Pharmacokinetics and Bacteriological Efficacy of Ticarcillin-Clavulanic Acid (Timentin) in Experimental Escherichia coli K-1 and Haemophilus influenzae Type b Meningitis

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The pharmacokinetics and bacteriological efficacy of ticarcillin and clavulanic acid administered individually or in combination were assessed in rabbits with experimental Escherichia coli K-1 and Haemophilus influenzae type b meningitis. The mean penetrations into the cerebrospinal fluid (CSF) of infected animals after a single dose of ticarcillin-clavulanic acid were approximately 11 and 28% for ticarcillin and clavulanic acid, respectively. In continuous-infusion experiments, the mean penetrations into CSF were 14.6 and 35% for ticarcillin and clavulanic acid, respectively, in rabbits with E. coli meningitis and 6.1 and 24%, respectively, in rabbits with H. influenzae meningitis. In animals that received a continuous infusion of the two drugs alone or in combination, the median CSF bactericidal titers for E. coli were <1:2, <1:2, and 1:2 for ticarcillin, clavulanic acid, and ticarcillin-clavulanic acid, respectively, and for H. influenzae the titers were <1:2, <1:2, and 1:4, respectively. The addition of clavulanic acid potentiated significantly the bacteriological efficacy of ticarcillin in reducing the number of bacteria in CSF of infected rabbits. Additional studies in animals and humans are required before recommendations can be made regarding the use of ticarcillin-clavulanic acid for treatment of meningitis.

The combination of ticarcillin and clavulanic acid (Timentin) has substantial in vitro activity against aerobic gram-negative bacteria, including beta-lactamase-positive Haemophilus influenzae type b and many members of the family Enterobacteriaceae (4, 6, 7, 13). These organisms are important meningeal pathogens. Although the pharmacokinetics and clinical efficacy of ticarcillin-clavulanic acid have been characterized in humans, there is relatively little information on the penetration of the two beta-lactams into cerebrospinal fluid (CSF) and on the efficacy of the combination in treating meningitis.

This study was undertaken to determine the concentrations in serum and CSF, bactericidal titers, and bacteriological effects of ticarcillin and clavulanic acid alone and in combination in rabbits with experimental meningitis caused by Escherichia coli K-1 and H. influenzae type b.

MATERIALS AND METHODS

Model of experimental lapine meningitis. New Zealand White male rabbits (2 to 3 kg) were prepared as previously described (2, 9). Briefly, a dental acrylic helmet was attached to the skull of each rabbit, and 3 days later the animals were anesthetized and placed in a stereotactic frame. A Quincke spinal needle was introduced into the cisterna magna and fixed in the frame. Bacteria were inoculated, and CSF samples were taken via the intracisternal needle during the treatment.

Inocula of 10^6 to 10^8 CFU of a beta-lactamase-producing strain of H. influenzae per ml and 10^6 to 10^8 CFU of E. coli per ml were used, and the injected volume was 0.2 ml. These inocula uniformly produced meningitis in the rabbits; larger inocula caused the death of 25 to 50% of the animals. Both organisms were isolated from the CSF of infants with bacterial meningitis.

Drug administration. Ticarcillin disodium and potassium clavulanate (provided by Beecham Laboratories, Bristol, Tenn.) were administered intravenously individually or in combination in a ratio of 30:1.

(i) Single-dose studies. A single dose of 160 mg of ticarcillin per kg (body weight) alone (4 rabbits) or in combination with 5.33 mg of clavulanic acid per kg (12 rabbits) was administered intravenously for 5 min.

(ii) Continuous-infusion studies. A loading dose of 160 mg of ticarcillin per kg (11 rabbits), 5.33 mg of clavulanic acid per kg (9 rabbits), or of the two drugs combined (12 rabbits) was followed by a continuous intravenous infusion (with a femoral venous catheter) of 160 mg of ticarcillin per kg per h, 5.33 mg of clavulanic acid per kg per h, or the two agents combined. The infusion was administered for 6 h with a constant infusion pump (model AS-5B; Auto-Syringe, Inc., Hooksett, N.H.). Infected rabbits were treated 14 to 16 h after the induction of meningitis.

