Optimal Times above MICs of Ceftibuten and Cefaclor in Experimental Intra-Abdominal Infections

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The duration of time that serum drug levels remain above the MIC (time above the MIC) for the pathogen has been shown to be the most significant parameter determining the efficacies of beta-lactam antibiotics. In the described study, we investigated the optimal time above the MIC of ceftibuten and cefaclor using a nonneutropenic mouse model of intra-abdominal infections caused by Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Streptococcus pneumoniae. The abilities of the drugs to protect mice against the organisms were determined in mouse protection tests, and the doses were fractionated to produce various dosing regimens with different times above the MIC. All drug-organism combinations showed a significant correlation ($r > 0.9$) between drug efficacy and the time above the MIC. Also, with ceftibuten treatment, the different dosing regimens that produced equal times above the MIC resulted in the same efficacy, whereas with cefaclor, an apparent dose-dependent effect was observed. These results showed that for a 100% recovery from $K$. pneumoniae and $E$. coli infections, the optimal times above the MIC with ceftibuten treatment were 2.2 and 1.6 h, respectively. Relatively high doses of both antibiotics were required to ensure recovery from $S$. pneumoniae infections. In vitro time-kill studies demonstrated that cefaclor exhibits a marked inoculum effect against the pathogens, and there was a concentration-dependent killing at a large inoculum size. On the other hand, ceftibuten showed no inoculum effect. It is suggested that optimization of both dose and time above the MIC appears to be necessary for the treatment of $S$. aureus infections with cefaclor, and this may apply to other beta-lactams that exhibit marked inoculum effects.

The therapeutic efficacies of antimicrobial agents are dependent on a variety of factors, including dosage. To optimize the efficacies of cephalosporins, studies in animals and trials in humans have been undertaken to compare the therapeutic outcomes resulting from different antibiotic dosing regimens. The results of such studies have consistently demonstrated that the major parameter correlating drug efficacy is the duration of time that the serum drug concentration exceeds the MIC (6, 8–10, 14, 18, 19, 24). However, there has been no study to determine the optimal time that drug levels should remain above the MIC in any dosing interval.

Ceftibuten, an orally active cephalosporin, has been demonstrated to have antibacterial activity in vitro against a wide range of gram-negative and certain gram-positive bacteria (11, 22). Its activity against common respiratory tract pathogens was found to be superior or comparable to those reported for other oral cephalosporins (12). Cefaclor has been shown to be effective in the treatment of respiratory tract, urinary tract, and skin infections and also has a broad range of activity against gram-positive and gram-negative bacteria (4, 13).

The study described here was undertaken to investigate, using an experimentally induced infection in mice, the optimal duration of time that the concentrations of ceftibuten and cefaclor in serum should remain above the MICs for representative gram-positive and gram-negative organisms to ensure treatment efficacy.

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MATERIALS AND METHODS

Antibiotics and bacteria. Ceftibuten was supplied by Schering Corp. (Kenilworth, N.J.), and cefaclor was purchased from Sigma Chemical Co. (St. Louis, Mo.). The doses of the antibiotics were prepared in distilled water and are expressed as milligrams per kilogram of body weight. The following organisms were used: $Escherichia$ coli ATCC 25922, Staphylococcus aureus ATCC 29213, Streptococcus pneumoniae ATCC 10813, and Klebsiella pneumoniae ATCC 13883. The MICs and MBCs of the antibiotics for the test organisms were determined by a standard microdilution technique (21).

Mouse protection tests. Swiss Webster female mice each weighing approximately 25 g were obtained from Taconic Laboratories (Germantown, N.Y.). The minimum lethal doses (MLDs) and the 50% lethal doses (LD$_{50}$s) for the test organisms and the dose of the antibiotic required to protect 50% of the infected animals (PD$_{50}$) were determined by the procedures described below.

