Pharmacokinetics of Cefamandole and Ampicillin in Experimental Meningitis

HARRY N. BEATY,†* AND ELWANDA WALTERS

Department of Medicine, University of Washington, Seattle, Washington 98105

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The penetration of cefamandole and ampicillin into the cerebrospinal fluid of rabbits with and without pneumococcal meningitis was evaluated. In normal animals, a mean maximum concentration of 0.22 ± 0.13 µg of cefamandole per ml was measured in the spinal fluid after a dose of 150 mg/kg given intramuscularly; with 25 and 50 mg/kg doses, no antibiotic was detected in the cerebrospinal fluid. With ampicillin, in intramuscular doses of 200 and 300 mg/kg, the mean maximum concentrations encountered in the cerebrospinal fluid were 1.59 ± 0.4 and 1.47 ± 0.44 µg/ml, respectively. Penetration of cefamandole, and to a lesser extent ampicillin, was increased after 24 h of experimental meningitis. With cefamandole, the concentration of drug in the cerebrospinal fluid exceeded the usual inhibitory concentration for Haemophilus influenzae only with the 150 mg/kg dose. After 48 h of meningitis, there was a trend toward higher levels of antibiotic in the cerebrospinal fluid, but the difference between animals infected 24 versus 48 h was not statistically significant. In animals with meningitis, serum concentrations after 150 mg of cefamandole per kg and both ampicillin doses studied were 32 to 38% lower than the serum levels achieved in normal rabbits after identical doses of antibiotic.

Cefamandole is a new semisynthetic cephalosporin antibiotic that recently was released for clinical use. Its major advantage over most other cephalosporins is a significantly expanded spectrum of antibacterial activity. In the course of its evaluation, particular interest developed in its potential usefulness in the treatment of infections caused by Haemophilus influenzae. Not only is cefamandole more active against most strains of Haemophilus than other cephalosporins (5), but it is fully active in vitro against the β-lactamase-producing isolates that are resistant to ampicillin (9). Most strains are inhibited by cefamandole concentrations of 0.5 µg/ml or less.

In spite of several studies of experimental meningitis which showed that cefamandole penetrates inflamed meninges well enough to achieve levels of antibiotic in the cerebrospinal fluid (CSF) that exceed inhibitory concentrations for H. influenzae (1, 9, 8), cefamandole appears to have a minor role in the treatment of meningitis caused by these organisms. Some patients respond satisfactorily to treatment (3), but in others the CSF may not be sterilized despite apparent in vitro susceptibility of the infecting strain and evidence of adequate penetration of antibiotic (10).

This study was undertaken to compare the penetration of ampicillin and cefamandole into the CSF of rabbits with and without pneumococcal meningitis. Results previously were reported in part (H. N. Beaty and E. Walters, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., abstr. no. 315, 1975) and are presented now in more detail, because the animal model employed offers some advantages over others in use and the unexpected finding of significantly lower blood levels of antibiotic in infected animals may have important implications.

MATERIALS AND METHODS

Meningitis model. The model of pneumococcal meningitis used in this study has been described previously (6). Briefly, rabbits are anesthetized and infected immediately after a cisternal tap by intravenous inoculation of a suspension of 10⁷ colony-forming units of Streptococcus pneumoniae type III per ml. At the time of the tap, 0.5 ml of a 0.125% suspension of sterile gastric mucin is injected into the cistern. From 60 to 90% of rabbits infected in this manner develop meningitis, i.e., pneumococci are recovered from the CSF 24 h after the intravenous injection of organisms. Animals with meningitis have progressively severe clinical disease which, if untreated, almost invariably results in death within 48 to 96 h.

Experimental design. Experiments were carried out on three major groups of animals.

