Influence of Four Modes of Administration on Penetration of Aztreonam, Cefuroxime, and Ampicillin into Interstitial Fluid and Fibrin Clots and on In Vivo Efficacy against Haemophilus influenzae

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The extravascular penetration and bactericidal activity of aztreonam, cefuroxime, and ampicillin against β-lactamase-positive and -negative Haemophilus influenzae strains were compared in a rabbit model. All groups of animals received an identical total dose of 100 mg of either antibiotic per kg given by four different intravenous modes of administration including a single large injection, four intermittent injections, a continuous infusion, and an injection followed by an infusion. Aztreonam had a higher degree of penetration in interstitial fluid and fibrin clots and was the most effective agent against β-lactamase-positive and -negative H. influenzae. A single large injection of either drug resulted in significantly higher peak levels and higher initial area under the curves of concentrations of drugs in serum, the interstitial fluid, and fibrin clots than those by other modes of administration. Continuous infusions of antibiotics resulted in poor in vivo bactericidal activity. Other modes of administration exhibited good antibacterial activity within the first 6 h of the study.

Therefore, a single large injection of aztreonam resulted in a much more rapid killing of H. influenzae than that by injection of the other drugs. Aztreonam and cefuroxime showed good in vivo stability to β-lactamase produced by H. influenzae while ampicillin was rapidly hydrolyzed in vivo.

The influence of the mode of administration on the penetration and efficacy of antimicrobial agents is still a matter of controversy (2, 4, 20, 33). Some investigators have suggested that the most effective mode of treatment should provide tissue concentrations of antibiotic continuously in excess of the minimal concentration inhibiting the pathogen (13, 23). Continuously maintained bactericidal levels are particularly important to effect cures in cases of invasive infections such as bacterial endocarditis (35), meningitis (24), or infections in neutropenic or cancer patients (8, 9, 14, 37), in whom host defenses may be impaired. Other investigators have demonstrated that these constant bactericidal levels of drug may not be necessary (7, 28, 29) and that frequent administration of antibiotics at short intervals is as effective as constant infusion, since it results in significant accumulation of drugs in interstitial fluid (17) and tissues (1, 4).

Any mode of administration producing higher penetration into extravascular sites may be clinically advantageous, since most infections, including those caused by Haemophilus influenzae, occur outside the bloodstream. It is likewise difficult to define the influence of the concentration obtained in the extravascular compartment on the bactericidal activity of antibiotics since most investigators have only evaluated the pharmacology of antibiotics (2, 11, 33), or have studied the bacteriological outcome of therapy (9, 14, 23), without specifically analyzing tissue penetration of drugs and the kinetics of bacterial killing at the site of infection.

In an effort to clarify these issues, we compared simultaneously the tissue penetration and bactericidal activity of three β-lactam antibiotics including a monobactam (aztreonam), acephalosporin (cefuroxime), and a penicillin (ampicillin) against β-lactamase-positive and -negative H. influenzae strains. We used a rabbit model involving the insertion of preformed fibrin clots into subcutaneous tissues (1). In this model the bacteria inserted in the clots are in direct contact with the antibiotic alone, without major intervention from host defenses. Fibrin also has the advantage of representing a regular constituent in inflammatory reactions of infected tissues. We have also studied the influence of β-lactamase production by bacteria into the fibrin clots on the levels and efficacy of antibiotics. (This study was presented in part at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy [Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 284, 1983].)

MATERIALS AND METHODS

H. influenzae strains. A β-lactamase-positive strain of H. influenzae C158 serotype b originally isolated at the Centre Hospitalier de l'Université Laval from the cerebrospinal fluid of a child with meningitis and a β-lactamase-negative strain of H. influenzae ATCC 10211 serotype b were used. The MIC and the MBC of antibiotics against both strains were determined as previously described (5) by a microdilution method in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) with an inoculum of 10⁶ CFU/ml.