(i) LD$_{50}$ and MLD determinations. $E$. coli, $S$. aureus, and $K$. pneumoniae were cultured for 20 h at 37°C in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.). $S$. pneumoniae was cultured in Mueller-Hinton broth supplemented with NAD and lyzed horse blood (Difco). The cultures were serially diluted by using 10-fold dilutions. All strains except $S$. pneumoniae were suspended in 8% mucin (Sigma). $S$. pneumoniae was diluted in Mueller-Hinton broth alone, since the organism is sufficiently virulent for mice and does not require mucin to induce disease. A 0.5-ml volume of each culture was injected intraperitoneally into groups of 10 mice each. The LD$_{50}$ for each test organism was calculated from the cumulative mortalities on day 6 from an infectious dose-percent mortality.
the organisms were diluted in 8% mucin to obtain the MLD for each infection. Ten mice were treated subcutaneously at 1 h after infection with 0.2 ml of a single dose of antibiotic. For example, 0.06- to 32-mg/kg doses of cefitabutin were used to treat mice with E. coli infection. Seven or more doses of an antibiotic were tested. The PD\textsubscript{50}s were evaluated from the survival rate recorded on day 6 following the infection. This was determined by interpolation from a curve of percent survival versus dose of antibiotic administered to infected mice. Following the same method, the 25, 75, and 100% protective doses were also calculated.

**Pharmacokinetic study. (i) Drug administration and sample collection.** Pharmacokinetic studies of the test antibiotics in serum after administration of a single dose were determined in mice. Each antibiotic was administered subcutaneously to 10 groups of six mice each. Blood was obtained from the mice in the separate groups by cardiac puncture at 0, 0.08, 0.125, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, and 4 h following drug administration. The blood was centrifuged for 10 min at 2,000 \( \times \) g, and the serum was transferred into polypropylene tubes, frozen, and maintained at \(-70^\circ\)C until it was analyzed.

(ii) Sample analysis. The samples were analyzed by high-performance liquid chromatographic (HPLC) methods. Sample treatment involved the addition of 100 \( \mu \)l of internal standard to a 100-\( \mu \)l sample, and plasma proteins were precipitated by the addition of 1 ml of acetonitrile. The mixture was vortexed and centrifuged at 2,000 \( \times \) g, and the aqueous phase was transferred to a clean tube containing 2.5 ml of dichloromethane. The mixture was vortexed and centrifuged. A total of 25 \( \mu \)l of the upper aqueous layer was injected into the HPLC system through a Waters sample processor (WISP 710A; Waters Associates, Milford, Mass.). Sample detection was done with a Waters model 440 absorbance detector with a fixed wavelength at 254 nm. Chromatographic separations for cefaclor and cefitabutin samples were accomplished on a reversed-phase 5-\( \mu \)m C\textsubscript{18} column (150 by 3.9 mm; Waters). The mobile phase for cefitabutin analysis consisted of acetonitrile and 0.15 M ammonium acetate solution (0.7:99.3 [vol/vol]; pH 7.0) delivered through a Waters chromatographic pump (model 6000 A) at a flow rate of 1.1 ml/min. Cefadroxil (10 \( \mu \)g/ml) was used as the internal standard.

Chromatograms were registered on an integrator (HP 3396 series 11; Hewlett-Packard, Avondale, Pa.). The interday and intraday coefficients of variation were less than 4% at concentrations of both 5 and 40 \( \mu \)g/ml. The assay detection limit was 0.4 \( \mu \)g/ml, and the linearity ranged from 0.5 to 60 \( \mu \)g/ml. The mobile phase for the HPLC assay of cefitabutin consisted of acetonitrile and 0.1 M phosphate buffer (8:92 [vol/vol]; pH 5.6) at a flow rate of 1.2 ml/min, and cefotaxime (6 \( \mu \)g/ml) was used as the internal standard. The limit of detection was 0.2 \( \mu \)g/ml, and the assay linearity range was 0.5 to 20 \( \mu \)g/ml. The intraday coefficients of variation were 6.27 and 2.96% at 1 and 10 \( \mu \)g/ml, respectively, while the interday precisions were 9.0 and 6.07% at 1 and 10 \( \mu \)g/ml, respectively.

