Penetration of Cefazolin, Cephaloridine, and Cefamandole into Interstitial Fluid in Rabbits

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We compared the penetration of three cephalosporins into interstitial fluid. Interstitial fluid was obtained in rabbits from Silastic tissue cages. Cefazolin, cephaloridine, and cefamandole were administered by the intramuscular route (30 mg/kg per injection). Peak blood levels and interstitial concentrations were studied after a single injection. Interstitial levels were also compared in a three-injection study (one injection every 12 h) and in a cumulative effect study (six injections), in which the interval between injections was established for each drug on the basis of its common therapeutic use. After a single injection, cephaloridine activity was detected more rapidly and attained higher levels than the other two drugs within the first 4 h. However, 2 h after the third injection, cefazolin levels in tissue fluid were higher than with cephaloridine. Cefamandole consistently gave the lowest interstitial levels. With all three drugs, detectable concentrations were present in interstitial fluid at a time when no detectable antibiotic was found in serum. In the six-injection study, the interstitial levels obtained with cefazolin were significantly higher than those observed with the other drugs. Our data suggest that cefazolin is a drug of choice due to its high extravascular levels.

Cephalosporins appear to have comparable in vitro antibacterial activity, although susceptibility to inactivation by bacterial penicillinase may vary from drug to drug. In some instances, however, the antibacterial activity of one cephalosporin derivative has been shown to be superior to another against a few susceptible bacterial species. To our knowledge, it is difficult to demonstrate through clinical studies—on the sole record of survival of patients after an infectious process—that one cephalosporin derivative is therapeutically more effective than the others. The preferred agent should combine the best pharmacokinetic and antibacterial features, i.e., high blood concentrations, long serum half-life, large apparent volume of distribution, low rate of side effects, possible administration through the intramuscular (i.m.) route, and the lowest minimal inhibiting concentrations.

The purpose of our study was to provide new pharmacokinetic data for clinical choice. After i.m. injections, the concentrations of three cephalosporins were compared in an extravascular space. An animal model was used to obtain interstitial tissue fluid. Cefazolin, cephaloridine, and cefamandole were selected for study on the following grounds: (i) Cefazolin has been described as the preferred agent for both therapeutic and economic reasons (10, 12; W. M. M. Kirby, J. B. DeMaine, and W. S. Serill, Postgrad. Med. J. 47:41, 1971), but some authors have questioned its diffusibility because of its high level of serum protein binding (15). (ii) Cefamandole pharmacology is similar to that of cephalothin (8), but this drug has been shown to be active in vitro against some Enterobacter and indole-positive Proteus strains that are not usually susceptible to other cephalosporins and to be more effective against Haemophilus species (11). (iii) Cephaloridine has been reported to be less than 30% bound in human serum (14) and is frequently used in prophylaxis.

MATERIALS AND METHODS

Animal model and interstitial tissue fluid. The investigations were carried out in female rabbits (Fauve de Bourgogne; weight range, 3.8 to 4.2 kg). Interstitial fluid was obtained as described by Chisholm et al. in dogs (7). "Tissue cages" were made from Silastic tubing (Silastic drains, Scurasil 72035, Rhône Poulenc Medical, Paris). Each tissue cage, provided with four holes (5-mm diameter), was 40 mm long and 13 mm in diameter and was closed at each end by vulcanization with self-vulcanizing silicone elastomer (Scurasil 20-350, Rhône Poulenc Medical, Paris). The abdominal skin of each rabbit was shaved, swabbed with merthiolate and then with absolute alcohol, and draped. After infiltration
with 2% lidocaine, an incision (40 to 50 mm long) was made in the skin. A subcutaneous space was made by dissection, and the tissue cage was inserted beneath the skin. The incision was closed with surgical silk and protected with dressing. After 4 weeks, the animals were used for the experiment.

Exchanges between plasma and tissue cage fluid were studied after injection of $^{82}\text{Br}$ (20 μCi), either intravenously or into tissue cage fluid. Normal renal function was assessed by determination of serum creatinine in all rabbits.

Antibiotics. Cefamandole nafate, sodium cefazolin, and cephaloridine in 1-g ampoules for injection and standard laboratory powders for assay were supplied by Eli Lilly and Co.