Rabbit model. As previously described (6), New Zealand White female rabbits (weight, 2 to 3 kg) were given an intramuscular injection of 20 mg of chlorpromazine per kg. Both flanks were shaved and swabbed with iodine and alcohol. The skin was anesthetized with a 2% lidocaine, and a 4-cm incision was made. After blunt dissection of the skin, four to six clots were placed in each subcutaneous pocket to limit clustering. Autoclips (18 mm) were applied to close the incision. A scalp-vein needle (23 gauge), inserted in the marginal vein of the left ear, served for infusion of antibiotics. A Harvard infusion pump was used to ensure a steady rate of flow. Another scalp-vein needle (21 gauge), placed in the central vein of the right ear, was used to collect blood samples.

* Corresponding author.
Antibiotic regimens. An identical dose of 100 mg of aztreonam (Squibb Co.), cefuroxime (Glaxo Laboratories), or ampicillin (Ayerst Laboratories) per kg was administered by four different intravenous modes of administration: a single large injection of 100 mg/kg over 30 min (termed bolus), four injections of 25 mg/kg over 30 min given every 6 h for 24 h (intermittent injections), an infusion of 100 mg/kg given over 24 h (continuous infusion), and a single injection of 25 mg/kg of the antibiotic followed by a constant infusion of 25 mg/kg over 23.5 h (bolus plus infusion). Four rabbits were used for each mode of administration. Blood, interstitial fluid, and fibrin clots were removed aseptically before the administration of antibiotics and at 0.5, 1, 2, 3, 4, 6, 12, and 24 h after the beginning of the infusion. In animals given intermittent injections, samples were removed at 0.5, 1, 2, 3, 4, 6, 6.5, 12, 12.5, 18, 18.5, and 24 h. One fibrin clot was sampled at each time interval, and the pharmacokinetics and efficacy of antibiotics were analyzed in the same animal. Interstitial fluid was collected by impregnating three paper disks in subcutaneous pockets without blood contamination.

Preparation of noninfected and infected fibrin clots. A sterile solution of 3% bovine fibrinogen (Sigma Chemical Co., St. Louis, Mo.) was entangled with 20% rabbit serum, 0.5% hemin (Eastman Kodak Co., Rochester, N.Y.), and 0.5% IsoVitalex (Becton Dickinson and Co., Cockeysville, Md.). This solution was supplemented with 5% brain heart infusion broth (sterile with an inoculum of 5 × 10^7 CFU of either strain of H. influenzae per ml) and distributed in a 2-ml volume into siliconized test tubes (13 by 100 mm). One-tenth milliliter of bovine thrombin (250 U/ml; Parke Davis Co., Detroit, Mich.) was added to each tube. The resulting clots were then gently removed, dialyzed in sterile saline solution at 37°C for 60 min, and immediately inserted subcutaneously in rabbits. In our model, the size, volume, and exchange surface (34) of extravascular sites were identical in all animals. The initial concentrations of proteins in fibrin clots and interstitial fluid were similar (<15% of protein content in serum).

Determination of drug concentrations. Clots were weighed and homogenized in a 1% solution of trypsin (Difco) in a volume equal to the weight of the clot. Trypsin had no effect on antibiotic activity and did not significantly influence the bacterial count. Dissolved clots, serum, and interstitial fluid samples were bioassayed by a conventional agar diffusion method with Escherichia coli MB 3804 as the assay organism and tryptic soy agar (Difco) as the medium. The lower limit of detection was 0.1 µg/ml for all drugs. Standard solutions were prepared by diluting known amounts of antibiotics in rabbit serum for serum: in trypsinized fibrin clots for clots, and in Krebs-Ringer solution (22) for interstitial fluid. The degree of protein binding of antibiotics to rabbit serum was determined by an equilibrium dialysis technique (3).

β-Lactamase stability and in vivo efficacy. The in vivo stability of the three antibiotics to hydrolysis by β-lactamase during the four therapeutic regimens was determined by comparison of drug levels in fibrin clots infected with β-lactamase-negative H. influenzae ATCC 10211 and β-lactamase-positive H. influenzae C-158.

As previously described (4), the efficacy of the various regimens was evaluated by analysis of the bacterial content of infected clots at each timed interval. Appropriate dilutions of trypsinized clots were inoculated on chocolate agar followed by incubation at 37°C for 24 h. Since 0.1-ml samples were spread over the entire surface of a 140-mm agar plate drug carryover was not a problem.