(iii) Pharmacokinetic analysis. The pharmacokinetic parameter estimation rate constant, elimination half-life, apparent volume of distribution, and area under the serum drug concentration-time curve were calculated by using a one-compartment model for cefaclor and a two-compartment model for cefitabutin, with first-order elimination, by nonlinear least-squares techniques (PCNONLIN, version 3.0; Statistical Consultants, Lexington, Ky.). Compartment model selection was based on visual inspection of the fit and evaluation of the correlation between observed and calculated values. Drug levels were measured after the administration of multiple doses to confirm the calculated accumulations of the drug in vivo. The accumulation factor was 1/(1 - e\(^{-kt}\)), where \( k \) is the elimination rate constant and \( t \) is time. For each drug, the kinetic profiles were determined at two dose levels to establish whether the kinetics were dose dependent.

**Pharmacodynamic studies.** The PD\textsubscript{50} and the other percent protective doses of the drugs were fractionated to produce different dosing regimens for each drug-organism combination. For example, the dosing regimens with a PD\textsubscript{50} were the PD\textsubscript{50} given as a single dose, and the dosing regimens with PD\textsubscript{50/2}, PD\textsubscript{50/4}, PD\textsubscript{50/8}, and PD\textsubscript{50/16} were the PD\textsubscript{50} administered 2, 4, 8, and 16 times, respectively. The dosing regimens were administered such that the serum drug levels were maintained above the MIC for the pathogen throughout the dosing period of the regimen. For example, a 64-mg/kg PD\textsubscript{50} of cefaclor against S. aureus was administered as regimens of 64 mg/kg as single doses, 32 mg/kg 2 times, 16 mg/kg 4 times, 8 mg/kg 8 times, and 4 mg/kg 16 times with intervals of 1.0, 0.75, 0.53, and 0.35 h, respectively. The antibiotics were administered subcutaneously in 0.2-ml volumes starting at 1 h following the organism inoculation as described above. Thirty mice were treated with each dosing regimen, and 10 mice served as the control group. The cumulative percent survival of the treated animals was recorded on day 6 following the infection. Plots of percent survival versus time the above MIC were generated.

**In vitro studies.** In vitro time-kill studies were performed in Mueller-Hinton broth with concentrations of cefaclor ranging from 0.25 to 128 times the MICs for S. aureus and K. pneumoniae, which were tested separately. The inoculum sizes of the organisms used to generate the time-kill curves were approximately 10\(^6\) and 10\(^8\) CFU/ml. Aliquots were removed from each tube and were serially diluted for determination of the CFU at 0.3, 6, and 9 h following inoculation.

**Statistical analysis.** Linear regression analysis was used to determine the pharmacokinetic parameter-effect relationships. Student's t test was used to compare the activities of both drugs against the same organism, as measured from the PD\textsubscript{50} determination.

**RESULTS**

**MIC and mouse protection test determinations.** The MICs and the results of the mouse protection tests with cefaclor and cefitabutin in combination with the test organisms are given in Table 1. The MICs and MBCs did not differ by more than one dilution for the drug-organism combinations. In general, when comparing both drugs, the in vitro susceptibilities of the organisms reflected the in vivo drug activity.

**Pharmacokinetics.** The pharmacokinetics of the antibiotics in mice were best described by a one-compartment model for cefaclor and a two-compartment model for cefitabutin. Cefitabutin was rapidly absorbed, with peak levels in serum achieved within 5 min, while the peak concentration of cefaclor in serum occurred in 12 min. The pharmacokinetic parameters obtained following administration of single subcutaneous doses of 64 and 52 mg of cefaclor per kg are given in Table 2. Following administration of a single subcutaneous dose of 256 mg of cefitabutin per kg, the derived kinetic parameters were as follows: \( K_{10} \) 3.03 h\(^{-1}\); \( K_{12} \) 0.653 h\(^{-1}\); \( K_{21} \) 2.345 h\(^{-1}\); \( \alpha \) 4.42 h\(^{-1}\); \( \beta \) 1.61 h\(^{-1}\); \( V \) 0.352 liter/kg; \( t_{1/2,\alpha} \) 0.43 h (where \( K_{10}, K_{12}, \) and \( K_{21} \) are absorption and distribution rate constants, \( \alpha \) and \( \beta \) are elimination rate constants, and \( V \) is the apparent volume of distribution).
TABLE 1. Mouse protection tests with cefaclor and ceftibuten against some microorganisms

