In Vivo Activity of Cefquinome against *Escherichia coli* in the Thighs of Neutropenic Mice

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Cefquinome is a cephalosporin with broad-spectrum antibacterial activity, including activity against enteric Gram-negative bacilli such as *Escherichia coli*. We utilized a neutropenic mouse model of colibacillosis to examine the pharmacodynamic (PD) characteristics of cefquinome, as measured by organism number in homogenized thigh cultures after 24 h of therapy. Serum drug levels following 4-fold-escalating single doses of cefquinome were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The pharmacokinetic (PK) properties of cefquinome were linear over a dose range of 10 to 640 mg/kg of body weight. Serum half-lives ranged from 0.29 to 0.32 h. Dose fractionation studies over a 24-h dose range of 2.5 to 320 mg/kg were conducted every 3, 6, 12, or 24 h. Nonlinear regression analysis was used to determine which pharmacodynamic parameter best correlated with efficacy. The free percentage of the dosing interval that the serum levels exceed the MIC ([C<sub>max</sub>/MIC] and 45% for the free-drug area under the concentration-time curve from 0 to 24 h [f<sub>T>MIC</sub>]).

In Vivo

Cefquinome is a fourth-generation cephalosporin which has been developed solely for veterinary use. It shows antimicrobial activity against a broad spectrum of Gram-positive and Gram-negative bacterial species and is stable against chromosomally and plasmid-encoded β-lactamases that are produced by a majority of clinically important bacteria (1, 2). It has shown excellent activity against all the *Enterobacteriaceae*, with MIC<sub>90</sub> ranging from 0.12 μg/ml (3).

*Escherichia coli* is a Gram-negative bacillus belonging to the *Enterobacteriaceae* family. All mammals, poultry, and birds are susceptible to colonization with *E. coli*, and it can be readily transmitted from animals to humans (4, 5). This organism becomes a permanent part of the normal microflora of the gastrointestinal tract. However, pathogenic *E. coli* strains can cause gastroenteritis, urogenital disease, septicaemia, and pleural infections in both humans and animals (6, 7). These strains constitute a significant risk to human and animal health worldwide.

We chose to characterize the pharmacodynamics (PDs) of cefquinome in the neutropenic murine thigh infection model (i) to elucidate the pharmacokinetic (PK)-PD parameter that best correlates with the efficacy of cefquinome and (ii) to determine the PK-PD parameter and the magnitude of the PK-PD parameter predictive of efficacy. It is proposed that these parameters might be used with MIC<sub>90</sub> data for the treatment of infections caused by *E. coli* to provide a rational approach to the design of dosage schedules that optimize efficacy with respect to bacteriological as well as clinical cures.

MATERIALS AND METHODS

Antimicrobial agent. Cefquinome was obtained from commercial sources as a powder (purity, 83.097%). Test solutions of the antimicrobial agent were freshly prepared prior to use.

Bacterial strains. Four *E. coli* strains (65, 195, 172, and 203) isolated from pork and chicken carcasses and *E. coli* ATCC 25922 were evaluated in this study. The organisms were grown, subcultured, and quantified in Mueller-Hinton broth (Guangdong Huankai Microbial Sci. & Tech. Co., Ltd., Guangzhou, China) and Mueller-Hinton agar (Guangdong Huankai Microbial Sci. & Tech. Co., Ltd.).

In vitro susceptibility studies. MIC values were determined by a broth microdilution assay according to Clinical and Laboratory Standards Institute (CLSI) reference methods (8). Determinations were performed in duplicate on three separate occasions. Final results are expressed as the geometric means of these results.

Animals. Six-week-old, specific-pathogen-free, female ICR/Swiss mice (Medical Experimental Animal Center of Guangdong Province, Guangzhou, China) weighing between 24 and 27 g were used for all studies. All animal studies were approved by the Animal Research Committees of South China Agriculture University.

Animals were maintained in accordance with American Association for Accreditation of Laboratory Animal Care criteria (9).