Specimen processing. Serial blood from an indwelling femoral artery catheter and CSF samples from the intracisternal needle were obtained before and at 0.25, 0.5, 1, 2, 4, and 5 h after a single drug dose and before and at 2, 4, and 6 h during constant drug infusion. Blood and CSF were immediately cultured quantitatively on chocolate agar plates for H. influenzae and on eosin-methylene blue for E. coli. Serum and CSF were kept frozen at −70°C until the antibiotics and antibacterial titers were measured, usually within 2 weeks. No substantial reduction in antibiotic concentrations resulted from storage.

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High-pressure liquid chromatography. Ticarcillin and clavulanic acid concentrations were measured with a Waters liquid chromatograph equipped with an M450 UV absorption detector, a WISP automatic injector, and an M640 integrator (Waters Associates, Inc., Milford, Mass.). The two compounds were extracted from serum samples (0.4 ml) by a two-phase procedure involving acetonitrile precipitation and methylene chloride concentration.

Clavulanic acid was quantitated by preparing the imidazole derivative in a modified form (1, 3).

Ticarcillin and clavulanic acid were analyzed on a reverse-phase 30-cm µ-Bondapak C18 steel column (Waters) at detector wavelengths of 229 and 313 µm for ticarcillin and clavulanic acid, respectively. The mobile phase for ticarcillin was 30% methanol in 70% aqueous 0.05 M sodium biphosphate (pH 2.1 with phosphoric acid), while that for the clavulanic acid-imidazole derivative was 5% methanol in 95% aqueous 0.05 M sodium biphosphate (pH 2.1 with phosphoric acid). The flow rates through the column were 1.5 and 2.0 ml/min for ticarcillin and clavulanic acid, respectively.

All specimens were quantitated by measuring the peak heights and comparing these values with those of the standard curve. The lowest reliable concentrations measured were 1 µg/ml for ticarcillin and 0.25 µg/ml for clavulanic acid. Retention times for ticarcillin and clavulanic acid were 3.6 and 2.1 min, respectively, for each high-pressure liquid chromatographic separation mode.

The intra-assay precision of the methods was assessed for both compounds by measuring a known standard extracted and analyzed 10 times daily. Both compounds demonstrated good assay reproducibility, with coefficients of variation of 5.4 and 2.1% for ticarcillin and clavulanic acid, respectively.

Susceptibility studies. The MICs and MBCs for the two test strains were determined by a microtiter technique with serial twofold dilutions in Mueller-Hinton broth for E. coli K-1 and in Mueller-Hinton broth with Supplement C (Difco Laboratories, Detroit, Mich.) for H. influenzae. The inoculum was approximately 10^8 CFU/ml. After 20 h of incubation at 37°C, the wells were inspected; the MIC was defined as the lowest concentration of antibiotic that inhibited visible growth. Each clear well was subcultured onto blood agar plates for E. coli and chocolate agar plates for H. influenzae, and these plates were incubated for another 18 h. The MBC was defined as the lowest concentration of antibiotic that killed 99.9% of the original inoculum. Susceptibility studies were performed by using ticarcillin and clavulanic acid at a ratio of 30:1.

Bacteriostatic and bactericidal titers. Bacteriostatic and bactericidal titers in blood and CSF for the test strain that caused meningitis (inoculum size, 10^3 organisms) were determined by a microtiter technique similar to that used for MIC and MBC measurements except that serum and CSF were used instead of standard solutions of drugs (8, 9). The diluent was Mueller-Hinton broth (for E. coli) or Mueller-Hinton broth plus Supplement C (for H. influenzae).

Pharmacokinetic analysis. The antibiotic concentrations in serum and CSF measured after single-dose administration were fitted to a regression line by the least-mean-squares method. The half-life in serum and CSF was calculated by dividing the natural logarithm of 2 by the slope of the line. The area under the concentration-time curve (AUC) in serum and CSF was obtained by successive trapezoidal approximations from time 0 to infinity. The percentage of antibiotic penetration into CSF was defined as CSF concentration/serum concentration × 100 for the constant-infusion studies and as AUC in CSF/AUC in serum × 100 for the single-dose studies.

Statistical analysis. Values are expressed as the mean ± standard error of the mean. Comparison of a single parameter between two groups was performed by using the two-tailed Student t test.

RESULTS

Susceptibility studies. The ticarcillin MIC and MBC for the test E. coli K-1 were 256 and 512 µg/ml, respectively, and were both reduced to 16 µg/ml after clavulanic acid was added. The ticarcillin MIC and MBC for H. influenzae type b were 16 and 32 µg/ml, respectively, and were reduced to 1 and 2 µg/ml, respectively, after clavulanic acid was added.