Group I. In group I, six to eight uninfected rabbits were studied at a time. Half were given cefamandole intramuscularly (i.m.) in doses of either 25, 50, or 150 mg/kg. The remainder received either 200 or 300 mg...
of ampicillin per kg. It was usually possible to obtain two 0.5- to 1.0-ml portions of CSF at designated times within the next 2 h. Blood for determination of serum levels of antibiotic was drawn each time a sample of CSF was secured. A sufficient number of animals was studied to obtain four to six samples of both CSF and serum 15, 30, 60, and 120 min after each of the dosages indicated.

Group II. In group II, animals were infected in groups of six to eight, according to the procedure outlined previously. CSF was obtained for culture 24 h later, and half the animals were given either 25, 50, or 150 mg of cefamandole per kg i.m. The remainder received either 200 or 300 mg of ampicillin per kg. In the manner described for experiments in group I, a number of samples of CSF and serum were obtained 15, 30, 60 and 120 min after injection of the various doses of antibiotic. Data from specimens obtained from animals that had not developed meningitis, i.e., in which the CSF was sterile at 24 h, were not included in the results.

Group III. In group III, rabbits were also infected in groups of six to eight. Spinal fluid was obtained for culture 24 h later, and animals proven to have meningitis were studied 48 h after the induction of infection. Half received 150 mg of cefamandole per kg i.m.; the rest were given 300 mg of ampicillin per kg. Serum and CSF were obtained at the time intervals and in the manner described for groups I and II.

Laboratory studies: (i) Routine studies. Spinal fluid was cultured on blood agar plates that were incubated overnight in 5% CO₂ at 37°C. Pneumococci recovered from CSF were identified by standard techniques. Serum and CSF collected for antibiotic assays were kept on ice or frozen until studies were done, which was always within 24 h. Erythrocyte counts were performed on each CSF sample, and those with counts high enough to represent greater than 1% contamination were discarded.

(ii) Antibiotic determinations. Concentrations of antibiotic were measured by the agar-well diffusion technique of Bennett et al. (2). Nutrient agar (1.5%) seeded with Bacillus subtilis was poured into a leveled, sterile plate (60 by 30 cm). After solidification of the agar, wells 4.5 mm in diameter were punched in the surface at measured intervals. Spinal fluid or serum to be tested, along with the appropriate standards, were added to the wells. Three to five replicates were made of each unknown and standard sample. Plates were incubated at 37°C for 18 h, and the diameter of the zones of inhibition around each well was measured. For each unknown sample and standard, the mean diameter of the zones of inhibition measured was determined. Antibiotic concentrations were extrapolated from a standard curve calculated as described by Bennett et al. (2). For serum assays, antibiotic standards of 4, 8, 16, 32, and 64 µg/ml were prepared in pooled rabbit serum. Serum samples obtained from animals receiving high doses of antibiotic were diluted 1:2 or 1:6 to use the standard curve. For CSF determinations, antibiotic standards ranging from 0.125 to 4.0 µg/ml were prepared in phosphate-buffered saline, which proved in separate studies to be a suitable diluent. The minimal detectable concentration of both antibiotics in the CSF was 0.1 µg/ml.

(iii) Statistical methods. The statistical method employed for comparison of groups of samples was the t-test for independent means. Significance was based on P ≤ 0.1.

RESULTS

The objective of the experimental design outlined above was to compare the penetration of cefamandole and ampicillin into the CSF of uninfected rabbits and animals with 24 and 48 h of experimally induced pneumococcal meningitis. The working hypothesis was that there would be little penetration of antibiotic, even at high doses, in the absence of infection, but that concentrations of antibiotic in the CSF would increase progressively with infection and increasing intensity of inflammation.

