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Penetration of Trovafloxacin into Cerebrospinal Fluid in Humans following Intravenous Infusion of Alatrofloxacin

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A single-dose study was conducted to determine concentrations of trovafloxacin (CP-99,219) achieved in the cerebrospinal fluid (CSF) relative to those in the serum of healthy subjects after intravenous infusion of alatrofloxacin (CP-116,517), the alanyl-alanyl prodrug of trovafloxacin. Twelve healthy subjects were administered single doses of alatrofloxacin at a trovafloxacin equivalent of 300 mg as an intravenous infusion over 1.0 h. CSF samples were taken by lumbar puncture at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 24 h after the start of the infusion; each subject was sampled at only one time point. Serum samples were taken from each subject at the time of CSF collection. A mean concentration of 5.8 μg of trovafloxacin per ml was present in serum 1.0 h after the start of the infusion. CSF/serum ratios ranged from 0.14 to 0.33 in the postdistribution phase (5 to 24 h postinfusion), with a mean ratio of 0.25. The most common adverse events were dizziness, nausea, and rash and were mild or moderate in intensity. The potency of trovafloxacin against susceptible organisms, coupled with its rapid penetration of CSF following the intravenous administration of alatrofloxacin, suggests that it may be useful in the treatment of bacterial meningitis in humans.

Alatrofloxacin is an L-Ala–L-Ala prodrug which is rapidly hydrolyzed to trovafloxacin, a fluoroquinolone of the fluoronaphthyridine class. Currently available quinolones such as ciprofloxacin, norfloxacin, and ofloxacin show a broad spectrum of antibacterial activity, especially against gram-negative organisms. However, these derivatives can be inadequate against some gram-positive organisms (11). A new fluoroquinolone antimicrobial agent with improved activity against gram-positive organisms would constitute a significant advance over the available quinolones. Trovafloxacin appears to demonstrate the desired potency against gram-positive organisms (4) while maintaining excellent activity against gram-negative organisms (1). In addition, trovafloxacin also has potent activity against anaerobic organisms (7).

Trovafloxacin cannot be administered intravenously because of its poor water solubility. Alatrofloxacin, however, is rapidly hydrolyzed to trovafloxacin in animals and human whole blood and thus offers a practical intravenous dosage form. Human pharmacokinetic studies have demonstrated that trovafloxacin is extensively distributed to the tissues with a long terminal half-life of approximately 10 h, which would allow single daily dosing. No significant laboratory biochemical or hematologic abnormalities were found, and tolerance was acceptable for doses of up to 300 mg (8).

The potent antimicrobial activity and long terminal half-life of trovafloxacin, which would permit single daily dosing, suggest that it is an ideal drug for central nervous system (CNS) bacterial infections with susceptible organisms. However, knowledge of the CNS penetration characteristics of trovafloxacin and its attainable concentrations in CSF is necessary to evaluate its potential efficacy against CNS bacterial infections. In this study, the concentrations of trovafloxacin in CSF and serum following a single intravenous infusion of alatrofloxacin, as well as the safety of intravenous administration of alatrofloxacin, were evaluated.

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MATERIALS AND METHODS

Subjects. Healthy males and nonfecund females, aged 18 to 50 years, were recruited for this study. The protocol was approved by a local Institutional Review Board, and all subjects provided written and oral consent. No more than 28 days prior to inclusion in the study, subjects underwent a screening process including a medical history, physical examination, 12-lead electrocardiogram, chemistry, hematology, and urinalysis. Subjects were required to have no history of clinically significant allergic, hematological, renal, endocrine, pulmonary, cardiovascular, hepatic, psychiatric, or neurologic disease, known hypersensitivity or intolerance to quinolone antibiotics, or any drug or alcohol dependence within the last 3 years. Subjects were also required to test negative for alcohol and prescription or recreational drugs, to have no intent to donate blood for 1 month after completion of the study, and to be within 20% of the ideal body weight for their age, gender, height, and frame according to the 1983 Metropolitan Life Insurance height and weight tables (4a).

