ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1997, p. 2083–2088 Vol. 41, No. 10
0066-4804/97/$04.00+0

Pharmacokinetic and Pharmacodynamic Models of the Antistaphylococcal Effects of Meropenem and Cloxacillin In Vitro and in Experimental Infection

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Received 22 April 1996/Returned for modification 9 September 1996/Accepted 14 July 1997

The efficacies of meropenem (MPM) and cloxacillin (CLC) against two *Staphylococcus aureus* strains were established in vitro. A pharmacodynamic model equation, based on the concept that the killing rate depends on concentration and time, was fitted to the numbers of CFU. The parameters of the equation are maximum killing rate, time point of maximum killing, and 50% effective concentration (EC_{50}). The EC_{50}s for the two strains were 0.047 and 0.040 mg/liter, respectively, for MPM and 0.105 and 0.121 mg/liter, respectively, for CLC. Calculated values of the parameters were used to predict the numbers of CFU at exponentially decreasing concentrations in vitro as well as in an experimental infection model. The prediction for in vitro conditions gave a satisfactory fit (R^2, between 0.862 and 0.894). In vivo the numbers were predicted with the assumption that killing rate in vivo is proportional to that in vitro (R^2, between 0.731 and 0.973). The proportionality factor ranged between 0.23 and 0.42; this variation was due mainly to covariation with growth rates in control animals, without other significant differences between antibiotics or strains.

One of the goals of developing pharmacokinetic and pharmacodynamic models for antibacterial drugs is their potential usefulness for predicting clinical efficacy. In previous work (2, 4, 7, 8) we used the maximum killing rate in vitro in the presence of a constant concentration of an antibiotic as a single parameter for the antibacterial effect. This parameter was then used to predict the effect in an in vivo model with nonconstant concentrations. Although a good correlation could be shown between the predicted and the observed effects, this model did not take into account that even at a constant concentration in vitro the killing rate is not constant but is time dependent. Therefore, a new pharmacodynamic model for the in vitro effect that takes into account this time relation was developed. In the present study this model was used to predict the antistaphylococcal effects of meropenem (MPM) and cloxacillin (CLC) at nonconstant concentrations in vitro and in vivo. MPM was chosen because it is a parenterally administered carbapenem antibiotic with an exceptionally broad spectrum of antibacterial activity, characterized by a bactericidal effect against gram-positive as well as gram-negative bacteria; it is stable in the presence of β-lactamase. It was compared to CLC, because CLC has been shown to be an effective β-lactam antibiotic for the treatment of *Staphylococcus aureus* infections. The in vivo experiments were carried out with irradiated granulocytopenic mice to minimize the effects of host factors.

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MATERIALS AND METHODS

Antibiotics. The antibiotics used in this study were MPM (activity, 87.72%; ICI, Macclesfield, United Kingdom) and CLC (activity, 90.5%; Beecham, Amstelveen, The Netherlands). Stock solutions of MPM and CLC were prepared fresh in phosphate-buffered saline (PBS; pH 7.5).

Microorganisms. The organisms used in this study were *S. aureus* ATCC 25923 (strain 1) and *S. aureus* 42D (strain 2), which originated from clinical material. Samples drawn from 18-h cultures were stored at −70°C. Before each experiment, aliquots were rapidly thawed in a water bath at 37°C and were diluted 1:1,000 in brain heart infusion (BHI) broth.

Animals. Female Swiss mice (specific pathogen-free; weight, 20 to 25 g) were obtained from Broekman Institutes, Someren, The Netherlands.

Experiments in vitro. (i) MIC determinations. MICs were determined in a twofold dilution series with an inoculum of approximately 5×10^5 CFU/ml in BHI broth (Oxoid, Basingstoke, England).

(ii) Short-term growth at constant and decreasing concentrations in vitro. In all experiments the bacteria were in the logarithmic phase of growth at the time of exposure to the antibiotic. After 1 h (strain 2) or 2 h (strain 1) at 37°C in a shaking water bath 50-ml aliquots were pipetted into 100-ml flasks and MPM or CLC at various concentrations was added. The culture flask was incubated at 37°C in a shaking water bath for the next 6 h. A continuous dilution system (Peristaltic 2000; Verder, Vleuten, The Netherlands) was connected to the flasks. Continuous dilution was ensured by pumping BHI broth at a constant rate (between 47.5 and 48 ml/h) into the culture flask and extracting the same volume simultaneously at the same rate (3). For experiments performed with a constant concentration, the concentration of the antibiotic in the inflowing BHI broth was the same as that in the culture, ranging from 0.016 to 8 mg/liter. For experiments performed with decreasing concentrations, the inflowing medium contained no antibiotic; initial concentrations in these experiments ranged from 0.063 to 8 mg/liter. A clearance of 48 ml/h from the culture at a volume of 50 ml resulted in an exponential elimination rate of 0.955 h^{-1}, corresponding to a half-life of 44 min, for the microorganism as well as the antibiotic. At various times 0.1-ml samples were taken, PBS was added to obtain appropriate dilutions, six 10-μl drops were placed on diagnostic sensitivity plates (DST agar; Oxoid), the plates were incubated overnight at 37°C, and the numbers of CFU were counted.

