New In Vitro Kinetic Model for Evaluating Bactericidal Efficacy of Antibiotics

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A new in vitro model was devised for evaluating the bactericidal activity of antibiotics under dynamic conditions. This model accurately reproduced the observed serum levels of antibiotics after intravenous dosing. The apparatus consists of two vessels which correspond to the central and peripheral compartments of a two-compartment open model. The volume of medium in each vessel and flow rates of media were determined from the pharmacokinetic parameters calculated from the observed serum levels selected for simulation. The bactericidal activity of cefazolin against strains of Escherichia coli and Klebsiella pneumoniae showing different minimal inhibitory concentrations was investigated with the apparatus simulating serum levels after intravenous injection, and the bactericidal activity was evaluated with respect to the relationship between the minimal inhibitory concentration and the serum levels.

Previously (10) we described an in vitro model designed to simulate stepwise antibiotic serum concentrations for assessing the activity of antibiotics in humans. This previous model simulated serum concentrations in a noncontinuous fashion. In this study, we devised a new kinetic model based on the two-compartment open model (5) whereby antibiotic concentrations can be followed continuously to simulate the variation in antibiotic concentrations in the blood stream after intravenous (i.v.) bolus dosing of humans.

MATERIALS AND METHODS

Antibiotics. The following antibiotics were used: cefazolin (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan), cefamandole (Eli Lilly & Co., Indianapolis, Ind.), cefuroxime (Glaxo Research Ltd., Greenford, England), and cefotaxin (Merck Institute for Therapeutic Research, Rahway, N.J.).

Bacterial strains and media. Test strains of Escherichia coli and Klebsiella pneumoniae were of clinical origin. The minimal inhibitory concentrations (MICs) of the test drugs were determined by the conventional agar dilution method, using 100-fold dilutions of overnight cultures in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) as inocula. The dilutions were spot-inoculated onto heart infusion agar (Difco Laboratories, Detroit, Mich.) containing graded concentrations of the test drugs. The MIC was estimated after incubation for 20 h at 37°C.

Determination of pharmacokinetic parameters. Generally, serum concentrations of antibiotics after i.v. injection are well described by the two-compartment open model. In this theory, the drug concentration-time equation is as follows:

\[ C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]  

where \( A \) is the extrapolated concentration of the \( \alpha \) phase at \( t = 0 \); \( B \) is the extrapolated concentration of the \( \beta \) phase at \( t = 0 \); \( C \) is the serum concentration at time \( t \); \( \alpha \) is the first-order elimination rate constant of the \( \alpha \) phase; \( \beta \) is the first-order elimination rate constant of the \( \beta \) phase; and \( t \) is the time after injection.

Serum concentrations simulated in the kinetic model were analyzed by the two-compartment open model, and the following pharmacokinetic parameters were determined by Marquardt's nonlinear least-squares regression analysis, using a FACOM 230/38 digital computer (Fujitsu Co., Ltd., Tokyo, Japan): \( \alpha \) (hours\(^{-1}\)), \( \beta \) (hours\(^{-1}\)), \( K_{12} \) (hours\(^{-1}\)), \( K_2 \) (hours\(^{-1}\)), \( K_3 \) (hours\(^{-1}\)), and \( C_0 \) (A + B, micrograms per milliliter). \( K_{12} \) and \( K_{21} \) are transfer rate constants between the central and peripheral compartments, and \( K_3 \) is the elimination rate constant for the central compartment. \( C_0 \) is the apparent initial drug concentration. The parameters of antibiotics tested are given in Table 1. These parameters were used for calculation of flow rates, volumes of medium, and dilution factors in the model.

Kinetic model (i.v. bolus injection). A schematic illustration of the kinetic model is shown in Fig. 1. The apparatus consisted mainly of two glass flasks (B and C) connected with airtight silicon tubing and equipped with magnetic stirrers. A peristaltic pump conveyed the fresh medium from flask A to flask B. The apparatus was sterilized before use and set up in an incubator at 37°C. Sterile fresh medium was supplied from flask A to flask B (medium volume [b]) at a constant flow rate, \( F_b \) (milliliters per minute), by a peristaltic pump, \( P_b \). The medium was circulated between flasks B and C at a constant rate, \( F_t \) (milliliters per minute), by pump \( P_t \), and homogeneous mixing was ensured with magnetic stirrers. When any one of the parameters, \( F_t \), \( F_b \), \( b \), or \( c \), is fixed at a rate or volume appropriate to the apparatus size, the others can be calculated from the following equation, using the simulated pharmacokinetic parameters:

\[ F_t \cdot t = F_b \cdot t + b \]
Table 1. Pharmacokinetic parameters of antibiotics and flow rates and volumes for an apparatus simulating serum levels after a 1-g i.v. injection