Neutropenic mouse thigh model. The mice were rendered neutropenic (polymorphonuclear leukocyte count, <100/mm<sup>3</sup>) by injection of cyclophosphamide (Puboxin Biotechnology Co., Ltd, Beijing, China) intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) prior to experimental infection (10). The mice were infected by an intramuscular injection of 0.1 ml of inoculum in each thigh (four thighs per...
group per time point). Broth cultures of bacteria were grown to logarithmic phase overnight to an absorbance of 0.3 at 580 nm (UV-2550 spectrophotometer; Shimadzu, Kyoto, Japan). After a 1:10 dilution in fresh Mueller-Hinton broth, the bacterial counts of the inocula ranged from 10⁶ to 10⁷ CFU/ml.

Cefquinome was administered subcutaneously at various time points beginning at 2 h after infection. At specified time points, the animals were sacrificed by CO₂ asphyxiation. After the mice were sacrificed, the thighs were immediately removed and homogenized in 10 ml of 0.9% sterile iced saline. Serial 10-fold dilutions of the homogenized material were plated on Mueller-Hinton agar plates for CFU determination. Each symbol in the figures represents the data from two mice (four thighs).

**PK parameter determination.** The single-dose PK parameters of cefquinome were determined in individual infected neutropenic mice following administration of 10, 40, 160, and 640 mg/kg administered in 0.2-ml volumes by subcutaneous injection. Blood samples were obtained by retro-orbital puncture from each of three mice at the following time points: 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 3, and 4 h after dosing. The samples were then centrifuged for 5 min at 10,000 × g, and serum was removed. Cefquinome concentrations in the serum were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The lower limit of detection was 0.005 μg/ml. The intraday variation was less than 6%. Protein binding in the serum of neutropenic infected mice was analyzed using ultrafiltration methods (11). The degree of binding was measured using cefquinome concentrations of 10 and 100 μg/ml.

Pharmacokinetic constants, including elimination half-life, area under the concentration-time curve (AUC), and maximum serum concentration (Cmax), were calculated by using a one-compartment model with first-order absorption. For treatment doses for which no kinetics were determined, PK parameters were extrapolated from the values obtained in the PK studies described above.

**PD parameter determination.** Neutropenic mice were infected with the standard E. coli ATCC 25922 2 h prior to the start of therapy. Twenty dosing regimens were chosen to determine the impact of dose level and dosing interval on cefquinome efficacy. These 20 regimens comprised five total dose levels (2.5, 10, 40, 160, and 640 mg/kg/24 h). The five total dose levels were fractionated by using four dosing intervals (every 3, 6, 12, and 24 h). Groups of two mice were again used for each dosing regimen. At the end of the study, the mice were euthanized and the thighs were immediately processed for CFU determination.

**Data analysis.** The results of these studies were analyzed by using the sigmoid maximum-effect (Emax) PD model derived from the Hill equation:

\[ E = \left( \frac{E_{\text{max}} \times C^N}{EC_{50} + C^N} \right) \]

where \( E \) is the effect or in this case the log change in CFU per thigh, comparing treated mice and untreated controls after the 24-h period of the study; \( E_{\text{max}} \) is the maximum effect; \( C \) is the PK-PD parameter being examined (e.g., free percentage of the dosing interval that the serum levels exceed the MIC (%T > MIC)); free percentage of the AUC from 0 to 24 h [\( \frac{\text{AUC}_{0-24}}{\text{MIC}} \)]; or \( E_{\text{max}} \times \text{MIC} \); \( EC_{50} \) is the C value required to achieve 50% of the \( E_{\text{max}} \); and \( N \) is a sigmoidicity factor that controls the steepness of the curve. Nonlinear regression analysis was used to determine which PK-PD index best correlated with CFU/thigh at 24 h. The coefficient of determination (R²) was used to estimate the variance that could be due to regression with each of the PK-PD indices.

The best model for each data set was established by using the Akaike information criterion (12).

**RESULTS**

**In vitro susceptibility testing.** The MICs of cefquinome against the study organisms are shown in Table 1. The MICs for the five E. coli organisms studied varied 32-fold (range, 0.031 to 1.00 μg/ml). The MICs for the posttreatment isolates recovered from the mouse thighs did not change after exposure to cefquinome therapy.