Pharmacokinetics. (i) Single-dose studies. The serum and CSF concentration-time curves, half-life times, and AUC values for ticarcillin and clavulanic acid in uninfected rabbits and in E. coli- and H. influenzae-infected animals are shown in Fig. 1. The mean peak concentration of ticarcillin in CSF of uninfected rabbits was 4 µg/ml and increased to 11.4 and 17.8 µg/ml in E. coli- and H. influenzae-infected rabbits, respectively. We were unable to detect clavulanic acid in CSF of uninfected animals. In animals with E. coli meningitis, clavulanic acid was detectable in six of eight animals, and the mean peak concentration in these animals was 0.96 µg/ml. In the H. influenzae-infected rabbits, the mean peak
concentration in CSF was 1.42 μg/ml. The mean peak concentrations of the two drugs in CSF occurred between 0.5 and 1 h after the 5-min infusion.

The mean penetration of ticarcillin into CSF in uninfected rabbits, calculated as AUC in CSF/AUC in serum × 100, was 3.3%. The mean penetrations of ticarcillin into CSF of animals with E. coli and H. influenzae meningitis were 11.5 and 11.0%, respectively.

We were able to calculate the penetration of clavulanic acid into CSF only in six infected animals with detectable concentrations of clavulanic acid in CSF. The mean penetrations were 28.9% for E. coli-infected rabbits and 28.0% for H. influenzae-infected rabbits.

(ii) Continuous-infusion studies. During constant infusion, concentrations of ticarcillin and clavulanic acid in serum and CSF were similar at 2, 4, and 6 h, whether the drugs were administered individually or in combination. For purposes of analysis, therefore, the concentrations at each of the three times were combined for each agent. The mean concentrations of those two drugs in serum and CSF of E. coli- and H. influenzae-infected rabbits are shown in Table 1.

The mean penetrations of ticarcillin into CSF, calculated as the CSF concentration/serum concentration × 100 for all values during the 6-h study, were 14.6 and 6.1% in E. coli- and H. influenzae-infected rabbits, respectively (Table 1).

The mean penetrations of clavulanic acid into CSF, calculated from animals that had CSF samples with detectable concentrations, were 35.1 and 24% in rabbits with E. coli and H. influenzae meningitis, respectively (Table 1).

Bacteriological results. The effect of antimicrobial therapy on the number of bacteria in CSF was expressed as the mean difference between concentrations of the infecting organisms (Δ log10 CFU/ml) just before initiation of therapy and 5 h after single-dose administrations or 6 h after the onset of continuous-infusion experiments.

(i) E. coli K-1 meningitis. In untreated animals with E. coli meningitis, the bacterial count in CSF increased by +1.25 log10 CFU/ml in 6 h. After a single dose of ticarcillin and clavulanic acid, the change in E. coli concentration in CSF in 5 h was −0.23 log10 CFU/ml. After a 6-h constant infusion, ticarcillin alone resulted in a bacterial count increase of 1.06 log10 CFU/ml, clavulanic acid alone resulted in an increase of 1.84 log10 CFU/ml, and the two agents combined resulted in a mean decrease in the number of bacteria in CSF of 1.78 log10 CFU/ml (P = 0.004 in comparison with untreated or ticarcillin-treated animals). None of the animals had a sterile CSF culture at the end of the 6-h treatment (Fig. 2).

(ii) H. influenzae meningitis. In untreated H. influenzae-infected rabbits, the bacterial count in CSF increased by 0.81 log10 CFU/ml in 6 h. A single dose of ticarcillin and clavulanic acid reduced the H. influenzae count in CSF by 1.38 log10 CFU/ml (P > 0.05). Constant infusion of ticarcillin alone resulted in a decrease of 0.59 log10 CFU/ml, while constant infusion of clavulanic acid alone was associated with an increase of 1.17 log10 CFU/ml. After a 6-h constant infusion, the two agents combined resulted in a decrease in the CSF bacterial count of 2.78 log10 CFU/ml (P < 0.001 in comparison with untreated animals and P = 0.011 in comparison with ticarcillin-treated animals). One of the five ticarcillin-clavulanic acid-treated animals had a sterile CSF culture (the lowest detectable bacterial count was 5 to 10 CFU/ml of CSF) at the end of the 6-h treatment (Fig. 3).