<table>
<thead>
<tr>
<th>Drug*</th>
<th>$C_0$</th>
<th>$K_{1}$ (h⁻¹)</th>
<th>$K_{12}$ (h⁻¹)</th>
<th>$K_{21}$ (h⁻¹)</th>
<th>$a$ (h⁻¹)</th>
<th>$b$ (ml)</th>
<th>$c$ (ml)</th>
<th>$F_1$ (ml/min)</th>
<th>$F_2$ (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin (9)</td>
<td>232 (58.0) b</td>
<td>0.799</td>
<td>1.82</td>
<td>2.52</td>
<td>4.77</td>
<td>0.433</td>
<td>90.0</td>
<td>63.3</td>
<td>1.20</td>
</tr>
<tr>
<td>Cefamandole (13)</td>
<td>105</td>
<td>1.80</td>
<td>0.505</td>
<td>0.952</td>
<td>2.60</td>
<td>0.660</td>
<td>90.0</td>
<td>47.8</td>
<td>2.70</td>
</tr>
<tr>
<td>Cefuroxime (4)</td>
<td>109</td>
<td>1.21</td>
<td>1.08</td>
<td>1.74</td>
<td>3.41</td>
<td>0.618</td>
<td>90.0</td>
<td>55.9</td>
<td>1.82</td>
</tr>
<tr>
<td>Cefoxitin (6)</td>
<td>187</td>
<td>3.50</td>
<td>2.92</td>
<td>2.13</td>
<td>7.56</td>
<td>0.984</td>
<td>65.7</td>
<td>90.0</td>
<td>3.83</td>
</tr>
</tbody>
</table>

* Number in parentheses indicates reference number.

b In the case of a 0.5-g i.v. injection.

\[
F_1 = b \cdot K_1 \\
F_2 = c \cdot K_{21} = b \cdot K_{12}
\]

where $K_{12}$ and $K_{21}$ (hours⁻¹) are transfer rate constants between the central and peripheral compartments, and $b$ and $c$ are the volumes of flasks B and C, respectively. In this experiment, either $b$ or $c$ was fixed at 90 ml. Then the volumes of the flasks, $b$ and $c$, and the flow rates, $F_1$ and $F_2$, were calculated from the parameters $K_0$, $K_{12}$, and $K_{21}$ of the simulated antibiotics. The actual values of $b$, $c$, $F_1$, $F_2$, and the pharmacokinetic parameters used in this study are listed in Table 1. The antibiotic solution ($b$ milliliters) of concentration $C_0$ (Table 1) was added to flask B, and the drug-free medium ($c$ milliliters) was added to flask C. The test organism was inoculated into flask B, and pumps $F_1$ and $F_2$ were started. The samples were collected from the medium outlet, d, as shown in Fig. 1.

Viable count. The residual antibiotic in the samples was destroyed by adding $\beta$-lactamase, and the samples were diluted with sterile saline and plated for viable cell count by the conventional method (2).

RESULTS

Reproducibility of simulated serum levels. In this kinetic model, simulation of the concentrations of cefazolin, cefamandole, cefuroxime, and cefoxitin in human serum after an i.v. dose of 1 g was verified, and the accuracy and reproducibility of the drug levels were investigated. The simulated drug concentrations in the kinetic model were found to be accurate and to reproduce the observed serum levels of the antibiotics in humans. The accuracy and reproducibility were verified with each of the above-mentioned antibiotics; however, we present here only the data obtained for cefazolin (Fig. 2).

Control growth in the kinetic model. Control growth curves for *E. coli* no. 69 were compared in the kinetic models simulating human serum concentrations of the four test drugs in antibiotic-free medium. In these models, the inoculated medium was diluted with a new sterile medium at a different flow rate: i.e., 1.20 ml/min for cefazolin, 2.70 ml/min for cefamandole, 1.82 ml/min for cefuroxime, and 3.83 ml/min for cefoxitin. These growth curves were also compared with those in a routine static situation.

The control growth curves differed markedly in the kinetic situation and also differed from the control growth curves in the static situation (Fig. 3a). To assess the bactericidal efficacy of an antibiotic in the kinetic model, the dilution effect was adjusted by the following dilution factor, $F(t)$:

\[
F(t) = \frac{\alpha - \beta}{(\alpha - K_{21})e^{-\alpha t} + (K_{12} - \beta)e^{-\beta t}}
\]
where \( \alpha \), \( \beta \), \( K_{21} \), and \( K_{12} \) are pharmacokinetic parameters of the antibiotic and \( t \) is the time after the pumps were started. When each viable count was multiplied by the dilution factor, \( F(t) \), the control growth curves in the model simulating serum concentrations of different test antibiotics were identical to the curve in the static situation (Fig. 3b).

**Relationship of MIC and bactericidal activity.** The relationship of MIC and bactericidal efficacy of cefazolin in the kinetic model was investigated with strains of *E. coli*. The MICs of cefazolin against *E. coli* were 50 \( \mu \)g/ml for strain 519, 25 \( \mu \)g/ml for strain 590, and 3.13 \( \mu \)g/ml for strain 71.