The rate of bacterial killing in fibrin clots during the different regimens was compared by three mathematical parameters: (i) the variation between bacterial count in fibrin clots at the beginning and the end of therapy (log_10 CFU/g); (ii) the kinetics of bacterial killing over a 24-h period as described by Sande et al. (27) using the slope of least-squares regression line of surviving microorganisms; (iii) the killing index which was calculated by dividing the area under the curve of number of bacteria killed versus time by the number of bacteria at the beginning of therapy, and the time of therapy. Killing index percent (AUC log_10 bacteria killed × 100)/log_10 bacteria at the beginning × 24 h). This killing index was used to evaluate the overall bactericidal activity over a determined time period after administration of antibiotics.

Pharmacokinetic and statistical analysis. The area under the curve of concentration versus time (AUC) was obtained by the method of successive trapezoidal approximation from time zero to 24 h. The results are presented as mean ± standard deviation of four experiments. Statistics were performed by the Student t and F tests.

RESULTS

In vitro studies. The respective MICs and MBCs of aztreonam, cefuroxime, and ampicillin were 0.1, 0.1, and 128 µg/ml for H. influenzae C158 and 0.1, 0.1, and 0.4 µg/ml for H. influenzae ATCC 10211. The protein binding to rabbit serum was 50.3 ± 9.8 for aztreonam, 29.3 ± 8.0 for cefuroxime, and 15.0 ± 2.0 for ampicillin.

Antibiotic levels. The concentrations in serum, interstitial fluid, and noninfected fibrin clots and AUCs for the drugs during the different modes of administration are presented in Fig. 1 to 4.

(i) Bolus. Peak concentration of the three antibiotics in serum were observed at the end of the 30-min injection of 100 mg/kg (Fig. 1). The mean peak concentrations in serum were 297.0 µg/ml for aztreonam, 164.9 µg/ml for cefuroxime, and 163.8 µg/ml for ampicillin. The mean half-lives in serum were 0.65, 0.44, and 0.46 h, respectively. The levels of antibiotics in interstitial fluid paralleled those in serum during the first 4 h but were higher thereafter. The mean peak interstitial concentrations were 150.5, 100.0, and 82.4 µg/ml, respectively, at 30 min, and the mean half-lives were 1.2, 1.7, and 1.1 h, respectively. The mean peak fibrin clot concentrations were 26.7 µg/g for aztreonam at 4 h, 8.0 µg/g for cefuroxime at 1 h, and 8.2 µg/g for ampicillin at 2 h. The mean half-lives were 5.5, 2.9, and 2.1 h, respectively. The highest AUC value in fibrin clots was observed for aztreonam. Moreover, a concentration of 2.0 µg of this antimicrobial agent per g of clots was still detectable at 24 h.

(ii) Intermittent injections. Intermittent administration of aztreonam, cefuroxime, and ampicillin produced peak levels in serum of 52.6, 30.3, and 25.7 µg/ml, respectively, and identical mean trough levels of 0.1 µg/ml (lowest limit of sensitivity of the assay) (Fig. 2). The mean peak interstitial fluid concentrations obtained by injections of aztreonam (33.6 µg/ml) were significantly higher than those observed after injections of cefuroxime (15.6 µg/ml) and ampicillin (11.6 µg/ml) (P < 0.01). After 6 h of each injection, the mean trough concentrations in interstitial fluid were 2.8 µg/ml for aztreonam, 0.1 µg/ml for cefuroxime, and 0.1 µg/ml for ampicillin. After intermittent injections of aztreonam, fibrin clot levels increased progressively to reach a maximum level of 6.2 µg/g at 18.5 h. This accumulation of drugs in fibrin clots was not observed with cefuroxime and ampicillin which reached respective peak concentrations of 1.4 µg/ml at 2 h and 0.4 µg/g at 1 h.

(iii) Continuous infusion. With continuous infusion, a
steady-state concentration of antibiotics in serum was achieved within 2 h of infusion (Fig. 3). The mean maximum levels in serum were 9.2 µg/ml for aztreonam, 4.3 µg/ml for cefuroxime, and 2.8 µg/ml for ampicillin. At 1 h, levels of aztreonam in interstitial fluid were in equilibrium with those of serum. Respective peak levels of 2.8 and 1.7 µg of cefuroxime and ampicillin per ml were reached at 4 h. Those initial levels decreased steadily thereafter to achieve final concentrations of 0.5 and 0.6 µg/ml. The levels of drugs in fibrin clots increased progressively to achieve steady-state levels at 12 h for aztreonam, at 3 h for cefuroxime, and at 2 h for ampicillin. At the end of the continuous infusion, concentrations of each drug in fibrin clots were similar to those in interstitial fluid.

