Effect of Pooled Human Cerebrospinal Fluid on the Postantibiotic Effects of Cefotaxime, Ciprofloxacin, and Gentamicin against Escherichia coli

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The killing and postantibiotic effects (PAE) of cefotaxime, ciprofloxacin, and gentamicin against Escherichia coli were determined in Mueller-Hinton broth (MHB) and pooled human cerebrospinal fluid (CSF). MICs performed in MHB and CSF were within one dilution for all antimicrobial agent-organism combinations. At two times the MIC, CSF significantly (P < 0.05) increased the duration of the PAE compared with MHB when cefotaxime, ciprofloxacin, and gentamicin were used against all strains tested. This effect occurred despite similar reductions in bacterial growth in both fluids after the 2-h antimicrobial agent exposure. We conclude that pooled human CSF markedly increases the PAE of cefotaxime, ciprofloxacin, and gentamicin against E. coli compared with MHB, without affecting bacterial killing.

The practice of designing antimicrobial agent dosing regimens to maintain concentrations in serum above the MIC for common organisms within the antimicrobial spectrum of activity for the majority of the dosing interval has recently been questioned (1). Investigators suggest that pharmacodynamic parameters such as the kinetics of bacterial killing and the postantibiotic effect (PAE) provide better parameters than the MIC and MBC in describing the time course of antimicrobial activity. Although the PAE (time period of no growth of the target organism after removal of the antimicrobial agent [2, 15]) has been investigated for over 40 years (12), it has only been recently that researchers have realized the clinical significance of this phenomenon. For agents such as aminoglycosides that display concentration-dependent bacterial killing, a prolonged and dose-dependent PAE (17), and activity at subinhibitory concentrations, new dosage regimens have been suggested (6, 14).

The majority of in vitro data regarding the PAE have been performed with standard microbiological media such as Mueller-Hinton broth (MHB) (13, 17). However, the PAE requires study in fluids such as serum, urine, and cerebrospinal fluid (CSF) to more fully assess its biological significance. We have recently described major effects on the PAE by human serum (3) and human urine (16), and this study represents preliminary observations of effects of pooled human CSF.

One clinical (G1307) and one reference (ATCC 25922) strain of Escherichia coli were used. The clinical isolate was obtained from an adult patient with meningitis. Isolates were frozen (−70°C) in skim milk and transferred onto blood agar plates monthly. At weekly intervals, isolates were subcultured onto fresh blood agar plates and streaked for purity.

The antimicrobial agents used included cefotaxime (Hoechst-Roussel Pharmaceuticals, Montreal, Canada), ciprofloxacin (Miles Pharmaceuticals, Etobicoke, Canada), and gentamicin (Schering Corp. Ltd., Pointe-Claire, Canada). Stock solutions of antimicrobial agents were prepared from standard powders and stored at −70°C until use. On the day of use, antimicrobial agent concentrates were diluted into appropriate media and used the same day.

Cation (25 mg of CaCl2 per liter, 12.5 mg of MgSO4 per liter)-supplemented MHB (pH 7.2 to 7.4) (Difco Laboratories, Detroit, Mich.) was used for MIC and PAE studies. Colony counts were performed by using Trypticase soy agar (Scott Laboratories, Fiskeville, R.I.) supplemented with 5% defibrinated sheep blood (blood agar).

CSF was collected over a 2-year period through collaboration with the departments of Clinical Chemistry and Clinical Microbiology in the two teaching hospitals (Health Sciences Centre and St. Boniface General Hospital). Samples from both pediatric and adult inpatients and outpatients were obtained and pooled. The pH of the pooled CSF was approximately 7.8.

In order to remove antimicrobial agents which may have been present, the CSF was passed through Bactec NR16 (Becton Dickinson Canada Inc., Mississauga, Canada) culture vials which contained only nonionic absorbing resin and cation-exchange resin and lacked medium and sodium polyanethol sulfonate. After being passed through the resins, the CSF was pooled again. To assess the effectiveness of the antimicrobial agent removal procedure, 100 μl of pooled CSF, both pre- and post-antimicrobial agent removal, was added to blood agar plates that were seeded with approximately 107 CFU of E. coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Enterococcus faecalis (ATCC 29212), and clinical strains of group B Neisseria meningitidis and group B streptococci. In addition CSF was analyzed for aminoglycoside (amikacin, gentamicin, netilmicin, and tobramycin) concentrations by using a TDX Analyzer (sensitivity, 0.1 mg/liter) (Abbott Laboratories, Ltd., Mississauga, Canada). CSF samples for the presence of aminoglycoside were run in duplicate.