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MLD(^a) (LD(_{50}))</th>
<th>Drug(^b)</th>
<th>MIC (µg/ml)</th>
<th>PD(_{50}) (mg/kg(^c) [95% confidence limit])</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus ATCC 29213</td>
<td>1 × 10(^6) (10)</td>
<td>Cefaclor</td>
<td>2</td>
<td>64 (45.6–82.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefaclufen</td>
<td>128</td>
<td>&gt;512</td>
</tr>
<tr>
<td>S. pneumoniae ATCC 10813(^d)</td>
<td>2.5 × 10(^2) (100)</td>
<td>Cefaclor</td>
<td>2</td>
<td>140 (98–182)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefaclufen</td>
<td>8</td>
<td>1,024 (717–1,331)</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>5.5 × 10(^4) (1,000)</td>
<td>Cefaclor</td>
<td>8</td>
<td>ND(^e)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefaclufen</td>
<td>0.5</td>
<td>2 (1.4–2.6)</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 13883</td>
<td>1 × 10(^6) (100)</td>
<td>Cefaclor</td>
<td>1</td>
<td>2 (1.4–2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefaclufen</td>
<td>0.06</td>
<td>0.125 (0.088–0.163)</td>
</tr>
</tbody>
</table>

\(^a\) In CFU/mouse.
\(^b\) Drug was administered 1 h after infection.
\(^c\) The PD\(_{50}\) was calculated on day 6.
\(^d\) Mucin was not used to induce infection.
\(^e\) ND, not determined.

is the distribution phase, \(\beta\) is the elimination phase, \(V\) is the volume of distribution, and \(t_{1/2}\) is the elimination half-life. At sampling times of 0.25, 0.50, and 1.0 h following administration of a 4-mg/kg dose, the experimental and calculated concentrations of ceftibuten in serum were 4.6 ± 0.80 and 4.75, 2.48 ± 0.25 and 2.35, and 0.66 ± 0.20 and 0.69 µg/ml, respectively (experimental concentrations are means ± standard deviations). The generalized equation for levels in serum for the curve fitting corresponding to the 4-mg/kg dose was \(C_t = 8.39e^{-4.42t} + 2.97e^{-1.61t}\), where \(C_t\) is the concentration at time \(t\). The kinetic equation was used to predict the concentrations of drug achievable in serum following administration of a 4-mg/kg dose, and these were found to be indistinguishable from the experimental values. These results indicate that the kinetics of both cefaclor and ceftibuten in mice are dose independent. This is in agreement with the dose-independent kinetics also observed for both drugs in humans (1, 16, 26).

**Pharmacodynamics.** Figure 1 shows the relationship between the duration of time that the serum ceftibuten levels exceeded the MIC for *K. pneumoniae* (time above the MIC) and the percent survival of the infected mice. It is evident that, for each dose level, the longer the time above the MIC produced by each regimen is, the greater the drug efficacy is, and the correlation was \(r > 0.98\). It is also apparent that the different dosing regimens that produce equal times above the MIC result in equal efficacies, indicating that the maximal bactericidal effect is concentration-independent. Similar results were obtained with the ceftibuten-*E. coli* combination (Fig. 2). An evaluation of the relationship between the time above the MIC and the efficacy of ceftibuten against *S. aureus* and *S. pneumoniae* was not made because of the relatively high protective doses of ceftibuten that were required (Table 1).