Single i.m. injection. A 30-mg/kg dose of cefamandole, cefazolin, or cephaloridine was injected into the thigh of five rabbits for each drug. Interstitial fluid was withdrawn from the tissue cage by needle aspiration at 30 min and at 1, 2, 4, 8, and 12 h. Blood samples were collected from a femoral catheter at 0.5, 1, 2, 4, 6, and 8 h.

Cumulative effect study. Three 30-mg/kg doses of cefamandole, cefazolin, or cephaloridine were injected i.m. every 12 h (respectively, at 0 h, 12 h, and 24 h) in rabbits for each drug. Six injections of each antibiotic were made to each rabbit. Interstitial fluid samples were collected just before the next injection.

Cumulative saturation effect study in simulated therapeutic conditions. A 30-mg/kg dose of cefamandole, cephaloridine, or cefazolin was injected i.m. every 6, 8, and 12 h, respectively, in five rabbits for each drug. Six injections of each antibiotic were made to each rabbit. Interstitial fluid samples were collected just before the next injection.

Blood samples. Blood samples were first allowed to clot and then centrifuged at 3,500 rpm for 15 min; the serum was stored in interstitial fluid, at −30°C. Standards for the assay of serum samples were prepared in normal rabbit serum. Standards for the assay of interstitial fluid samples were prepared with rabbit serum diluted threefold in 0.15 M phosphate buffer (pH 7.4). Concentrations of antibiotics were determined by diffusion in nutrient agar according to the method of Bennett et al. (4) with Bacillus subtilis ATCC 6553 as the test organism.

Protein binding. Protein binding was determined by an ultrafiltration method using 100% pooled rabbit serum adjusted to physiological temperature and pH, with an antibiotic concentration of 20 μg/ml (3).

Serum half-life. For the pharmacokinetic analysis, the serum half-life of each antibiotic was calculated from the serum concentrations obtained after intravenous injection at the time when blood levels declined exponentially during the late elimination phase. The formula used was $T_{1/2} = \ln2/Ke$, where $\ln2$ is the natural logarithm of 2 and $Ke$ is the slope of the regression line determined by the method of least squares (9).

Statistical analysis. Statistical studies were carried out by analysis of variance using time as the block factor (16). A significant interaction appeared between time and antibiotics. The Student's $t$-test was used to compare, two by two, the interstitial antibiotics levels at 1, 2, 12, and 26 h. For that test, the residual variance and its degree of freedom were used. The Student's $t$-test was also used to compare, two by two, the interstitial concentrations obtained at 2 and 26 h with each drug in the cumulative effect study.

RESULTS

Interstitial fluid. Mean values of main biological components of the fluid were similar to those obtained by Calnan et al. (6). Initial concentrations of total protein in the cage fluid, which were similar to those in serum, declined (after approximately 3 weeks) to a steady level of 30% of serum protein concentrations (Table 1).

Studies with $^{82}\text{Br}$. Rapid diffusion of $^{82}\text{Br}$ from plasma to tissue fluid and vice versa was demonstrated: when injected intravenously, $^{82}\text{Br}$ appeared in tissue fluid within 5 min. Equilibrium was obtained at approximately 60 min. After injection into the Silastic cage, $^{82}\text{Br}$ was detected within 3 min in plasma.

Single i.m. injection. After a 30-mg/kg i.m. dose, peak blood levels occurred at 0.5 h for cefazolin, cephaloridine, and cefamandole. Peak levels in serum (± standard deviation) were significantly higher for cefazolin (35.10 ± 1.27 μg/ml) than for cephaloridine (25.83 ± 1.04 μg/ml) and cefamandole (18.77 ± 6.44 μg/ml). Average concentrations of the three drugs in serum are shown in Fig. 1. No drug activity was detectable in serum 12 h after i.m. injection with all three antibiotics.

Interstitial fluid concentrations achieved after a single i.m. injection of each drug are shown in Fig. 2. Cephaloridine appeared more rapidly in interstitial fluid and gave significantly higher levels than the other two drugs within the first 4 h. Twelve hours after i.m. injection, all three antibiotics were still detectable in interstitial fluid; interstitial fluid levels at that time were significantly higher for cefazolin than for the other two derivatives ($P < 0.01$).