(iii) Pharmacodynamic model of bacterial growth and killing. In the presence of β-lactam antibiotics the growth rates of the microorganisms are not constant but change with time (2): at any given concentration the killing rate increases with time to a maximum and then decreases again. Moreover, a certain fraction of the inoculum is not inhibited, at least at intermediate concentrations, resulting in regrowth after some duration of exposure. In a previous study (3) growth and killing curves for *S. aureus* in the presence of MPM and CLC at constant concentrations in vitro could be fitted to a biexponential model (according to an equation of the general form $q(e^{-r1t} - e^{-r2t})$. Since the calculated values of the two exponential coefficients $r1$ and $r2$ were found to be practically identical, a monoexponential model was used in the present study, according to an equation

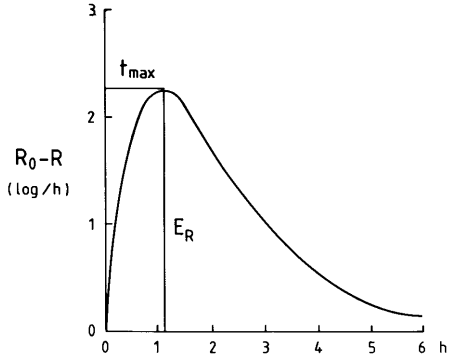

FIG. 1. Graphic representation of the pharmacodynamic model of the relationship between the rate of killing and time t according to equation 1 (see Materials and Methods).

of the general form qte^{-rt}. Substituting characteristics of bacterial killing the following equation is then derived:

$$R_0 - R = e^1 E_R \frac{t}{t_{max}} e^{-t/t_{\max}} \quad (1)$$

In this equation the net rate of killing is represented by $(R_0 - R)$, in which R_0 is the growth rate in the absence of antibiotic and R is the growth rate in the presence of antibiotic; e is the base of natural logarithms; E_R is the maximum value of $(R_0 - R)$ (previously used as a single-effect parameter [3]), and $t_{\max}$ is the time point corresponding to E_R. In Fig. 1 this mathematical model is represented by a graph, showing the relation between net killing rate, expressed as $\log_{10}$ per hour, and time. This graph may be seen as representing the distribution of survival times of individual bacteria (corrected for doubling time) for a given concentration; $t_{\max}$ is thus related to the mean survival time and E_R is related to the fraction of the inoculum that survives in the presence of the antibiotic (see below).

Integration of equation 1 leads to the equation for the actual numbers of CFU (N):

$$\log N = \log N_0 + R_0 t - \int_0^t e^1 E_R \frac{t}{t_{\max}} e^{-t/t_{\max}} dt \quad (2)$$

where N_0 is the number of CFU at time zero. Since equation 1 represents the distribution of survival times, the graph of the actual numbers of CFU may represent the cumulative distribution of survival times for a given constant concentration. The value of E_R is equal to the difference in slope between the curve for the control and the steepest part of the killing curve.

Equation 2 can be developed formally to equation 3:

$$\log N = \log N_0 + R_0 t - e^1 E_R [t_{\max} - (t_{\max} + t) e^{-t/t_{\max}}] \quad (3)$$

From this equation it follows that the difference in log numbers between the control culture and a culture in the presence of the antibiotic approaches a constant value, i.e., $-e^1 E_R t_{\max}$. This value represents the fraction of the inoculum that proliferates in the presence of the antibiotic.

Equation 3 was used to estimate $t_{\max}$ and E_R for various constant concentrations. Since the error in bacterial counts is proportional to the number, least-squares nonlinear regression analysis of the log numbers of CFU was performed (NONLIN; SYSTAT, version 5.0; Systat Inc., Evanston, Ill.).