The in vivo pharmacodynamic profile of the cefaclor-\(K. pneumoniae\) combination showed a similar correlation between the time above the MIC and drug efficacy. Also, in this combination, an increase in dose led to an increase in efficacy which was accompanied by a corresponding increase in the time above the MIC (Fig. 3). Administration of different doses of cefaclor to *S. aureus*-infected mice showed a correlation between the time above the MIC and efficacy at a fixed dose (\(r > 0.9\)) (Fig. 4). However, unlike previous observations, it is apparent that different dosing regimens with equal times above the MIC produced different efficacies, with a larger dose eliciting greater efficacy. This suggests a dose-dependent activity. A time above the MIC that produces equivalent degrees of efficacy for the different doses would be expected to result in an overlap of the profiles, as was observed earlier. This observation with the cefaclor-*S. aureus* combination did not appear to be in agreement with the concept that the killing activities of *S. pneumoniae* ATCC 13883.

**FIG. 1.** Relation between time above the MIC and the therapeutic efficacy of ceftibuten against *K. pneumoniae* ATCC 13883. The lowest datum points in each profile represent the drug administered as a single dose, as indicated. The second and third datum points represent the regimen given as equally divided doses of 2 and 4 times, respectively. There was a correlation \(r > 0.9\) in the relations. The optimal time above the MIC for 100% protection with the drug was 2.2 h. •, 0.5 mg/kg; □, 1 mg/kg; △, 4 mg/kg.

### TABLE 2. Pharmacokinetic parameters of cefaclor following subcutaneous administration of single doses of the drug to mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>(C_{\text{max}}) (µg/ml)</th>
<th>(t_{1/2}) (h)</th>
<th>(V) (liter/kg)</th>
<th>AUC (µg · h/ml)/dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>71.51</td>
<td>0.22</td>
<td>0.55</td>
<td>0.57</td>
</tr>
<tr>
<td>32</td>
<td>31.22</td>
<td>0.21</td>
<td>0.58</td>
<td>0.52</td>
</tr>
</tbody>
</table>

\(^a\) \(C_{\text{max}}\), maximum concentration of drug in serum; \(t_{1/2}\), elimination half-life; \(V\), volume of distribution; AUC, area under the concentration-time curve.
ceftibuten is 80 mg/kg; △, 40 mg/kg; ○, 64 mg/kg; ▲, 96 mg/kg; ▲, 160 mg/kg.

Ceftibuten demonstrates an inoculum effect with the gram-negative bacterium K. pneumoniae. In vitro time-kill experiments with an inoculum size of 5.5 × 10⁵ CFU showed that the maximal bactericidal effect was achieved by the drug at the MBC (Fig. 6A). With a larger inoculum size (5.5 × 10⁶ CFU), however, the maximal bactericidal effect was attained at 64 times the MIC (Fig. 6B). It is interesting that the small inoculum size used in vitro was close to the MLD for the organism used in the in vivo studies.

No in vitro inoculum effect could be demonstrated with ceftibuten against either E. coli or K. pneumoniae, because the MICs of the drug remained unchanged when they were tested with small and large inoculum sizes.

**DISCUSSION**

From the results of the mouse protection tests (Table 1), it is evident that ceftibuten is markedly more active than ceftibuten against E. coli and K. pneumoniae infections. This is consistent with the results of in vitro MIC susceptibility tests, which were also in agreement with the results presented in earlier reports (11, 12, 22).

The results of the present investigation demonstrate that there is a high linear relation between the duration of time that the serum drug levels exceed the MIC and the efficacies of the drug. This is in consonance with other reports of the pharmacodynamic characteristics of beta-lactam antibiotics (14, 24). The results of the treatment of mice with K. pneumoniae infection with ceftibuten showed that for 100% recovery of the infected animals, the optimal duration of time that the serum drug levels should remain above the MIC for K. pneumoniae is 2.2 h (Fig. 1). The results with the ceftibuten-E. coli combination showed an optimal duration of 1.6 h (Fig. 2).