**PKs.** The time courses of the mean serum cefquinome concentrations in infected neutropenic mice following single subcutaneous doses of 10, 40, 160, and 640 mg/kg are shown in Fig. 1. These concentrations were used to calculate the various pharmacokinetic parameters listed in Table 2. The decline in the concentrations was best described by a one-compartment model with first-order absorption. Over the dose range studied, kinetics were linear, with the elimination half-lives showing little change with dose escalation. The elimination half-lives varied from 0.29 to 0.32 h. The kinetics at these doses were linear for both Cmax and AUC. Protein binding values at 10 and 100 μg/ml were less than 8%, with a mean of 7.4%.

**Dose fractionation studies.** We determined which PK-PD in-

![FIG 1 Serum cefquinome concentrations in infected neutropenic mice after a single subcutaneous dose.](https://aac.asm.org/FIG.png)


dex correlated best with efficacy by relating the number of bacteria in the thigh at the end of 24 h of therapy with the %\(T_{>\text{MIC}}\), \(fC_{\text{max}}/\text{MIC}\) ratio, and \(f\text{AUC}_{0-24}/\text{MIC}\) ratio for each of the dosage regimens studied. At the start of therapy, mice had 6.55 ± 0.35 \(\log_{10}\) CFU/thigh of \(E.\) coli. The organisms grew at rates of 1.39 ± 0.34 \(\log_{10}\) CFU/thigh after 24 h in saline-treated control mice. The relationships between the antibacterial effects and each of the PD parameters for cefquinome with \(E.\) coli ATCC 25922 are shown in Fig. 2. The strongest relationship was seen when results were correlated with the %\(T_{>\text{MIC}}\) with an \(R^2\) value of 73%. Correlation with the other parameters was not nearly as strong \((f\text{AUC}_{0-24}/\text{MIC}, R^2 = 45\%\), and \(fC_{\text{max}}/\text{MIC}, R^2 = 13\%)\). Consideration of levels of bound or unbound drug did not appreciably affect the relationship between efficacy and %\(T_{>\text{MIC}}\) (data not shown). The above-described studies indicate that treatment efficacy was dependent upon the dosing intervals studied and suggest that efficacy was driven by the %\(T_{>\text{MIC}}\) index.

**Magnitude of %\(T_{>\text{MIC}}\) index for efficacy.** The dose-response curves with 4-hourly dosing of cefquinome for multiple strains of \(E.\) coli are shown in Fig. 3. At the start of therapy, mice had 6.84 ± 0.12 \(\log_{10}\) CFU/thigh of \(E.\) coli. The organisms grew at a rate of 1.81 ± 0.04 \(\log_{10}\) CFU/thigh after 24 h in saline-treated control mice. The magnitude of the %\(T_{>\text{MIC}}\) associated with the doses required to produce a static effect or reduce the numbers of CFU by 1 and 2 \(\log_{10}\) over 24 h are listed in Table 1; the %\(T_{>\text{MIC}}\) varied from 25.86 to 31.57 for stasis, 32.17 to 43.41 for a 1-log kill, and 40.71 to 62.93 for a 2-log kill. The maximum killing observed for the \(E.\) coli ATCC 25922 was 4.05 ± 0.47 \(\log_{10}\) CFU/thigh.

**DISCUSSION**

Clinical and experimental studies indicate that suboptimal antibiotic dosage regimens may be a significant risk factor for emergence of resistance (13, 14). Hence, it is important to optimize dosages, not only with respect to achieving a therapeutic effect but also with respect to minimizing resistance development. Antimicrobial pharmacokinetic-pharmacodynamic (PK-PD) studies are useful to identify the therapeutic potential of a drug through the integration of the PK properties, in vitro potency (MIC), and outcome (15, 16). These approaches may be useful to guide the dosage of antibiotics and the development of susceptibility breakpoints in order to predict efficacy, limit toxicity, and reduce the development of organism resistance (17).