The relationship between the estimated values of E_R and the concentration could be described by a Hill equation:

$$E_R = S \frac{C^{s_1}}{C^{s_1} + EC_{50}^{s_1}} \quad (4)$$

in which C is the concentration, EC_{50} is the concentration at which E_R is 50% of its maximum value S, and s_1 is a constant of the equation that determines the steepness of the concentration-effect curve. Moreover, the relationship between the reciprocal values of $t_{\max}$ and the concentration could also be described by a Hill equation. Therefore,

$$t_{\max} = T \frac{C^{s_2} + EC_{50}^{s_2}}{C^{s_2}} \quad (5)$$

in which EC_{50} is the concentration at which $t_{\max}$ is twice its minimum value T, and s_2 is a constant of the equation. Preliminary analysis, performed for the two strains and the two antibiotics, indicated that EC_{50} for E_R and for $t_{\max}$ converged to nearly identical values.

Substitution of equations 4 and 5 into equation 3 leads to a model equation describing the concentration-dependent time course of the numbers of CFU. The final regression model used only five parameters, namely, S, T, s_1, s_2, and EC_{50}. This model was fitted to the total data for one strain and one antibiotic.

The pharmacodynamic model according to equation 2 was then used to predict the effect of exponentially declining concentrations in vitro as follows. Equations 4 and 5 were substituted into equation 2 by using the parameter values calculated at constant concentrations, and $C_0 e^{-0.955t}$ was substituted for C (C_0 is the initial concentration) by using the elimination rate constant of the in vitro system. Then the integral was numerically calculated for each time point with a 28S Advanced Scientific Calculator (Hewlett-Packard, Corvallis, Oreg.).

Experiments in vivo. (i) Thigh infection model in granulocytopenic mouse. For the in vivo studies the stored culture was diluted 1:20 in BHI broth to obtain a suspension of about 5×10^7 CFU/ml. Mice were rendered granulocytopenic by total-body irradiation consisting of 6 Gy delivered by a 6-MV linear accelerator (SL 75/6; Philips, Eindhoven, The Netherlands). The effect was maximal on day 5 after irradiation, when the number of granulocytes was about 0.07×10^9/liter. On that day, *S. aureus* 1 and 2 were simultaneously injected into the two thigh muscles of a mouse. The bacteria were allowed to grow in the thighs for 1 h, and then 80 mg of MPM or CLL per kg of body weight was administered subcutaneously. Control mice received saline. Two animals each, one treated and one untreated, were killed at 0.25, 1, 2, 4, 5, 6, 8, and 10 h after administration of the drug or saline. The thighs were isolated from the femur and were homogenized for 30 s in 5.0 ml of sterile ice-cold PBS in a tissue homogenizer (Ystrall type X-1020; International Laboratoriums Apparate, Dottingen, Germany). Tenfold dilutions of the muscle homogenate were made in PBS, and appropriate dilutions were plated to obtain bacterial counts.

(ii) Pharmacokinetics. The pharmacokinetics of the antimicrobial agents in plasma after administration of a single dose were established. Antibiotics were administered subcutaneously at a dose of 80 mg/kg. Blood samples were collected by cardiac puncture with heparinized syringes after the animals had been killed by exposure to 100% CO_2. The samples were centrifuged at $1,500 \times g$ for 10 min at room temperature, the plasma was removed, and the drug concentration was measured on an agar gel (Oxoid, pH 7.0); the indicator organisms were *Escherichia coli* NIHJ for MPM and *Bacillus stearothermophilus* var. *calidolactis* for CLC. Appropriate twofold dilutions of the samples were prepared with pooled murine plasma. Standards were prepared in the same way, ranging from 0.4 to 0.025 μg/ml for MPM and from 0.5 to 0.032 μg/ml for CLC. The temperature for overnight incubation was 30°C for *E. coli* NIHJ and 56°C for *B. stearothermophilus* var. *calidolactis.*

(iii) Protein binding. Binding of MPM and CLC in murine plasma was determined by equilibrium dialysis in a Dianorm dialyzer (Diachema A.G., Zürich, Switzerland) (1). The dialysis chambers had a volume of 1.0 ml and were separated by a membrane measuring 4.5 cm^2. One chamber contained 0.7 ml of plasma and the other one contained a solution of the antibiotic in saline at concentrations ranging from 5 to 50 mg/liter. The chambers were placed in a rotator (Scientific Industries, Springfield, Mass.), and dialysis was carried out at 16 rpm at 37°C for 4 h. The concentrations in the two chambers were measured as described above.

