Role of Serum Protein Binding and Multiple Antibiotic Doses in the Extravascular Distribution of Ceftizoxime and Cefotaxime

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The extravascular penetration of ceftizoxime and cefotaxime was studied in a rabbit subcutaneous Visking chamber model. Four rabbits, implanted with four chambers each, received each drug intramuscularly at a dose of 50 mg/kg every 3 hours for eight doses. Serum drug concentrations were measured after the eighth dose, and extravascular (chamber) concentrations were measured after the first and eighth doses. Cefotaxime (93% bound to rabbit serum proteins) demonstrated a much lower peak chamber-to-peak serum percent penetration after the first dose (20/163 = 13%) than did the less-bound (32%) ceftizoxime (21/52 = 40%, P < 0.002). Similarly, the ratio of the chamber fluid area under the curve to the serum area under the curve was significantly lower for cefotaxime (15%) than for ceftizoxime (44%, P < 0.002) after the first dose. Both agents approached equilibrium conditions between the intravascular and extravascular space by the eighth dose, and the ratios of chamber area under the curve to serum area under the curve of cefotaxime (76%) and ceftizoxime (79%) were similar. The peak-to-peak percent penetration of ceftizoxime (54%) was still significantly higher than that of cefotaxime (41%, P < 0.01), although the chamber concentration of ceftizoxime (66.2 μg/ml) was considerably higher than that of cefotaxime (28.2 μg/ml). This study illustrates (i) dampened peak-to-trough antibiotic level fluctuation seen at extravascular sites as compared with measured serum concentrations, (ii) the large differences in extravascular penetration between single- and multiple-dose studies, and (iii) the importance of serum protein binding in the delay, but not the prevention, of extravascular drug distribution.

The role of antibiotic binding to serum protein in the pharmacokinetics of extravascular distribution and in the interpretation of studies of extravascular drug penetration is important (9). Many investigators including ourselves, have attempted to judge whether agents with high or low levels of serum protein binding have superior extravascular penetration based on a comparison of peak extravascular-to-peak serum concentration ratios after a single antibiotic dose (1, 5-8, 11). The purpose of this report is to (i) describe the extravascular distribution of two extended-spectrum cephalosporins in a rabbit model after single and multiple systemic administrations and (ii) demonstrate that the major effect of a high level of serum protein binding is to delay the distribution of drug to the extravascular space, presumably by decreasing the concentration of free drug available for diffusion.

MATERIALS AND METHODS

Animal model. The rabbit model used has been described previously (8). Tissue fluid chambers made of Visking tubing (Union Carbide Corp., Chicago, Ill.), tied off at one end and occluded at the other end by a cork through which tubing from a 21-gauge Butterfly intermittent infusion set (Abbott Hospital Products, Inc., North Chicago, Ill.) had been passed, were surgically implanted in the subcutaneous space of the back of 3.0- to 3.4-kg female New Zealand white rabbits while the latter were anesthetized with 10 mg of xylazine hydrochloride (Cutter Laboratories, Inc., Shawnee, Kans.) plus 60 mg of ketamine hydrochloride (Bristol Laboratories, Syracuse, N.Y.). Four of these chambers were implanted in each animal, and then each rabbit was rested for 3 to 5 days before being studied. Immediately before the antibiotic study, the chambers were percutaneously filled with 3 ml of sterile pooled rabbit serum.

Antibiotic administration. Cefotaxime (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.) and ceftizoxime (Smith Kline & French Laboratories, Philadelphia, Pa.) were given intramuscularly at a dose of 50 mg/kg each every 3 h for eight doses. A total of four animals were studied in a crossover fashion with half of the animals receiving cefotaxime first and half ceftizoxime. The animals were rested for 2 days between studies. Sixteen data points were collected from...
properties of antimicrobial agents have attempted to determine advantages or disadvantages of serum pro-

TABLE 1. AUCs and AUCC for cefotaxime and ceftizoxime after the first and eight doses of each drug

<table>
<thead>
<tr>
<th>Drug</th>
<th>AUCS</th>
<th>AUCC (% of AUCS) after:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dose 1</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>14.19</td>
<td>2.17(15.3)</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>5.36</td>
<td>2.36(44)</td>
</tr>
</tbody>
</table>

* Measured after dose 8.

FIG. 1. Mean (± standard deviation) cefotaxime concentration in serum and in extravascular chambers (first and eighth doses).

the extravascular spaces at each sampling-time point for each agent.

**Specimen collection.** The fluid in the extravascular chamber was sampled by percutaneous aspiration of 0.25 ml at 0, 30, 60, 90, 120, and 180 min after the first and eighth doses (the chambers were refilled to 3 ml of serum after the first dose was completed by replacing the amount removed during this initial sampling). These samples were diluted (1:2) with 0.5 ml of pooled rabbit serum before antibiotic assay. Blood was sampled for serum concentration measurements at 0, 15, 30, 45, 60, 120, and 180 min after the eighth dose.

