Application of Mathematical Model to Multiple-Dose Experimental Chemotherapy for Fatal Murine Pneumonia

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Two beta-lactam antibiotics, cefazolin and cefmenoxime, were administered for 7 days to mice with pneumonia caused by Klebsiella pneumoniae by using dosage regimens that would simulate multiple dosing in usual clinical treatments at dosing intervals of 8 or 12 h. Viable numbers of the bacteria in the lungs were measured at 12- or 24-h intervals. The mathematical model established in a previous single-dose study was applied in this study to explain the time courses of the changes in bacterial count over 7 days. However, because the error in viable count measurements was larger than that in the previous study, the time course of the changes in mean viable count was not regular and the viable count reduction rate changed during multiple dosing, and therefore it was difficult to explain the time course by repeated application of the mathematical model described previously. This study suggests that the changes in pharmacokinetic and pharmacodynamic parameters during multiple dosing need to be considered.

In a previous report (3), we applied a mathematical model to the bacterial killing curves observed during experimental treatments by using two beta-lactam antibiotics, cefazolin and cefmenoxime, and a fatal murine pneumonia model. The drugs were administered to the infected mice by using dosage regimens that resulted in serum antibiotic levels that simulated those in humans after single-drip infusions. The mathematical model explained the bacterial count at time t after dosing by using the pharmacokinetic and pharmacodynamic parameters.

Continuing along the same lines as the previous study, which was considered to be a simulation of single dosing in humans, we performed the present study as a simulation of multiple dosing in humans. By considering multiple dosing with interval time t, the model described above can be applied repeatedly by taking the bacterial count at time t after the previous dose as the initial count for the succeeding dose. We can thus predict the time at which complete clearance of bacteria will be achieved if the numerical values of the pharmacokinetic and pharmacodynamic parameters do not change throughout the dosing period.

For each drug we determined two intervals, one that would produce and one that would not produce complete clearance in several days, and tested for the feasibility of applying the mathematical model to multiple dosing.

MATERIALS AND METHODS

Animals. A total of 150 male DDY mice were infected with Klebsiella pneumoniae B-54 with an initial inoculum of 500 times the 50% lethal dose, that is, \(5 \times 10^6\) CFU per mouse, and were then subjected to antibiotic therapy 24 h later. These mice were 3 weeks old and weighed about 12 g.

Bacteria. K. pneumoniae B-54 was isolated from a patient with very severe pneumonia in 1978. The 50% lethal dose of this strain was \(9.7 \times 10^4\) CFU per mouse in this experimental pneumonia model.

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Antibiotics. Cefazolin (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) and cefmenoxime (Takeda Chemical Industries Ltd., Osaka, Japan) were used. The MICs of cefazolin and cefmenoxime against K. pneumoniae B-54 were measured by the agar well method. The MIC of cefazolin was 1.56 µg/ml, and the MIC of cefmenoxime was 0.013 µg/ml.

Antibiotic assay. Cefazolin and cefmenoxime in plasma samples were assayed by the agar well method with Escherichia coli NIHJ as the test organism. The sensitivity was about 0.1 µg/ml, and the coefficient of variation from replicate analyses between days was ±7.0%, as in the previous study (3).

Doses and dosage regimens. Table 1 shows the dosing regimen which was established in the previous study (3) to enable simulation of the time course of the changes in serum antibiotic levels following a single-drip infusion in humans. In the present study, this regimen was used repeatedly to simulate multiple-drip infusions in humans. The total dose per regimen in mice corresponded to 17.0 mg of cefazolin per kg of body weight and 20.3 mg of cefmenoxime per kg administered to humans by single-drip infusion over 1 h. By using these doses and the human pharmacokinetic parameters obtained in the previous study, serum drug levels (c) in mice at time t after drip infusion are approximated by

\[ C_{\text{cefazolin}} = 17.0 \left( 1.71 \exp(-2.17t) + 2.78 \exp(-0.40t) \right), \]

\[ C_{\text{cefmenoxime}} = 20.3 \left( 1.82 \exp(-2.58t) + 0.72 \exp(-0.69t) \right). \]

We set up two treatment groups for each drug: dosing twice a day at 12-h intervals and dosing three times a day at 8-h intervals. Thirty-six mice were allocated to each of the four treatment regimens.