(iv) Bolus plus infusion. The bolus plus infusion regimen resulted in an initial peak serum concentration of 50.6 µg/ml for aztreonam, 41.2 µg/ml for cefuroxime, and 26.5 µg/ml for ampicillin (Fig. 4). These levels decreased progressively to achieve respective levels of 6.2, 1.3, and 1.0 µg/ml. Interstitial fluid levels paralleled those of serum during the first 6 h and decreased to achieve levels at 24 h of 5.2, 0.7, and 0.5 µg/ml. The levels of drugs in fibrin clots at 24 h were similar to those observed in interstitial fluid.

Stability of antibiotics to β-lactamase in fibrin clots. Levels

**FIG. 1.** Mean concentrations of aztreonam (A), cefuroxime (B), and ampicillin (C) and AUCs in serum, interstitial fluid, and noninfected fibrin clots after a single bolus injection of 100 mg/kg over 30 min. Each point gives the mean value for four rabbits, and the vertical bars indicate the standard deviation. AUC₀₋₂₄ indicates the AUC from 0 to 24 h.

**FIG. 2.** Mean concentrations of aztreonam (A), cefuroxime (B), and ampicillin (C) and AUCs in serum, interstitial fluid, and noninfected fibrin clots after intermittent injections of 25 mg/kg given every 6 h.
of the three antibiotics in serum and interstitial fluid were identical between groups of animals with noninfected and infected fibrin clots. Concentrations of the respective antibiotics in fibrin clots infected with \( \beta \)-lactamase-negative \( H. \) \( influenzae \) ATCC 10211 were similar to those observed in noninfected fibrin clots. The in vivo \( \beta \)-lactamase stability of antibiotics, which was determined by the comparison of AUC values of the three antibiotics in fibrin clots infected with \( H. \) \( influenzae \) ATCC 10211 (\( \beta \)-lactamase negative) and with C158 (\( \beta \)-lactamase positive), are presented in Table 1. The presence of \( \beta \)-lactamase did not influence the AUCs of aztreonam and cefuroxime, following the four regimens.

Both drugs showed good stability against the \( \beta \)-lactamase produced in fibrin clots by \( H. \) \( influenzae \). In contrast, the AUCs of ampicillin in fibrin clots infected with C158 were lower than those in fibrin clots infected with the \( \beta \)-lactamase-negative strain ATCC 10211 (\( P < 0.001 \)). The residual concentrations of ampicillin in infected fibrin clots following the different regimens are shown in Fig. 5. In fibrin clots infected with the \( \beta \)-lactamase-producing strain, ampicillin was rapidly hydrolyzed by the \( \beta \)-lactamase in a few hours, particularly during continuous infusion since no ampicillin could be detected.

**In vivo bactericidal activity.** The comparative in vivo
TABLE 1. In vivo β-lactamase stability of antibiotics over 24 h with the four modes of administration determined by comparison of AUCs of antibiotics in fibrin clots infected with H. influenzae ATCC 10211 or C158

<table>
<thead>
<tr>
<th>Mode of administration and H. influenzae strain</th>
<th>β-Lactamase</th>
<th>AUC ± SD (μg/ml per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aztreonam</td>
</tr>
<tr>
<td>Bolus ATCC 10211</td>
<td>–</td>
<td>271.0 ± 4.5a</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>264.1 ± 5.3</td>
</tr>
<tr>
<td>Intermittent ATCC 10211</td>
<td>–</td>
<td>96.1 ± 6.0a</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>98.5 ± 0.9</td>
</tr>
<tr>
<td>Continuous ATCC 10211</td>
<td>–</td>
<td>93.4 ± 2.0a</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>85.5 ± 21.0</td>
</tr>
<tr>
<td>Bolus plus infusion ATCC 10211</td>
<td>–</td>
<td>128.5 ± 3.8a</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>128.7 ± 5.9</td>
</tr>
</tbody>
</table>

*Not significant by Student's t test.
+ P < 0.001.