Concentrations of cefamandole and ampicillin in normal animals. The highest serum concentration of cefamandole measured after doses of 25 and 50 mg/kg i.m. was at 15 min (Table 1). The peak occurred at 30 min after a dose of 150 mg of cefamandole per kg and both

<table>
<thead>
<tr>
<th>Antibiotic dose (mg/kg)</th>
<th>Body fluid</th>
<th>Conc (µg/ml) at: ( t ) a</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
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<tr>
<td>Cefamandole</td>
<td></td>
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</tr>
<tr>
<td>25</td>
<td>Serum</td>
<td>73.6 ± 6.6</td>
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<tr>
<td></td>
<td>CSF</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>Serum</td>
<td>86.1 ± 18.4</td>
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<tr>
<td></td>
<td>CSF</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>Serum</td>
<td>269.4 ± 17.6</td>
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<tr>
<td></td>
<td>CSF</td>
<td>0</td>
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<tr>
<td>Ampicillin</td>
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</tr>
<tr>
<td>200</td>
<td>Serum</td>
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<tr>
<td></td>
<td>CSF</td>
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<tr>
<td>300</td>
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<td>216.0 ± 33</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>0.14 ± 0.06</td>
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* Each value represents mean ± standard error of data from four to six animals.
the ampicillin doses. Cefamandole was detectable in the CSF of normal rabbits only with the 150-mg/kg dose, which produced a mean peak serum concentration of 274.8 µg/ml.

In contrast, ampicillin was present in the CSF of all animals tested after both 200- and 300-mg/kg doses. The CSF concentrations of ampicillin were almost identical with the two dosages, despite the fact that 300 mg of drug per kg produced significantly higher serum levels.

Concentrations of cefamandole and ampicillin after 24 h of meningitis. The penetration of cefamandole into the CSF was significantly greater 24 h after induction of pneumococcal meningitis (Fig. 1). However, the peak concentrations, which were measured 30 to 60 min after administration of antibiotic, were only 0.12 ± 0.07 (standard error) and 0.28 ± 0.06 µg/ml. The highest level measured after 150 mg/kg was 0.87 ± 0.29 µg/ml at 60 min, and it was significantly higher than the concentrations achieved with lower doses of cefamandole.

With ampicillin, the CSF concentrations measured in infected animals at 30 min were significantly higher than those in normal controls. However, only the peak level achieved with 200 mg of ampicillin per kg was significantly greater than the peak concentration in uninfected animals because of a large standard error of the mean among animals receiving 300 mg/kg.

The concentrations of cefamandole in serum from infected animals receiving 25 and 50 mg of drug per kg did not differ from levels found in normal animals. However, neither the 150-mg/kg dose of cefamandole nor either of the ampicillin regimens produced levels of antibiotic in the serum of rabbits with meningitis that were as high as in controls. In each instance, the mean peak serum level (172.0 ± 18 µg of cefamandole per ml; 98.9 ± 12 and 164.3 ± 21 µg of ampicillin per ml) was 62 to 68% of the peak level seen in normal animals.

Concentrations of cefamandole and ampicillin after 48 h of meningitis. In animals infected 48 h before administration of the drug, cefamandole at 150 mg/kg and ampicillin at 300 mg/kg were the only doses evaluated. With both drugs, the CSF levels of antibiotic were significantly higher than the levels achieved in normal animals at each time interval (Fig. 2). However, the trend toward higher peak concentrations 48 h after infection rather than at 24 h did not achieve statistical significance.

Again, serum concentrations were significantly lower than those encountered in normal animals receiving the same dose of drug. Appropriate data from experiments in all groups of animals are depicted for easy comparison (Fig. 2). There was no difference in the measured serum concentrations of antibiotic in animals infected for 24 rather than 48 h; but levels in both groups were significantly lower than those found in normal rabbits.

Experiments with animals in groups II and III always had an internal control, because 10 to 40% of the animals inoculated did not develop meningeal infection. These animals were studied in the same way as infected animals, because it was not possible to determine whether they had meningitis before administration of antibiotics for assessment of penetration into the CSF. When results of cultures were known, antibiotic data from animals with sterile CSF were not analyzed with those from rabbits with docu-
mented meningitis. However, it was possible to compare the serum concentrations of antibiotic in these animals with those produced by equal doses of drugs in animals of group I and those in groups II and III that had meningitis. In all instances, animals that did not develop infection had serum levels equal to those found in normal rabbits.