Prior numerous in vitro and in vivo models and clinical trials with cephalosporins have suggested that antibacterial effects best correlate with the duration that drug concentrations exceed the MIC of the microorganism (%\(T_{>\text{MIC}}\)) and that the magnitude of the %\(T_{>\text{MIC}}\) predictive of cephalosporin efficacy ranges from 25 to 70%, depending upon the defined therapeutic endpoint (18–20). Data from the present studies of multiple dosing regimens indicate that the %\(T_{>\text{MIC}}\) is the PK-PD index that best predicts the efficacy of cefquinome. The mean free-drug %\(T_{>\text{MIC}}\) required to produce a static effect against \(E.\) coli varied from 25.86 to 31.57% of the dosing interval, which was lower than observed in previous studies with other cephalosporins against various \(E.\) coli strains. For enteric Gram-negative bacilli such as \(E.\) coli or Klebsiella pneumoniae, a net bacteriostatic effect was observed for cephalosporins when free-drug concentrations in serum were above the MIC for as little as 35 to 40% of the dosing interval, and antibacterial effects appeared to plateau when concentrations were above the MIC for 60 to 70% of the dosing interval (18, 21). In the current investiga-

**FIG 2** Relationships between %\(T_{>\text{MIC}}\), \(fC_{\text{max}}/\text{MIC}\), and \(f\text{AUC}/\text{MIC}\) and the change in the \(\log_{10}\) number of CFU/thigh of \(E.\) coli ATCC 25922. The lines are the best model fits of the data. \(R^2\) is the correlation coefficient.

**FIG 3** Dose-response curves for cefquinome against various strains of \(E.\) coli. Each symbol represents the mean data from two mice (four thighs).

<table>
<thead>
<tr>
<th>Cefquinome dose (mg/kg)</th>
<th>(C_{\text{max}}) (mg/liter)</th>
<th>(T_{\text{max}}) (h)</th>
<th>AUC (mg h · liter(^{-1}))</th>
<th>(t_{1/2}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>640</td>
<td>598</td>
<td>0.35</td>
<td>591</td>
<td>0.31</td>
</tr>
<tr>
<td>160</td>
<td>164</td>
<td>0.33</td>
<td>155</td>
<td>0.32</td>
</tr>
<tr>
<td>40</td>
<td>49.5</td>
<td>0.31</td>
<td>44.1</td>
<td>0.31</td>
</tr>
<tr>
<td>10</td>
<td>13.6</td>
<td>0.27</td>
<td>11.9</td>
<td>0.29</td>
</tr>
</tbody>
</table>

\(a\) \(C_{\text{max}}\) maximum concentration of drug in serum; \(T_{\text{max}}\) time to maximum concentration of drug in serum; AUC, area under the concentration-time curve; \(t_{1/2}\) half-life.
tion, dosing regimens that produced %T>MIC in a similar range produced a 2-log drop. Analysis of the results of the experiments shows that a 2-log drop was not achieved until the %T>MIC had reached 40.71 to 62.93%. Those studies were similar to those for the activities of ceftolozane (57.8%) and ceftaroline (50%) against E. coli (22, 23). In addition, the %T>MIC required to produce a 1-log drop was varied from 32.17 to 43.41% of the dosing interval. This value is similar to those for the activities of other cephalosporins against E. coli. Previous studies found that cefotaxime, ceftriaxone, ceftazidime, cefadroxil, cefpime, cefpirome, cefotaxime, ceftobiprole achieved 1-log drop against E. coli, with %T>MIC of 32 to 57 (19, 22, 24–26).

In summary, cequinom is a potent cephalosporin resulting in more than a 2-log kill over 24 h in the thighs of neutropenic mice for E. coli. The %T>MIC required for stasis was less with cequinome for E. coli than observed with other cephalosporins. The data for cequinome in the mouse models suggest that animal dosage regimens should supply free-drug %T>MIC of cequinome for 50 to 60% of the interval for E. coli. However, it is apparent that optimal cequinome PK-PD targets are not achieved in pigs, sheep, and cattle at current recommended doses (1 to 2 mg/kg). These studies suggest that cequinome, if used for treatment of E. coli infection with an MIC₉₀ of 0.2 µg/ml (27), would benefit from larger (2 to 3 mg/kg) and more frequent (thrice daily) doses than are commonly used in clinical practice.

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REFERENCES