Both pre- and post-antimicrobial agent removal CSF samples were analyzed for electrolytes (sodium, potassium, chloride, calcium, magnesium, and phosphate), glucose, and total protein. Reference strains E. coli ATCC 25922, S. aureus ATCC 25923, and P. aeruginosa ATCC 27853 and clinical strains of group B streptococci and group B...
Table 1. Biochemical analysis of CSF

<table>
<thead>
<tr>
<th>Test (normal serum value [per liter])</th>
<th>CSF value</th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (135–147 mmol)</td>
<td>140</td>
<td>146</td>
<td>66</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Postassium (3.5–5.3 mmol)</td>
<td>2.8</td>
<td>2.9</td>
<td>0.7</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Chloride (95–110 mmol)</td>
<td>130</td>
<td>130</td>
<td>57</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>Calcium (2.10–2.60 mmol)</td>
<td>1.20</td>
<td>1.12</td>
<td>0.56</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Magnesium (0.8–1.1 mmol)</td>
<td>1.0</td>
<td>1.17</td>
<td>0.22</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Phosphate (0.81–1.45 mmol)</td>
<td>0.50</td>
<td>0.47</td>
<td>0.18</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>Glucose (3.6–6.1 mmol)</td>
<td>2.2–3.7</td>
<td>4.1</td>
<td>1.7</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Total protein (60–80 g)</td>
<td>0.2–1.5</td>
<td>2</td>
<td>2</td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

* Approximate value observed in healthy humans (4, 5, 7–9).

* CSF-MHB, CSF (post-antimicrobial agent removal) with MHB added to a final concentration of 90% CSF–10% MHB.

**N. meningitidis** grew in both pre- and post-antimicrobial agent removal CSF samples; however, they grew slowly compared with growth in MHB. Thus, MHB was added to CSF to enhance growth (90% CSF–10% MHB [vol/vol]). All MIC and PAE experiments were performed with the modified CSF solution (90% CSF–10% MHB).

Determination of MIC was made by the microdilution broth method with doubling dilutions as described by the National Committee for Clinical Laboratory Standards (11).

PAE determinations were performed after a 2-h exposure to the test antimicrobial agent. The concentrations of cefotaxime, ciprofloxacin, and gentamicin tested were two times the MIC, which represents clinically achievable concentrations in the CSF. Multiples of the MIC were tested by using values obtained in MHB or CSF. For each antimicrobial agent-concentration combination in MHB, experiments were performed in triplicate. PAE experiments in CSF were performed only a duplicate because of limited quantities of CSF. All experiments were performed in a shaking water bath (Labline Instruments Inc., Melrose Park, Ill.) at 200 rpm. All PAE studies were performed with cultures of the organism in the logarithmic phase of growth at time of exposure to antimicrobial agents (10). To obtain such a culture, two or three colonies were inoculated into 50 ml of MHB or 10 ml of CSF-MHB and incubated at 37°C for approximately 12 to 14 h. Before each experiment, 5 ml of this culture was transferred into 45 ml of MHB or 1 ml was transferred into 9 ml of CSF-MHB (optical density at 580 nm, <0.05) and allowed to grow for several hours until the optical density at 580 nm reached 0.3. All optical densities were measured with a Spectronic 1201 Spectrophotometer (Milton Roy, Rochester, N.Y.). The culture was then diluted 1:10 by the addition of 1 ml of this suspension to 9.0 ml of the antimicrobial agent-containing (test) or control medium. This procedure resulted in a final concentration of approximately 10^6 to 10^7 CFU/ml. This point was defined as the initial time of exposure to the antimicrobial agent. Counts of CFU were performed immediately to verify the original inoculum. After 2 h of exposure, the antimicrobial agent was removed with a 1:100 dilution by the addition of 0.1 ml of antimicrobial agent-organism solution into 9.9 ml of prewarmed MHB. The following controls were maintained during experiments: (i) a growth control prepared in a manner similar to that used for the test solution without exposure to antimicrobial agents, and (ii) a residual antimicrobial agent control. The residual antimicrobial agent control was used to ensure the 1:100 dilution reduced the antimicrobial agent to inactive concentrations. The growth control and the residual antimicrobial agent control were treated in the same manner as the test solution. Counts of CFU were made for the test culture and growth control before exposure to antimicrobial agents, after 2 h of exposure, and after dilution to remove the antimicrobial agent. Thereafter, all cultures including the test, growth control, and residual antimicrobial agent control were assessed for growth every 60 min until marked turbidity was noticed. Serial 10-fold dilutions were prepared by using sterile cold 0.85% NaCl. Aliquots of 0.01 and/or 0.1 ml of the appropriate dilutions were plated by using a spread plate technique. Plates were read after 18 to 24 h of incubation at 37°C. The PAE was measured as previously described (10). The following calculation was used to quantitate the PAE: PAE = T – C, where T = time (minutes) required for the count of CFU in the test culture to increase 1 log_{10}, unit above the count immediately after dilution and where C = time (minutes) required for the count of CFU in the control to increase 1 log_{10} unit above the count immediately after dilution.