**DISCUSSION**

One advantage of the model used in these studies is that animals are infected with an intravenous inoculum. This more closely mimics the pathogenesis of meningitis in humans, and avoids some of the potential artifacts created by introducing massive numbers of bacteria directly into the cisternal system. The intracisternal injection of a small amount of gastric mucin increases the tropism of organism in the bloodstream for the central nervous system, but does not lessen the advantage of an intravenous inoculum. Detailed studies of meningeal inflammation in this model have shown that the contribution of mucin to the total process is minimal (4).

The detailed characterization of the inflammatory process in and around the brain of these animals is another advantage of this model. It has been shown that the mass of granulocytes in the meninges increases progressively during the first 72 h after infection (4). Many studies, including the one reported herein, document that antibiotics penetrate the "blood-brain barrier" more effectively when meningitis is present. Korzeniowski and co-workers showed that the concentration of cefamandole in the CSF of patients with meningitis was highest when the glucose concentration was low and the protein was high (3); in experimental meningitis, these findings are correlated with increased intensity of inflammation.

Many studies of penetration of antibiotics into the CSF in experimental meningitis are conducted within 18 h of infection. In the model used in this study, the inflammatory mass at 18 h is modest in comparison with that which can be measured later in the course of infection. Nevertheless, at all doses studied, the penetration of cefamandole into the CSF was significantly increased after 24 h of meningitis (Fig. 1).

Unfortunately, the hypothesis that the concentration of antibiotic would increase in direct relationship to the intensity of inflammation was not proven unequivocally. There was a trend toward higher concentrations of both ampicillin and cefamandole in the CSF of animals that had had meningitis for 48 h rather than 24 h (Fig. 2). However, the observed differences did not reach an arbitrarily assigned level of statistical significance. This might have been overcome by studying more animals or administering antibiotics still later in the course of infection. At least the feasibility of studying the pharmacokinetics of drugs at various times in the course of experimental meningitis was documented by these experiments.

The unexpected finding of significantly reduced serum levels of antibiotics in rabbits with meningitis is interesting. The phenomenon was observed only with the 150-mg/kg dose of cefamandole, but was seen with both the ampicillin regimens employed. A number of hypotheses

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**FIG. 2.** Concentrations of cefamandole (a) and ampicillin (b) in the serum and CSF of normal rabbits and animals after 24 or 48 h of pneumococcal meningitis. Cefamandole was administered i.m. in a dose of 150 mg/kg; the ampicillin dose was 300 mg/kg. Symbols: ○, normal animals; O, after 24 h of meningitis; △, after 48 h of meningitis. Each data point represents the mean of four to six observations; the vertical bar indicates the standard error of the mean.
could be formulated to explain these findings. The possibility of a systematic error in performing the studies seems unlikely, because the phenomenon was observed in groups of animals studied over a period of months, using different lot numbers of antibiotic, and calculations of antibiotic doses were checked carefully. More importantly, the differences were seen consistently within groups of animals that were studied concurrently; the critical determinant being whether they developed meningitis after an intravenous inoculum of organisms or not.

It is possible that the blood levels of antibiotic are lower in animals with infection because of increased renal blood flow and concomitant increase clearance of drug secondary to high fever. Because the discrepancy is seen only with higher doses of antibiotic, a simultaneous alteration in protein binding would have to be postulated. An increased volume of distribution of antibiotic, again associated with altered protein binding, could explain this phenomenon. Lower-than-normal blood levels of gentamicin observed in human volunteers with fever induced by the administration of endotoxin were presumed to be on this basis when increased renal clearance of the drug could not be documented (7).

Studies of this type call attention to the fact that much of our knowledge of clinical pharmacology of antibiotics is derived from studies of healthy volunteers. The pharmacokinetics of many drugs may be drastically altered by disease, so studies of the type reported here should be expanded to determine the mechanisms responsible for the reduced serum levels and to assess their potential clinical significance.