Measurement of bacterial counts. At the start of treatment and at 11 time points, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, and 168 h, after the initial dose, three mice per treatment regimen were sacrificed by exsanguination and the lungs were aseptically removed. Each lung was homogenized with aseptic saline solution, which was used at eight times the lung volume. After homogenization the number of viable bacteria was measured by the quantitative culture method with a detection limit of 10 CFU per lung. The coefficient of
TABLE 1. Dosing regimen for mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total dose (mg/kg)</th>
<th>Dose (mg/kg) administered subcutaneously at the following times after the initial dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>126</td>
<td>16</td>
</tr>
<tr>
<td>Cefmenoxime</td>
<td>84</td>
<td>20</td>
</tr>
</tbody>
</table>

* The multiple subcutaneous injection in mice was established in the previous study (3) to simulate the serum concentration time course in humans when the drugs were given by single-drip infusion over 1 h. Repetition of this regimen was considered to simulate multiple drip infusions in humans. By using the differences between AUCs for mice and those for humans, the total doses for mice were converted to the corresponding doses for humans; 17.0 and 20.3 mg/kg for cefazolin and cefmenoxime, respectively.

variation from replicate measurements within a day was about ±5.0%, in log_{10} CFU.

The mathematical model and estimation of parameters. The mathematical model for single dosing was established in the previous study (3) as

\[ \ln(C_t/C_{00}) = K_pf - KD^n \sum_i (A_i/B_i[1 - \exp(-B_i/E)]) \]  

where \( C_t \) and \( C_{00} \) are the bacterial counts at time \( t \) and \( t = 0 \) after the initial dose, respectively, \( K_p \) and \( D \) are the rate constants of bacterial growth and antimicrobial killing, respectively, \( K_f \) is the dose of the drug administered, \( n \) is the degree of antibiotic distribution into the tissue, \( E \) is the time lag between drug concentration time courses in serum and that in lung, and \( A_i \) and \( B_i \) are pharmacokinetic macro- and microconstants in a two-compartment model, respectively (\( i = 1, 2 \)).

The parameters were estimated in the previous study as \( K_p = 0.47 \) h^{-1} (in common with cefazolin and cefmenoxime), \( K_{cefazolin} = 0.40 \) h^{-1}, and \( K_{cefmenoxime} = 0.66 \) h^{-1}; \( n_{cefazolin} = 0.007 \) and \( n_{cefmenoxime} = 0.271 \); and \( E_{cefazolin} = 1.08 \) and \( E_{cefmenoxime} = 3.09 \). The pharmacokinetic parameters in a two-compartment open model were described above.

Equation 1 was modified by the insertion of the additional parameter \( M \) to represent the overall change in the successive killing phases caused by multiple dosing. The modified equation is

\[ \ln(C_t/C_{00}) = K_pf - MKD^n \sum_i (A_i/B_i[1 - \exp(-B_i/E)]) \]  

When \( t \rightarrow \infty \), the second term of the right side can be approximated by the following equation by using the area under the concentration-time curve from 0 h to infinity (AUC_{\infty})

\[ \ln(C_t/C_{00}) = K_pf - MKD^n \text{AUC}_{0-\infty} \]  

Similar to the above approximation, the case of 12-h interval can be shown as follows:

\[ \ln(C_t/C_{00}) = K_p(12 \times \text{dosing frequency}) - MKD^n \text{AUC}_{12-12} \times \text{dosing frequency} \]  

Given the above parameter values of \( K_p, K_f, n, A_i, \) and \( B_i \) (\( i = 1, 2 \)), the data of bacterial counts for each regimen were subjected to least-squares analysis by using equation 4 to give the estimates of \( M \).

If \( M = 1 \), the killing curve under multiple dosing is the complete repetition of the single wave obtained in the previous single-dose study, while \( M > 1 \) and \( M < 1 \) lead to faster and slower bacterial reductions, respectively.

FIG. 1. Time courses of observed and simulated bacterial killing by cefazolin with dosing twice a day at 12-h intervals.

RESULTS

Figures 1 through 4 are the plots of the bacterial counts in log_{10} CFU for each treatment. The vertical and horizontal axes represent log_{10} CFU and time after initial dose, respectively. The broken lines represent mean viable counts (log_{10} CFU) from three mice with associated standard deviations (SDs).

Changes in observed bacterial counts. The initial mean log_{10} CFU was 6.97 in all the treatment groups, and good agreement was noted with the value of 6.94 obtained in the previous study (3).

Twice-daily treatment with cefazolin produced little bacterial count reduction, and treatment three times a day reduced the number of bacteria to a residual mean log_{10} CFU of 1.64 on day 7. In contrast, both twice and three times daily treatment with cefmenoxime gave rapid reduction, yielding residual mean log_{10} CFUs of 1.48 and 1.39, respectively, on day 4. The killing curves for cefmenoxime showed a characteristic shape with three phases; a steep reduction on day 1 followed by a slacking off during days 2 and 3 and another

FIG. 2. Time courses of observed and simulated bacterial killing by cefmenoxime with dosing twice a day at 12-h intervals.
Each three at fazolin case treatment group, which resulted in reduction at the later 2.17 (1.47); 0.8. at stabilized for cefmenoxime the interval, 3.07 (1.75); 2.4 and the solid waves are those obtained by using the estimated M values.