Following the administration of different doses of ceftibuten to S. aureus-infected mice at any equal time above the MIC, the higher dose consistently produced greater survivorship. The implication is that the maximal bactericidal effect of the concentration increase was not attained. Earlier recommenda-
This is based with the observed dose-dependent have postantibiotic could be even at that the drug inoculum effect experiments antibiotics was shown that antibiotics. required for the control; *, 1/2 counts (5, 7). The results of the in vitro susceptibility and time-kill experiments revealed that cefaclor exhibits a marked inoculum effect with S. aureus. It is therefore most probable that the lack of a maximal concentration effect of cefaclor, even at a peak serum drug concentration-to-MIC ratio of 55, could be attributed to the pronounced inoculum effect of the drug with the organism. However, it is also possible that the postantibiotic effect of cefaclor could have contributed to the observed dose-dependent efficacy of the drug against S. aureus. This is based on reports (3, 25) that beta-lactam antibiotics have postantibiotic effects with S. aureus and that the duration of the effect is known to be concentration dependent. The dependence of cefaclor activity on inoculum size was further shown in the time-kill experiments with K. pneumoniae. In K. pneumoniae-infected animals, however, a relatively small inoculum size of 10^6 CFU as the MLD was required, and the in vivo pharmacodynamic profile showed no dose-dependent effect. This reflects the concentration-independent killing observed in vitro with a small inoculum size.

The explanations for the observed inoculum effect have not yet been defined. One explanation is that an organism may be less susceptible when it is present in large numbers because of the combined production of β-lactamases (2, 20), or, alternatively, preferential affinity for some penicillin-binding proteins may be involved (23). It is deducible from our results that the optimal duration of time that cefaclor levels in serum should be maintained above the MIC for S. aureus or K. pneumoniae to ensure drug efficacy depends on a dose that may be deemed optimal. The optimal

**FIG. 5.** Time-kill curves of S. aureus ATCC 29213 with initial inocula of 1 x 10^6 CFU (A) and 2 x 10^6 CFU (B) following exposure to cefaclor at concentrations of from 1/2 to 128 times the MIC. The bacterial counts were determined by quantitative subcultures. (A) ○, control; ●, 1/2 x the MIC; □, 1 x the MIC; ■, 4 x the MIC; Δ, 16 x the MIC; ▲, 64 x the MIC; (B) ○, control; ●, 1 x the MIC; Δ, 4 x the MIC; ▲, 16 x the MIC; △, 64 x the MIC; ●, 128 x the MIC.

**FIG. 6.** Time-kill curves of K. pneumoniae ATCC 13883 with initial inocula of 5.5 x 10^5 CFU (A) and 5.5 x 10^7 CFU (B) following exposure to cefaclor at concentrations of from 1/2 to 128 times the MIC. The bacterial counts were determined by quantitative subcultures after 3, 6, and 9 h. (A) ○, control; ●, 1/2 x the MIC; □, 1 x the MIC; ■, 4 x the MIC; Δ, 16 x the MIC; ▲, 64 x the MIC; (B) ○, control; ●, 1 x the MIC; Δ, 4 x the MIC; ▲, 16 x the MIC; △, 64 x the MIC; ●, 128 x the MIC.
dose, on the other hand, appears to be a function of the degree of infection. Hence, optimization of both dose and time above the MIC appears to be necessary for clinical efficacy. It has been asserted that some antibiotics are often used in excessive and apparently illogical dosages (15, 17), but the results of the present study with cefaclor suggest that such high doses may be required, in some cases, to achieve maximum efficacy against infections caused by strains which exhibit a marked inoculum effect when tested in vitro with the drugs.

In conclusion, it is generally difficult to extrapolate results from animal model experiments to the clinical situation in humans. Since infections in humans are usually established for much longer periods of time than the pretreatment infection duration of 1 h used in the present study, caution should be exercised in the direct application of the determined time above the MIC to clinical situations. Knowledge of whether a cephalosporin exhibits a marked inoculum effect with a pathogen appears to be necessary in determining an optimal dosage requirement.

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