The efficacy of antibiotics against β-lactamase-positive and -negative H. influenzae is shown in Fig. 6 and 7. Within 12 h, the bacterial count in the untreated animals was 9 log₁₀ CFU/g of fibrin clots. Aztreonam was the most effective agent in reducing titers of H. influenzae (P < 0.01). Fibrin clots infected by both H. influenzae strains were sterilized within 12 h of bolus and 18 h of intermittent injections of aztreonam. Although less effective, continuous infusion and bolus plus infusion resulted in the respective reduction of 2.8 log₁₀ CFU/g and 3.7 log₁₀ CFU/g.

Administration of cefuroxime by a single bolus injection resulted in an initial decrease in bacterial count (<4 log₁₀ CFU/g) during the first 12 h and a progressive rise thereafter (>5 log₁₀ CFU/g). Intermittent injections of cefuroxime could only sterilize the fibrin clots infected with H. influenzae ATCC 10211. The decrease in the number of H. influenzae C158 was 4.5 log₁₀ CFU/g after the fourth injection. As with aztreonam, the response to the continuous infusion of cefuroxime was very slow during the first 2 h and resulted in a diminution of 1 log₁₀ CFU/g at 24 h.

As expected, administration of ampicillin was ineffective against β-lactamase-producing strain H. influenzae C158.
However, the rate of killing of *H. influenzae* ATCC 10211 was greatly influenced by the different regimens. After bolus injection and bolus plus infusion, *H. influenzae* titers were reduced by 5 and 4 log10 CFU/g, respectively. Intermittent injections reduced the count by 1 log10 CFU/g, and a continuous infusion reduced the count by 0.5 log10 CFU/g. The ratio of peak levels in fibrin clots and serum and the killing index (F = 0.078). A poor correlation between the time above the MIC and efficacy was observed (F = 2.52).

**DISCUSSION**

It is extremely hard to draw any conclusion from the literature on the issue of whether antibiotics should be administered by intermittent administration or by continuous infusion. The difficulty resides in the fact that most investigators have limited their animal or clinical studies to the pharmacokinetic aspects of antimicrobial agents, or else they have evaluated the clinical outcome of antibiotic therapy without specifically analyzing simultaneously the distribution of drugs at the site of infection. Both issues have only rarely been dealt with concurrently (15, 16, 18, 25, 28, 29).

In this study, we studied simultaneously the interrelation between the concentration of antibiotics in serum, interstitial fluid, and fibrin clots and their efficacy in vivo. We showed that a single large injection results in significantly higher peak levels and in higher initial AUCs of drugs in serum, interstitial fluid, and fibrin clots than other modes of administration. Continuous infusions of antibiotics resulted in poor bactericidal activity, whereas other modes of administration showed good antibacterial activity in the first 6 h of the experiments. Thereafter, a single large injection of aztreonam and ampicillin resulted in much more rapid killing of sensitive bacteria than did injection of cefuroxime. Injection

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**FIG. 6.** Comparative in vivo efficacies of aztreonam (A), cefuroxime (B), and ampicillin (C) against β-lactamase-negative *H. influenzae* ATCC 10211 by four different modes of administration. Each point gives the mean value for four rabbits, and the vertical bars indicate the standard deviation.