For 12-h intervals. The 12-h interval simulation represented by the dotted curves in Fig. 1 through 4 lay far above the observed counts, which decreased much faster. The solid simulation curve obtained by using the estimated values of M showed a better fit to the respective observed counts than the dotted line with M = 1 did. The estimated values were M = 1.369 for cefazolin and M = 1.335 for cefmenoxime.

For 8-h intervals. The 8-h interval simulation gave dotted simulation curves that differed much less from the observed values than those obtained in the 12-h interval simulation did. The estimates were M = 1.077 for cefazolin and M = 0.928 for cefmenoxime, giving the solid simulation curves which did not deviate greatly from the M = 1 simulation.

Since the change in the mean log10 CFU, on the other hand, did not exhibit a regular monotone time course, the simulation of solid waves did not give a good fit in the early half of the time course, even when the estimated M was used. However, from day 5 on, when complete bacterial removal was close to being attained, a smaller deviation of the solid simulation waves from the observed values was noted.

**DISCUSSION**

The mathematical model for multiple dosing described here is appropriate when the viable count log10 CFU decreases linearly with time. However, since no regular monotone reduction was observed, the simple repetition of the curve generated from the previous single-dose study (3) did not work satisfactorily in the case of multiple dosing. The reason for this appears to be that we attributed the change in the pharmacokinetic and pharmacodynamic parameters obtained in the previous single-dose study solely to M, a global degradation constant. Also, it was found to be difficult to interpret M as a function of dosing frequency or time.

The causes of nonmonotone changes in the mean observed values include, first, the large errors in log10 CFU; second, the small number of animals used, which led to somewhat unreliable mean values per measurement time point; and third, possible irregular changes in pharmacokinetic and pharmacodynamic parameters during multiple dosing.

Such changes in vivo antimicrobial activity during multiple dosing could be affected by the changes in the pharmacokinetic parameters AUC, maximum concentration of drug in serum, and duration of time levels exceed the MIC (t-MIC) and in the pharmacodynamic parameters postantibiotic effect, postantibiotic leukocyte enhancement, drug distribution into the tissue, and susceptibilities of organisms.

Concerning pharmacokinetic parameters, Drusano (2), Leggett et al. (4), and Vogelman et al. (10) showed that t-MIC is the most important parameter in the case of beta-lactams and gram-negative bacilli. As shown previously (3), t-MICs with *K. pneumoniae* were 10 h for cefazolin and 11 h for cefmenoxime; therefore, both drug treatments at 8-h intervals satisfied the requirements for the MIC for *K. pneumoniae* while neither of the treatments did so when...
they were conducted at 12-h intervals. This insufficiency may have caused the gaps in viable counts seen between treatments at 8- and 12-h intervals.

With respect to pharmacodynamic factors, Mattie and van der Voet (7) reported that the killing and growth rates were variable and could change during the dosing period. Vogelman et al. (10) showed the phenomena termed postantibiotic effect and postantibiotic leukocyte enhancement. Tuomanen et al. (9) reported phenotypic tolerance in the stationary phase of growth and stated that the antimicrobial effect generally appears after the onset of growth of microorganisms.

However, the changes in pharmacokinetic and pharmacodynamic factors during multiple dosing have not yet been studied.

During multiple dosing, host-dependent factors become important, in addition to the changes in pharmacokinetic and pharmacodynamic parameters. The K. pneumoniae-infected mice used in this study were nonneutropenic, and their susceptibilities to K. pneumoniae could affect the in vivo antimicrobial effects. For example Coonrod (1), MacDonald et al. (5), and Roosendaal et al. (8) described phagocytosis by neutrophiles as a host-dependent factor. Matsumoto et al. (6) have considered that histopathological findings of the infectious focus change from the initial stage of infection to the maturing and healing stages and histological changes become more complicated with the addition of chemotherapy.

Although no histopathological examinations were conducted in the present study, it is reasonably assumed that, if interindividual differences were increasingly enhanced in the course of multiple dosing with respect to the size of the infected lesion and the histopathological picture of the mice, errors in log_{10} CFU may have been magnified compared with the case of single dosing.

These host-related factors would naturally exert some influence on the pharmacokinetic and pharmacodynamic factors, leading to changes in the antibacterial effect and the nonmonotone time course of bacterial count.

In the mathematical model reported previously (3), parameter $E$ is related to the duration of the antimicrobial action, with larger $E$ values being associated with prolonged effects, while the parameters $K$ and $n$ are related to the strength of the effect, with larger values of $K$ and $n$ indicating stronger activity. To follow up changes in the individual parameters $K_b$, $K$, $E$, and $n$ during multiple dosing, more frequent bacterial counting concentrated in a shorter period will have to be repeated intermittently, unlike in the present experimental design for multiple dosing, which was based on measurement at equal intervals over a long duration. Furthermore, if the tissue drug levels could be determined along with the bacterial count, the substantial effect of the nonmonotone change, if any, would be explained.

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REFERENCES