(iii) Blood and CSF titers. The median blood and CSF bacteriostatic and bactericidal titers for the two infecting organisms are shown in Table 2.

DISCUSSION

Clavulanic acid has a significant potentiating effect on the in vitro activity of ticarcillin against gram-positive and gram-negative bacteria, including Staphylococcus spp., E. coli, Klebsiella spp., Proteus mirabilis, Citrobacter spp., Enterobacter spp., and H. influenzae (4, 6, 7, 13; R. Wise, Antimicrob. Newsl. 1:23–26, 1984). We selected for study two organisms that are common meningeal pathogens in infants and children and used the rabbit model of experimental meningitis.

### TABLE 1. Ticarcillin and clavulanic acid concentrations in serum and CSF of animals with experimental E. coli and H. influenzae meningitis during 6-h continuous infusions

<table>
<thead>
<tr>
<th>Drug</th>
<th>Pathogen</th>
<th>No. of animals</th>
<th>Mean ± SEM drug concn (μg/ml) in:</th>
<th>Mean ± SEM CSF/serum penetration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum</td>
<td>CSF</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>E. coli</td>
<td>12</td>
<td>480 ± 63</td>
<td>73.7 ± 22.1</td>
</tr>
<tr>
<td></td>
<td>H. influenzae type b</td>
<td>11</td>
<td>483 ± 85</td>
<td>23.9 ± 5.1</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>E. coli</td>
<td>10*</td>
<td>7.8 ± 1.6</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>H. influenzae type b</td>
<td>8*</td>
<td>7.7 ± 1.1</td>
<td>1.9 ± 0.4</td>
</tr>
</tbody>
</table>

* Only animals with detectable levels of clavulanic acid in CSF are included.
The mean penetration of ticarcillin into CSF, calculated from the results of the single-dose studies, was approximately 11% in animals with *E. coli* or *H. influenzae* meningitis. In the constant-infusion experiments, the penetrations of ticarcillin into CSF were 14.6 and 6.1% in *E. coli* and *H. influenzae*-infected rabbits, respectively. Blood cultures were only occasionally positive at the start of or during therapy; therefore, sepsis did not appear to be a factor contributing to the variations in drug concentration and penetration in CSF.

After a single dose, the mean penetration of clavulanic acid into CSF was approximately 28%, and after 6 h of constant infusion, the mean penetration was from 24 to 35%. It must be emphasized, however, that these results were based on measurable concentrations of clavulanic acid in CSF only; we did not average values from all animals. It is difficult to determine the significance of undetectable clavulanic acid in CSF because the reduction in the number of bacteria in CSF of animals with undetectable concentrations was comparable to that in animals with measurable concentrations.

Although ticarcillin and clavulanic acid were administered in a ratio of 30:1, considerable variations in the relative amounts of each component in serum and CSF were observed. The bacteriological and clinical effect of these findings is unknown but does not appear to be significant. This conclusion is based on our results indicating that the addition of clavulanic acid to ticarcillin contributed significantly to the bacteriological efficacy of ticarcillin in the experimental meningitis model. This synergistic effect was shown in the continuous-infusion studies, in which the number of *E. coli* and *H. influenzae* cells in CSF declined significantly after the addition of clavulanic acid.

In these experiments, the combination of ticarcillin and clavulanic acid appeared to be relatively ineffective for the treatment of experimental *E. coli* and *H. influenzae* meningitis, especially when results are compared with those of the new cephalosporins (5, 8-10). The inability to achieve median bactericidal titers in CSF greater than 1:2 for *E. coli* and greater than 1:4 for *H. influenzae* is a potential concern, because it has been shown that a minimum CSF bactericidal titer of 1:8 to 1:10 (9, 10) or CSF antibiotic concentrations 10 to 30 times greater than the MBC of the same antibiotic for the infecting agent (11, 12) are necessary to achieve satisfactory bacteriological results in this model. Ticarcillin and clavulanic acid treatment for longer than 6 h in the animals might have had a more significant bacteriological effect. The application of data derived from the animal model to humans with meningitis is problematical and can be undertaken only with caution. Additional studies in animals and humans are required before recommendations can be made regarding the use of ticarcillin-clavulanic acid for meningitis treatment.

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**LITERATURE CITED**