**FIG. 7.** Comparative in vivo efficacies of aztreonam (A), cefuroxime (B), and ampicillin (C) against β-lactamase-positive *H. influenzae* CL58 by four different modes of administration. Each point gives the mean value for four rabbits, and vertical bars indicate the standard deviation.
of 100 mg of cefuroxime per kg was very effective in the first 12 h, but as drug levels decreased below the MIC, regrowth of bacteria was observed. As demonstrated previously (4, 13) and confirmed in this study, high peak concentrations of antibiotics reached rapidly at the infected site are an important factor in the outcome of therapy. Fibrin clots are extremely hard to penetrate (1), and bolus injections probably allow rapid penetration of antibiotics into the core of the fibrin clots, thus inhibiting the microorganisms which otherwise could have been protected if drugs would have only penetrated the fibrin clots superficially. A large gradient between serum and tissue is probably necessary to allow antibiotics to penetrate all layers of infected tissues. This progressive diffusion of drugs has been demonstrated by other investigators who have used autoradiographic techniques to study the diffusion of labeled antibiotics into abscesses (19, 32). They have shown that there is a decreasing gradient in the concentration of drug toward the interior of tissues. Although the antibiotic levels were above the MIC at most time intervals, the other modes of therapy were less effective than was a single large injection. This observation also suggests that bacteria might have been protected in the core of the fibrin clots. This was especially true in the continuous infusion in which the gradients between serum and fibrin clot concentrations are very low and resulted in poor efficacy. These results are in accordance with results of our previous study in which the combination penicillin-gentamicin given by continuous infusion was less effective in reducing the titer of Streptococcus faecalis in fibrin clots than was a bolus injection (4). Continuous infusion may allow drugs to penetrate deeply in the fibrin, but this requires considerable time, thereby allowing bacteria to be maintained in high numbers in the fibrin clots. These numbers may prejudice antibiotic activity. Furthermore, in the presence of high bacterial counts, more β-lactamase may be produced at the site of infection and may neutralize antibiotics. This may explain why after continuous infusion, no ampicillin could be detected in the fibrin clots inoculated with the β-lactamase-producing H. influenzae. In contrast, after a single large injection, substantial amounts of ampicillin could still be detectable in the clots, even though the strain was resistant.

A continuously maintained concentration above the MIC for the pathogen may not be indispensable in cases of infection in which both host defenses and drugs are operative (7, 13, 28, 29). Schmidt et al. (28, 29) have shown that an acceptable delay of up to 8 h with levels below the MIC is acceptable in some types of infection. Eagle et al. (13) also have demonstrated that some microorganisms continue to be inhibited even after concentrations of antibiotics fall below the MIC. However, high initial tissue levels may be crucial in the initial phase of severe infections caused by bacteria with borderline MICs, especially β-lactamase-producing gram-positive microorganisms which secrete their β-lactamase outside the cell. To cure fulminant infections it is probably necessary to reach high antibiotic levels in tissue very rapidly. The value of the initial levels of antibiotics on the outcome of Streptococcus pneumoniae meningeitis has been stressed recently by McCracken et al. (23). Moreover, studies by Klastersky et al. (21) on aminoglycoside penetration into bronchial secretions have shown that intermittent injections can result in higher levels in the first 2 h after administration of the antibiotic than can continuous infusion, which did not achieve at any time satisfactory inhibitory activity against members of the family Enterobacteriaceae and Pseudomonas aeruginosa recovered in the bronchial tree. Antibiotic levels in bile have also been shown to be higher by the intermittent schedule than by continuous infusion (31). Using their experimental meningitis model in rabbits, Sande et al. (27) observed an enhanced bactericidal effect with intermittent infusion of penicillin early in the treatment period (only between the first and second hours of therapy), which is a reflection of higher levels in cerebrospinal fluid. In this study, during the remaining 6 h of treatment, the magnitude of bacterial killing (change in log titer) was identical in groups of animals receiving either intermittent or continuous dosages.

In our experiments we demonstrated that a continuous

### Table 2: Parameters for the evaluation of penetration and efficacy of antibiotics in fibrin clots infected with H. influenzae ATCC 10211