Study design. Subjects reported to the research facility at least 12 h prior to study drug administration and were observed for at least 24 h after dose administration. A single dose of alatrofloxacin equivalent to 300 mg of trovafloxacin was diluted to 3 mg/ml with 5% dextrose and administered to 12 subjects as intravenous infusions over 1.0 h. CSF samples of at least 2 ml were collected at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 24 h after the start of the infusion, and each subject was sampled at only one time point. Blood sufficient to yield 2.5 ml of serum was collected from each subject just prior to and 1.0 h after the start of the infusion and at the time of CSF collection. For CSF collection, each subject was required to lie on his or her side and have a small area of the lower back cleaned with antiseptic. Each subject was given a local anesthetic, and a needle was inserted into the spinal fluid sac for CSF collection. Following CSF collection, each subject was required to lie on his or her abdomen for several hours to reduce the possibility of headache.

Aliquots of at least 1 ml from each CSF sample were immediately frozen at −20°C until analysis. Additional aliquots from each sample were examined microscopically for cellular content to rule out a grossly contaminated sample.

Blood samples were collected into evacuated tubes and allowed to clot for 45 min at room temperature and then spun in a refrigerated centrifuge to separate the clot from the serum. The serum was transferred to plastic vials and immediately frozen at −20°C until analysis.

Assay. Trovafloxacin concentrations in serum and CSF were determined by reverse-phase, high-performance liquid chromatography with UV detection for serum and mass spectrography detection for CSF (10). The linear dynamic ranges of the trovafloxacin assays were 0.1 to 20 μg/ml of serum and 0.025 to 2.5 μg/ml of CSF.

For serum samples, standards were prepared with serum containing trovafloxacin in concentrations ranging from 0.100 to 20.0 μg/ml. Quality control (QC)

TABLE 1. Trovafloxacin concentrations in CSF and corresponding concentrations in serum following intravenous infusion of a single dose of alatrofloxacin equivalent to 300 mg of trovafloxacin

Subject no.	Time (h)	Drug (μg/ml) in:		Ratio[a]
		CSF	Serum	
1	1	0.14	5.2	0.03
2	2	0.33	3.5	0.09
3	3	0.91	6.6	0.14
13	4	0.57	3.8	0.15
5	5	0.61	2.6	0.23
6	6	0.51	2.3	0.22
7	7	0.75	2.3	0.33
8	8	0.56	4.0	0.14
9	9	0.49	2.2	0.22
10	10	0.51	1.7	0.30
11	11	0.35	1.3	0.27
12	24	0.20	0.8	0.25

[a] The mean and SD of the 5.0- to 24.0-h data are 0.25 and 0.06, respectively.

samples were prepared with serum containing trovafloxacin at concentrations of 0.30, 4.0, and 15.0 μg/ml. For serum analysis, CP-102,372 (Pfizer Inc., Groton, Conn.) served as the internal standard. The intra-assay precision (standard deviation [SD]/mean observed concentration) of the QC samples was ≤4.41% for trovafloxacin, and the intra-assay accuracy (mean observed concentration/nominal concentration) ranged from 92.6 to 105%.

For CSF samples, standards were prepared with human serum containing trovafloxacin at concentrations ranging from 25.0 to 2,000 ng/ml. QC samples were prepared with human CSF containing trovafloxacin at concentrations of 35.0, 350, and 2,000 ng/ml. For CSF analysis, CP-102,372 served as the internal standard. The analytical method was cross-validated for CSF by analyzing, on one occasion, calibration standards in duplicate and QC controls in replicates of five. The intra-assay precision was ≤3.66% for trovafloxacin. The intra-assay accuracy ranged from 86.7 to 96.9%.