To assess whether antimicrobial agents, which may have been present in the CSF as a result of patient treatment, had been removed after passing through nonionic and cationic resins, 100 µl of CSF was added to blood agar plates seeded with bacteria. CSF added to bacterium-seeded plates before passage through resins demonstrated a marked (50 by 50 mm) zone of inhibition for all tested isolates. After passing through resins, no zone of inhibition was observed with any isolate, suggesting that antimicrobial agents which were present prior to resin treatment had been removed. Analysis of aminoglycoside concentration in CSF after resin treatment by using the TDX Analyzer demonstrated that aminoglycosides, if present, were present at concentrations of <0.1 mg/liter. This level represents the lowest possible concentration which can be detected and suggests that resin treatment removed aminoglycosides present prior to resin treatment.

Table 1 represents the biochemical analysis of pooled human CSF. Note that the biochemistry of the CSF pre-resin treatment to remove antimicrobial agents was quite similar to values obtained with normal CSF. Treatment with resins removed significant amounts of all solutes except protein. Upon addition of MHB (10%) to CSF (post-antimicrobial agent removal), biochemical analysis revealed a composition of 90% CSF–10% MHB, which is similar to CSF pre-antimicrobial agent removal.

Results of MIC determinations are reported in Table 2. MICs determined in MHB compared with those in CSF were within a one doubling dilution for all antimicrobial agent-organism combinations.

Growth kinetics of the organisms measured in MHB and
CSF were very similar. In each case, the growth controls increased 1 log unit approximately 80 to 100 min after dilution. In addition, the residual antimicrobial agent controls included in both MHB and CSF experiments grew at the same rate as that of the growth controls, indicating that a 1:100 dilution was effective in removing the activity of the antimicrobial agents.

Results of PAE determinations for cefotaxime, ciprofloxacin, and gentamicin are displayed in Table 2. As shown, all experiments with MHB were performed in triplicate on separate days. Experiments with CSF were performed only twice because of limited CSF availability. With cefotaxime, PAEs obtained in CSF were significantly longer than those obtained in MHB in both strains. With ciprofloxacin, PAE determinations performed in CSF were significantly longer than those obtained in MHB. Experiments performed with gentamicin demonstrated significantly longer PAEs in CSF than in MHB, again with both strains. Although PAEs were profoundly longer with experiments performed in CSF compared with those in MHB, bactericidal activity was not different between fluids. After resumption of growth, treated cells in both MHB and CSF eventually reached the same growth rate compared with that of the controls.

In previous studies we have reported that biological fluids such as serum and urine can have profound effects on the PAE (3, 16). Human serum significantly increased the PAE of fluoroquinolones against S. aureus (3). This effect was not due to pH, and heat treatment of serum suggested that complement or some other heat-labile component has an important role in the PAE. Experiments performed with fluoroquinolones against E. coli in pooled human urine demonstrated that urine dramatically decreased the PAE (16). We attributed the majority of this effect to the reduced pH of urine. The present study represents preliminary results on the effects of pooled human CSF on the PAEs of cefotaxime, ciprofloxacin, and gentamicin against E. coli. It should be noted that the lack of an adequate amount of CSF despite a collection time of 2 years precluded extensive investigation.

This study demonstrates that pooled human CSF has a profound effect on the PAEs of cefotaxime, ciprofloxacin, and gentamicin against E. coli. Significant increases in PAE occurred in CSF compared with those in MHB despite similar antimicrobial agent effects in both fluids after the 2-h exposure time. Considering the significant increases in PAE in CSF compared with those in MHB, we must assume that some component(s) of CSF is acting in combination with the antimicrobial agents. This effect is not due to pH, since adjusting MHB to the same pH as CSF (pH 7.8) resulted in no change in PAE. Whether the addition of 10% MHB to the CSF to enhance bacterial growth affected the PAE is unclear, although we believe that the main effect of MHB addition was to replenish solutes in the CSF which were removed with the resin procedure.

CSF originates from the choroid plexus of the ventricles, and its composition is derived from filtration, differential absorption, and active secretion (5). Most constituents in the CSF are present in equal or lower concentrations than in plasma (4; 5; 7-9). The concentration of most of the CSF electrolytes varies with changes in concentrations in plasma, but some appear to be independent. Almost all the proteins normally present in CSF are derived from the serum, with the exception of the trace proteins which originate in the brain. Since the majority of the protein in the CSF is derived from the serum, one could speculate that the mechanism of the increased PAE due to CSF would be similar to that of serum. That is, some protein component(s) is interacting with antimicrobial agents. We are presently continuing to collect CSF in order to more fully elucidate the role of CSF in the PAE.

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REFERENCES