Table 1. Comparison of physicochemical properties of arterial blood and interstitial tissue fluid in rabbits

<table>
<thead>
<tr>
<th>Determination</th>
<th>Arterial blooda</th>
<th>Interstitial tissue fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/100 ml)</td>
<td>± 0.5</td>
<td>± 0.5</td>
</tr>
<tr>
<td>Albumin/globulins</td>
<td>0.92</td>
<td>2.23</td>
</tr>
<tr>
<td>Na (meq/liter)</td>
<td>138 ± 3</td>
<td>138 ± 4</td>
</tr>
<tr>
<td>K (meq/liter)</td>
<td>4.4 ± 0.3</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>Cl (meq/liter)</td>
<td>100 ± 2</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>7.45 ± 0.04</td>
<td>7.31 ± 0.03</td>
</tr>
<tr>
<td>Urea (g/100 ml)</td>
<td>0.038 ± 0.005</td>
<td>0.035 ± 0.006</td>
</tr>
<tr>
<td>Cells</td>
<td>100/ml</td>
<td></td>
</tr>
</tbody>
</table>

*a Mean values ± standard deviation.*

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Cumulative effect study. For all three compounds, interstitial concentrations measured 2 h after the third injection (26 h) were significantly higher than those obtained 2 h after the first injection (2 h) (Fig. 3).

The concentrations achieved in interstitial fluid at 1, 2, 12, and 26 h after the administration of three injections given, respectively, every 12 h were compared. The results of these comparisons are shown in Table 2.

Cefamandole always gave the lowest concentrations. As indicated above, cephaloridine produced higher levels at 1 and 2 h. Cefazolin, however, induced significantly higher concentrations 2 h after the third injection (26 h).

Cumulative and saturation effect in simulated therapeutic conditions. The effect on interstitial concentrations of six i.m. injections administered at various intervals for each antibiotic is reported in Fig. 4. A saturation effect seemed to occur with all three antibiotics: rapidly (two injections) with cephaloridine, at a level of approximately 5 µg/ml, and after the fifth injection for cefamandole and cefazolin, at levels of approximately 4 and 9.5 µg/ml, respectively.

Serum half-life. The half-life of the three drugs after intravenous injection in rabbits was 32 min for cefazolin, 24 min for cephaloridine, and 23 min for cefamandole. The correlation coefficients of the regression lines plotted on a semilogarithmic scale with a straight line were ≥ 0.99.

Protein binding. The protein binding of the three antibiotics measured in 100% rabbit serum under simulated physiological conditions was 92.5% for cefazolin, 39.5% for cephaloridine, and 88% for cefamandole.

DISCUSSION

The same dose, 30 mg/kg, was used for all three cephalosporins. In the first part of this experiment, the animals received the drug at identical time intervals in order to study the diffusion into interstitial fluid under the same conditions. In the second part, an attempt was made to reproduce human therapeutic conditions as closely as possible.

In the single injection study, the rapid penetration of cephaloridine (0.5 h) and the higher levels obtained at 1, 2, and 4 h can be explained by the low degree of protein binding of this agent. Many authors have emphasized the importance of protein binding in determining the distribution of antibiotics in vivo (2, 15). Our
determinations of the extent of protein binding in rabbits for cefazolin and cephaloridine are in the same range as those obtained by Nishida et al. (13). They are somewhat higher than in man. Barza and Weinstein (1) have shown an inverse correlation between the percentage of penetration into fibrin clots and the extent of binding of our penicillins. It appeared from their study that the degree of penetration is closely related to the relative levels of free drug in the blood. It is not well defined whether the peak level or the area under the curve of free drug is the most important determinant of penetration. In this part of our study, the very high concentrations observed with cefazolin in tissue fluid at 12 h after a single i.m. injection are somewhat at variance with what is generally accepted regarding the influence of protein binding on the diffusion of the drug. Cefazolin is highly bound, and Regamey et al. (14) have postulated a potential limitation of this drug related to its high level of serum protein binding. In our study, we have shown that cefazolin is likely to give high interstitial concentrations. Waterman et al. have demonstrated that binding of antibiotics by serum proteins does not restrict such agents to the intravascular space (19). The late high levels of cefazolin can be accounted for by its blood peak and half-life, which are, respectively, higher and longer than for cephaloridine. Cefamandole is relatively highly bound and gives the lowest blood levels. Consequently, this drug produces the lowest interstitial fluid concentration.