RESULTS

Thirteen healthy volunteers participated in this study. One subject experienced pain and edema at the site of infusion and was eliminated from the study. The mean weight of the remaining subjects (eight males and four females; mean age, 26.1 years) was 68.8 ± 11.6 kg (range, 49.1 to 88.2 kg).

Concentrations of trovafloxacin in CSF and serum following the infusion of alatrofloxacin are listed in Table 1. Analysis of the samples revealed a mean concentration of 5.8 ± 1.9 μg of trovafloxacin per ml of serum 1.0 h after the start of infusion. While levels in serum decreased rapidly after termination of the infusion, the concentration in CSF was relatively stable from 4 to 10 h (Fig. 1A). CSF/serum ratios ranged from 0.14 to 0.33 (Fig. 1B) over the postdistribution phase (5 to 24 h after the start of the infusion). The mean CSF/serum ratio ± SD during this period was 0.25 ± 0.06.

Adverse events judged to be related to the study drug and occurring in 10% or more of the subjects are listed in Table 2. The most common adverse events reported after administration of alatrofloxacin were dizziness, nausea, and pruritis or erythematous rash. Most of these cases were considered to be mild. Six moderate adverse events were reported, including injection site complication, dizziness, vomiting, and rash. The subject who experienced pain and edema at the site of infusion was the only subject to be eliminated from the study. No severe or serious adverse events, clinically significant laboratory abnormalities, or abnormal electrocardiogram results were observed.

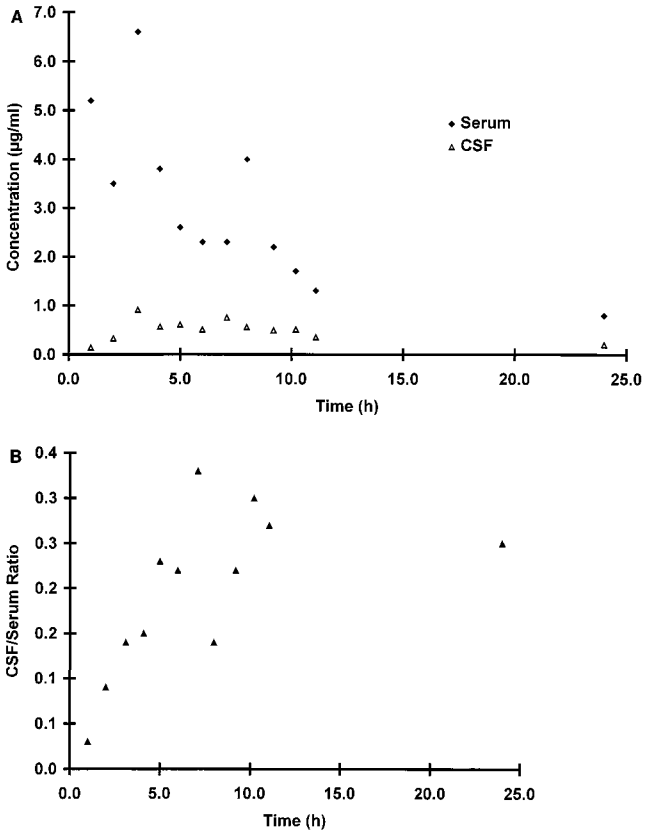

FIG. 1. (A) Concentrations of trovafloxacin in CSF and serum determined in subjects following intravenous infusion of a single dose of alatrofloxacin equivalent to 300 mg of trovafloxacin. (B) CSF/serum concentration ratios determined in subjects following intravenous infusion of an alatrofloxacin dose equivalent to 300 mg of trovafloxacin.

DISCUSSION

The current study investigated the penetration of human CSF by trovafloxacin following intravenous administration of alatrofloxacin. Trovafloxacin rapidly penetrated the CNS and maintained relatively steady levels over the course of 10 h. One subject was eliminated from the study due to pain and edema at the site of infusion. The most commonly reported adverse events were dizziness, nausea, and rash and were primarily mild in intensity. These results suggest that alatrofloxacin is an ideal compound for the intravenous administration of trovafloxacin.