The killing curves for cefazolin under conditions simulating serum concentrations in humans after a single 0.25-g i.v. dose are shown in Fig. 4a. The drug concentrations were maintained above the MICs for 5 min (strain 519), 30 min (strain 590), and 5.2 h (strain 71). The bactericidal effect of cefazolin was the most potent against *E. coli* 71, and no regrowth of bacterial cells was found even when the drug concentrations were decreased to near the MICs. Against strains 590 and 519 (MICs, 25 and 50 \( \mu \)g/ml, respectively), cefazolin showed only a partial bactericidal effect. In these strains the cell counts decreased temporarily, but after 2 h rapid growth resumed.

Under conditions simulating serum concentrations after a single 1-g i.v. dose (Fig. 4b), the drug concentrations were maintained above the MICs for 2 h (strain 519), 3.6 h (strain 590), and more than 8 h (strain 71). In this model, the bactericidal effect of cefazolin was also the most potent against *E. coli* 71. Moreover, the bactericidal effect of cefazolin against *E. coli* 519 and 590 was superior to that of the 0.25-g i.v. dose, the regrowth of these strains being prevented for 4 and 6 h, respectively. The results indicate that an increase in viable cell count is prevented for at least 2 h after the drug concentration is decreased below the MIC. Under static conditions at a concentration of 2\( \times \) the MIC, the viable cell counts decreased to a level of \( 10^2 \) to \( 10^3 \) colony-forming units/ml, and no regrowth was observed for 8 h.

**Bactericidal activity of cefazolin, cefamandole, cefuroxime, and cefoxitin by the kinetic model.** The bactericidal effects of the cephalosporins were studied in the model simulating serum concentrations in humans after a single 1-g i.v. dose. In this experiment, strains with typical susceptibility levels to the cephalosporins were used, i.e., *E. coli* 69 and *K. pneumoniae* 104.

Against *E. coli* 69, cefazolin, cefamandole, and cefoxitin were markedly bactericidal, with similar killing rates for the first 3 h; however, cefoxitin failed to suppress bacterial growth during the ensuing 5 h (Fig. 5a). The most prolonged effect was observed with cefazolin, and no significant growth of the bacterial cells occurred during the 8-h incubation. The killing curve obtained in this model showed that the bactericidal effect of cefoxitin was transitory and effective only during the initial 3 h.

The test antibiotics were similar in their bactericidal activity against *K. pneumoniae* 104. Cefazolin had the most potent killing effect, with a rapid and prolonged inhibition of bacterial growth. Cefuroxime and cefamandole caused a marked decrease in cell counts during the first 6 h of incubation; however, regrowth occurred.

Conversely, the killing effect of cefoxitin was
transitory and was only effective in the first 3 h, as was the case with *E. coli* 69.

**DISCUSSION**

In our previous paper (10), we reported on a kinetic model for investigating in vitro activity of antibiotics in concentration-time curves simulating drug serum levels under various conditions.

In the earlier kinetic model stepwise serum concentrations were simulated by adding the drug solution or by periodically diluting with the drug-free medium. The model was successfully used for assessing bactericidal efficacy in a dynamic situation (8, 12). However, when drug concentrations vary widely and change quickly, as is the case with serum levels in humans after an i.v. dosing, this model failed to fully simulate these fluctuations. To simulate human serum levels as closely as possible, we devised a new kinetic model based on the two-compartment open model. With this new model, drug concentrations were accurately reproduced and closely followed the actual dynamic situation.
It was apparent by our new model that gram-negative rods required a significant and definite time to initiate regrowth after exposure to cephalosporins. Parker and Marsh (11) observed the same phenomenon in gram-positive cocci treated with penicillin in a routine static situation and called it the recovery period. Eagle et al. (3) proposed a dosage schedule for penicillin therapy in infectious diseases based on the findings of Parker and Marsh. From a similar viewpoint, the kinetic model described in this paper can help to establish suitable dosage schedules for antibiotics.

More recently, Al-Asadi et al. (1) devised a new kinetic model for investigating in vitro activity of antibiotics under different concentration-time curves. In their model, drug concentrations in the culture compartments were mainly controlled by diffusion through a dialysis membrane. The model possesses apparent merit in that the dilution of bacterial cells is not accompanied by dilution of the medium. It is, however, difficult to simulate serum concentrations by various dosing routes because the penetration constant of the dialysis membrane is affected by the kind of membrane.

In the kinetic model of Grasso et al. (7), based on the theory of the one-compartment open model system, the dilution factor was considered negligible and was omitted. Our experiments indicated that the dilution factor is not negligible and that differences in control growth curves between the static and dynamic models do exist. The need for rigid correction of the defects of the Grasso kinetic model was obvious, as was the case with our kinetic model. In their model, observed viable cell count must be corrected by the following equation:

\[ F(t) = e^{K_r t} \] (6)

where \( K_r \) is the elimination rate constant and \( t \) is the time after the pump was started. The Grasso model simulated human serum antibiotic levels after oral or intramuscular dosing, and the bactericidal activity of antibiotics could be evaluated after correction of the observed viable cell counts by the dilution factor (6). Serum levels after i.v. drip infusion can also be simulated by a slight modification of the kinetic model described in this paper.

We are now preparing another mode which flow dialysis cells are connected to the model described here to assess the activity of antibiotics in the serum and tissue fluids under dynamic conditions.

LITERATURE CITED


