 10 \mu g/ml)\), but not in dextrose phosphate broth \((\text{MIC} = 1.25 \mu g/ml; \text{MBC} = 2.5 \mu g/ml)\). The influence of the medium on N-formimidoyl thienamycin susceptibilities has been previously reported by Eliopoulos and Moellering (2). Our data from the meningitis model suggest that the infecting E. coli K1 strain was not clinically tolerant because a large reduction in the CSF bacterial count occurred, despite the fact that only 20% of the CSF samples contained concentrations of N-formimidoyl thienamycin that exceeded the MBC (determined in Mueller-Hinton broth) of the pathogens.

Based on the results from these experimentally infected rabbits, N-formimidoyl thienamycin might be useful for therapy of meningitis caused by E. coli and other susceptible bacteria. Unpublished data from our laboratory showed that of 63 gram-negative enteric bacillary strains from CSF cultures of neonates (from the Neonatal Meningitis Cooperative Studies), all were inhibited by 0.6 \( \mu g/ml \), and all of 30 group B streptococcal strains from CSF were inhibited by 0.02 \( \mu g/ml \) of N-formimidoyl thienamycin per ml. Because these microorganisms are the principal pathogens of neonatal meningitis, it is possible that N-formimidoyl thienamycin will have a role in the treatment of this condition.

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**LITERATURE CITED**