In this study, quantifiable levels of trovafloxacin (≥0.2 μg/ml) were maintained in CSF over the 24 h of the study. Further

TABLE 2. Adverse events occurring in ≥10% of 12 subjects following intravenous infusion of alatrofloxacin

Adverse event	No. of patients (%)
Dizziness	6 (50)
Flushing	3 (25)
Headache	3 (25)
Hypoesthesia	2 (17)
Injection site reaction	2 (17)
Nausea	4 (33)
Pruritis	3 (25)
Rash	4 (33)
Vomiting	2 (17)

studies are necessary to determine the time course of complete elimination of trovafloxacin from the CSF following intravenous administration of alatrofloxacin.

The adverse events observed in this study are similar in type and incidence to those commonly associated with other quinolone antibiotics. Gastrointestinal symptoms such as nausea and vomiting are the most prevalent adverse events associated with the use of quinolones, while CNS effects such as dizziness and headache are slightly less common (12). Itching and erythema around the infusion site are also common with the intravenous administration of quinolone antibiotics (13). The occurrence of dizziness and headache in this study is potentially attributable to the lumbar puncture procedure itself. Additionally, the adverse events of nausea and vomiting might have been exacerbated by the prolonged bedrest required for the lumbar puncture procedure. Single and multiple oral doses of trovafloxacin have been well tolerated in previous studies (8, 9).

Trovafloxacin is more potent in vitro and in vivo than other quinolone derivatives against many gram-positive organisms implicated in bacterial meningitis in humans. In rabbit models of meningitis, trovafloxacin has demonstrated penetration of inflamed CSF and significant bactericidal activity (5, 6). To be effective against CNS bacterial infections, a drug must rapidly penetrate the CNS and achieve levels severalfold the MIC for 90% of the strains tested (MIC_{90}). In meningitis, inflammation might also affect drug penetration. In vitro, trovafloxacin has demonstrated potent activity against *Streptococcus pneumoniae* (MIC_{90} range, 0.06 to 0.25 μg/ml), *Haemophilus influenzae* (MIC_{90}, 0.015 μg/ml), and *Neisseria meningitidis* (MIC_{90} range, 0.001 to 0.008 μg/ml), the most common organisms implicated in bacterial meningitis in humans (1). The concentrations of trovafloxacin achieved in the CSF of subjects in this study were approximately 2 orders of magnitude greater than the MIC_{90} for *H. influenzae* and *N. meningitidis*, suggesting that alatrofloxacin is a potential candidate for treatment of CNS bacterial infections with susceptible organisms. More clinical trials are necessary to determine the efficacy of trovafloxacin against *S. pneumoniae* in humans.

In this study, the CSF of each subject was sampled at only one time point and all subjects received the same amount of study medication. Thus, it is possible that intersubject differences in body weight affected the pharmacokinetic profile of trovafloxacin in this study. However, as the SD from the mean weight of subjects was not considerably high, this factor most likely did not contribute significantly to the results.

To avoid intersubject variability, future studies might benefit from the sequential CSF sampling procedure utilizing an indwelling catheter, which removes the need for multiple needle insertions (3). The risks of this procedure are similar to those associated with lumbar puncture, which are well known. This technique has been used successfully over a period of 24 h (2). With this procedure, multiple CSF samples can be taken from a single subject, resulting in a more uniform profile. However, this technique has not been widely used and the pharmacokinetic profiles obtained are not as well understood as those obtained with the single sampling procedure.

In conclusion, trovafloxacin rapidly penetrated the CSF of healthy subjects following the intravenous infusion of alatrofloxacin and maintained relatively steady levels in CSF over several hours. These results suggest that alatrofloxacin is an ideal compound for the intravenous administration of trovafloxacin and possibly for the treatment of CNS bacterial infections in humans.

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