With all three drugs, late sustained interstitial levels were measured at a time (12 h) when no concentration of the drug was detectable in the blood. This finding has been described by many authors on similar animal models (1, 5, 7, 17, 18). Calnan et al. claimed that the fluid obtained under these conditions is indeed representative of body interstitial fluid (6). The presence of protein in the tissue fluid raises many questions. The extent to which cephalosporins bind to protein within the interstitial fluid has been demonstrated to be weak (19). It does not seem to affect the antibiotic behavior outside the vascular compartment (19). Our findings—and those of the aforementioned authors—

### Table 2. Cumulative effect study

<table>
<thead>
<tr>
<th>Drug pair</th>
<th>Conc (µg/ml) at:</th>
<th>1 h</th>
<th>2 h</th>
<th>12 h</th>
<th>26 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 h</td>
<td>12 h</td>
<td>26 h</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 h</td>
<td>12 h</td>
<td>26 h</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td></td>
<td>0.47 ± 0.27a</td>
<td>2.26 ± 0.80</td>
<td>4.30 ± 0.75</td>
<td>10.76 ± 3.4</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td></td>
<td>2.83 ± 0.50</td>
<td>4.25 ± 0.77</td>
<td>2.71 ± 0.59</td>
<td>6.52 ± 1.7</td>
</tr>
<tr>
<td>Degree of significance</td>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Cefazolin</td>
<td></td>
<td>0.47 ± 0.27</td>
<td>2.26 ± 0.80</td>
<td>4.30 ± 0.75</td>
<td>10.76 ± 3.4</td>
</tr>
<tr>
<td>Cefamandole</td>
<td></td>
<td>0.32 ± 0.27</td>
<td>0.85 ± 0.44</td>
<td>1.39 ± 0.71</td>
<td>2.92 ± 0.98</td>
</tr>
<tr>
<td>Degree of significance</td>
<td></td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td></td>
<td>2.83 ± 0.50</td>
<td>4.25 ± 0.77</td>
<td>2.17 ± 0.59</td>
<td>6.52 ± 1.7</td>
</tr>
<tr>
<td>Cefamandole</td>
<td></td>
<td>0.32 ± 0.27</td>
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<td>1.39 ± 0.71</td>
<td>2.92 ± 0.98</td>
</tr>
<tr>
<td>Degree of significance</td>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>P &lt; 0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> Injections, 30 mg/kg i.m., were given at 0, 12, and 24 h. Comparison, two by two, of the concentrations of cefazolin, cephaloridine, and cefamandole in interstitial fluid in rabbits. Each drug was administered to five rabbits.

<sup>b</sup> ± Standard deviation.

<sup>c</sup> NS, Not significant, P < 0.05.

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### FIG. 4. Cumulative effect of six i.m. injections of cefazolin (30 mg/kg every 12 h), cephaloridine (30 mg/kg every 8 h), and cefamandole (30 mg/kg every 6 h) on interstitial fluid concentrations measured just before the next injection in rabbits.
parallel the results reported by Barza et al. for fibrin clots rather than for their "interstitial fluid." We do not claim that this animal model closely approximates the physiological situation, but the tissue cage model provides some basis for comparison between different drugs in the same pharmacological group.

In the three-injection study (every 12 h), the cumulative effect of each drug in the interstitial fluid seems to be related to the late sustained levels. The higher interstitial levels of cefazolin over cephaloridine obtained 2 h after the third injection are probably due to a greater cumulative effect of cefazolin. It should be pointed out that this comparison was not carried out under strictly comparable conditions: the intervals between injections were identical, whereas the half-life of cephaloridine is shorter than that of cefazolin. In the six-injection study, cefazolin, administered in the same total dose, produced the highest interstitial concentrations over a much longer period of time. Therefore, within the limits of this animal study, it appeared that cefazolin might be the preferred agent in prophylactic treatment and probably in long-term therapeutic use.

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LITERATURE CITED