**Antibiotic assays.** Cefotaxime and ceftizoxime were assayed by high-pressure liquid chromatography (3, 4). Antibiotics were extracted from serum with a DEAE-Sephadex A-25 anion-exchange column. Samples (0.5 ml) were placed on the column, washed with phosphate-buffered saline (pH 7.2), and eluted with 5 ml of 1.0 M NaCl. A 100-μl portion of the protein-free eluate was directly injected into a Varian model LC5020 chromatograph (Varian, Walnut Creek, Calif.) with a Varichrom variable wavelength detector and a CDS 111L peak integrator. An analytical reverse-phase octadecylsilane column (Waters Associates, Inc., Milford, Mass.) was used for antimicrobial isolation. The mobile phase was 13% acetonitrile with 87% dilute acetic acid (pH 2.8) for cefotaxime and 18% acetonitrile with 82% dilute acetic acid for ceftizoxime. Both were run at a flow rate of 1.5 ml/min. The column eluate was monitored at 279 nm with 0.02 absorbance units as full-scale sensitivity.

**Protein binding.** The binding of cefotaxime and ceftizoxime to rabbit and human serum proteins was determined by ultracentrifugation as previously described (10).

**Calculations.** The area under the curve (AUC) was determined graphically with a compensating polar planimeter for each antibiotic in serum (AUCS) and in extravascular chambers (AUCC). Statistical significance was determined by the two-tailed Mann-Whitney U test. Equilibrium between the intravascular space and the extravascular chamber was defined as the time when the AUCC approached the AUCS.

**RESULTS**

The cefotaxime concentrations in the serum and extravascular space are shown in Fig. 1, and ceftizoxime concentrations are shown in Fig. 2. The AUCC and AUCC (first and eighth doses) are summarized in Table 1. Table 2 lists the peak chamber-to-peak serum concentration ratios for cefotaxime and ceftizoxime after the first and eighth doses.

Antibiotic binding to rabbit serum protein was 92.7% for cefotaxime (n = 7; range, 91.4 to 94%) and 32.2% for ceftizoxime (n = 4; range, 28.3 to 35.1%). Cefotaxime binding to human serum was 42.6% (n = 4; range, 37.6 to 46.3%), and ceftizoxime binding to human serum was 28.1% (n = 4; range, 22.5 to 35.6%). All results were at an antibiotic concentration of 40 μg/ml.

**DISCUSSION**

Studies on the extravascular penetration of antimicrobial agents have attempted to determine advantages or disadvantages of serum pro-
tein binding based on peak extravascular fluid-to-peak serum concentration ratios after a single dose. Because these determinations probably did not reach equilibrium and did not take into account protein binding in the extravascular space, conflicting results were often obtained, especially for highly protein-bound agents such as cefazolin (1, 11). We have previously discussed the need for multiple-dose studies to reach equilibrium, or a steady state, as well as the need for consideration of protein binding to antibiotic at the extravascular site to correctly interpret extravascular penetration experiments (5–9). The need to perform protein-binding studies with the serum and body fluid of the animal under study is illustrated by a recent report of Durack and Perfect (2). They found superior cerebrospinal fluid penetration of cefoperazone compared with cefotaxime after a single dose. No binding studies were performed, and, as seen from our investigation, it cannot be assumed that the lower protein binding of cefotaxime in humans will be the same in rabbits. Our study demonstrates that protein binding can affect extravascular penetration kinetics, especially after a single dose, and needs to be considered for the correct interpretation of experimental studies. The high level of serum protein binding of cefotaxime in rabbits (93%) as compared with the much lower binding of ceftizoxime (32%) allowed us to compare two similar extended-spectrum cephalosporin antibiotics to determine the effect of protein binding on extravascular distribution kinetics.

It can be seen from the tables and figures that the highly protein-bound cefotaxime achieved much lower extravascular fluid concentrations in relation to serum levels after the initial dose than did ceftizoxime, although the absolute peak level achieved was similar. The AUCC/AUCS ratio was only 0.15 for cefotaxime as compared with 0.44 for ceftizoxime after the first dose ($P < 0.002$). Also, the peak chamber level-to-peak serum level ratio was much lower for cefotaxime (0.125) than for ceftizoxime (0.395) after the first dose ($P < 0.002$). However, by the eighth dose both drugs approached equilibrium between the serum and the extravascular space, and these differences were no longer of such great magnitude. The two drugs still differed significantly ($P < 0.01$) in the peak chamber-to-peak serum concentration ratio, although there are inherent difficulties in using this determination as a measure of extravascular penetration (9). By the eighth dose there was no difference between the AUCC-to-AUCS ratio for cefotaxime and that for ceftizoxime ($P > 0.2$). Our data also confirm (12) the marked dampening effect on the peak-and-trough fluctuations of antibiotic concentrations achieved in extravascular fluid collections of 3 ml when compared with measured serum levels (Fig. 1 and 2). This effect is particularly apparent for the highly bound ceftizoxime.

This comparison of the extravascular penetration kinetics of cefotaxime and ceftizoxime again demonstrates that it is important to perform multiple-dose studies to determine antibiotic binding to serum proteins of the animal studied to correctly interpret the resultant data. Obviously, extension of these observations in the rabbit to humans must be done cautiously due to the very different serum protein binding in humans (ceftizoxime, 43%; cefotaxime, 28%), particularly for cefotaxime. This investigation clearly demonstrates that a high level of serum protein binding has the effect of slowing the rate of drug distribution to the extravascular space.

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LITERATURE CITED