<table>
<thead>
<tr>
<th>Antibiotics and mode of administration</th>
<th>AUC&lt;sub&gt;0-24&lt;/sub&gt; (clots/serum)</th>
<th>Peak level (clots/serum)</th>
<th>Time (h) above MIC</th>
<th>Log&lt;sub&gt;10&lt;/sub&gt; CFU/g at 24 h</th>
<th>Slope of killing line</th>
<th>Killing index (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aztreonam</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Bolus</td>
<td>0.98</td>
<td>0.10</td>
<td>24.0</td>
<td>−6.6</td>
<td>−0.27</td>
<td>76.6</td>
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<tr>
<td>Intermittent</td>
<td>0.49</td>
<td>0.10</td>
<td>24.0</td>
<td>−6.4</td>
<td>−0.25</td>
<td>72.5</td>
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<td>Continuous</td>
<td>0.61</td>
<td>0.11</td>
<td>23.0</td>
<td>−5.8</td>
<td>−0.12</td>
<td>33.2</td>
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<tr>
<td>Bolus plus infusion</td>
<td>0.51</td>
<td>0.11</td>
<td>24.0</td>
<td>−3.7</td>
<td>−0.15</td>
<td>47.5</td>
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<tr>
<td><em>Cefuroxime</em></td>
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<td>0.43</td>
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<td>Bolus plus infusion</td>
<td>0.34</td>
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<td>24.0</td>
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<td><em>Ampicillin</em></td>
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<td>Bolus</td>
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<td>14.4</td>
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<tr>
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<tr>
<td>Bolus plus infusion</td>
<td>0.21</td>
<td>0.02</td>
<td>22.9</td>
<td>−4.0</td>
<td>−0.14</td>
<td>47.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> AUC<sub>0-24</sub>, AUC from 0 to 24 h.

<sup>b</sup> Killing index was calculated by determining the AUC of bacteria killed versus time.
infusion of 100 mg/kg over 24 h produced levels in fibrin clots at equilibrium similar to those attained with intermittent injections of 25 mg/kg every 6 h, but the penetrability of fibrin during the infusion was slow and resulted in poor killing. These data are in accordance with results of previous investigations involving aminoglycosides (2, 4, 21) and penicillin in infected fibrin clots (4). Moreover, Van Ettal et al. (33) have found the same mean concentrations of oxacillin, gentamicin, and amikacin in a Visking chamber whether antibiotics were administered by intermittent injections or by continuous infusion. Although they observed that the extravascular concentration of ampicillin achieved after continuous infusion was greater than that observed by intermittent administration, our data show that continuous infusion of ampicillin produced levels in fibrin clots at equilibrium similar to those produced by intermittent injections.

The time to reach steady-state levels in fibrin clots during continuous infusion was 12 h for aztreonam, 3 h for cefuroxime, and 2 h for ampicillin, and was directly correlated to their respective degree of serum protein binding (50, 29, 13%). The half-life of these three antibiotics was also a major determinant of the time to reach steady state. Aztreonam has been shown to exhibit a high degree of penetration in fibrin clots compared with previously studied penicillins and cephalosporins (6, 15). Other investigators have also observed the good penetration of this novel monocyte β-lactam antibacterial agent in animal tissue (10) and in human interstitial fluid (36). Moreover, aztreonam has shown a good stability to β-lactamase produced by H. influenzae. Cefuroxime was also resistant to hydrolysis by the β-lactamase in this experiment, but its penetration into interstitial fluid and fibrin clots was lower than that of aztreonam. The persistence of high levels of aztreonam throughout the experiments cannot be explained by a reservoir effect caused by the protein present in either interstitial fluid or fibrin clots since the protein content in those was less than 15% the protein content in serum. In fact, aztreonam had slightly higher protein binding than did cefuroxime and ampicillin, and this should have limited its diffusion into these extravascular spaces.

In the first few hours after injection, drug levels in interstitial fluid paralleled those in serum. Similar results have been reported when ampicillin and cefuroxime were studied in interstitial fluid absorbed by cotton threads implanted under the muscle fascia (26). In contrast, the pharmacokinetics of antibiotics in tissue fluid obtained from tissue cages (11, 12), skin wound models (30), and dialysis units (33), were different; the levels of drugs did not parallel levels in serum and resembled our results for fibrin clots.

It is hard to evaluate the clinical significance of the results of our studies, and one must be careful before suggesting that large doses should be given at long intervals in the acute phase of infection instead of standard intermittent or continuous therapy. On the other hand, innovative approaches to therapy should be considered seriously, since it may, at the same time, improve efficacy and quality of care. As long as antibiotics are maintained at levels above the MIC in tissues, breakthrough bacteremia or recurrence of infection should be prevented (35). Large doses of antibiotics as initial therapy should be further investigated.

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LITERATURE CITED

graphic distribution studies with labelled penicillin, streptomycin, tetracycline, and cycloserine. *In M. Herold and Z. Gabriel (ed.). Antibiotics, advances in research, production and clinical use. Butterworths, London.