(iv) Pharmacodynamic model in vivo. For calculation of the predicted effect in vivo, the pharmacodynamic parameters derived from the experiments performed in vitro were used. Values of $(R_0 - R)$ were calculated for the actual concentrations in plasma, corrected for protein binding, and then the integral in equation 2 was estimated by the trapezoid method as the area under the curve for calculated $(R_0 - R)$. For the experiments in vivo it was further assumed (2, 4) that the killing rate would not be identical to that in vitro but would be proportional to it. Therefore, in equation 2 the integral was substituted by p times the integral. The model equation to fit to the numbers of CFU in the treated animals thus became

$$\log N = \log N_0 + R_0' t - p \int_0^t e^1 E_R \frac{t}{t_{\max}} e^{-t/t_{\max}} dt \quad (6)$$

in which R_0' is the growth rate in vivo for the controls. Because of the size of the inoculum in the in vivo experiments, exponential growth ended at a certain time point during the experiment. To obtain the best estimate of R_0' the following equation was used for the untreated animals:

$$\log N = (\log N_0 + R_0' t) \cdot (t \le t_s) + (\log N_0 + R_0' t_s) \cdot (t > t_s) \quad (7)$$

In this equation t_s is the time point at which exponential growth changes to a stationary phase. The terms $(t \le t_s)$ and $(t > t_s)$ are constraints, taking the value of 1 if they are true and 0 if they are false. Equations 6 and 7 could be fitted simultaneously for each experiment by nonlinear regression analysis, leading to estimates of R_0', t_s, and p.

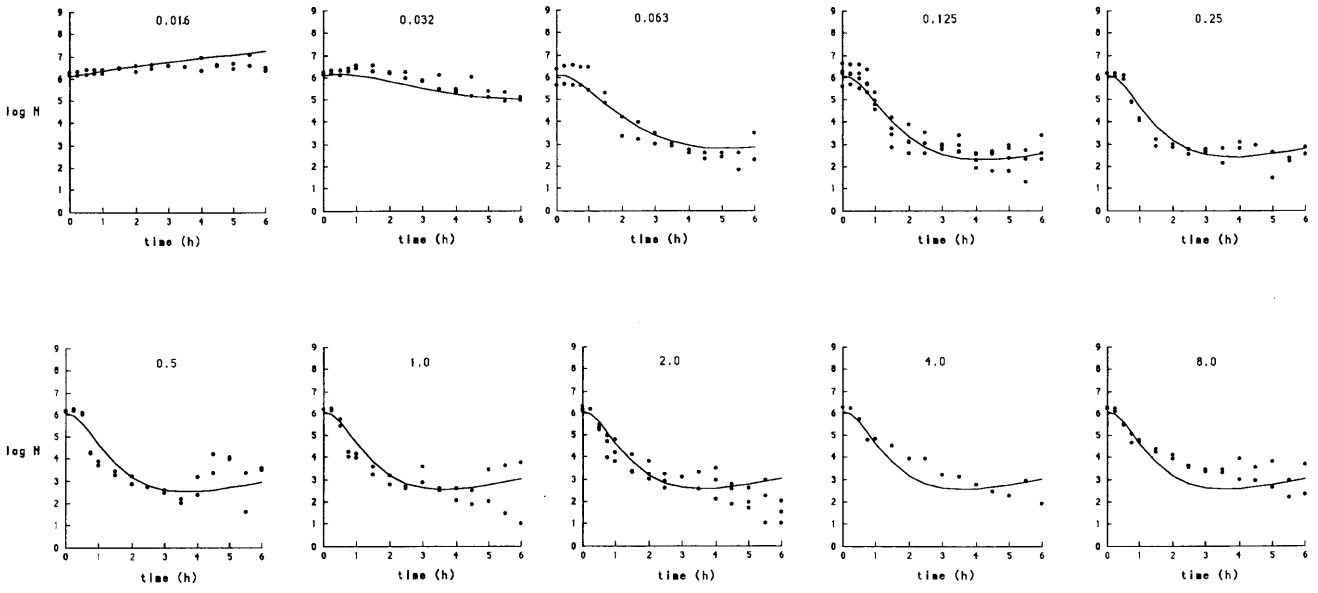

FIG. 2. Numbers of *S. aureus* 1 organisms in the presence of MPM at various concentrations in vitro (the concentrations of MPM are indicated at the top of each panel). Continuous lines connect estimates calculated according to equation 3 (see Materials and Methods).

RESULTS

Experiments in vitro. (i) MIC determinations. The MIC of MPM was 0.063 mg/liter for both strains, that of CLC was 0.25 mg/liter for both strains.

(ii) Antibacterial effect at constant concentrations in vitro. The results of one nonlinear analysis, for MPM and *S. aureus* 1, are presented in Fig. 2. A similar pattern was seen for both strains and both antibiotics. The mean value of R_0 for strain 1 is 0.333 (standard error of the mean, 0.011) $\log_{10}$/h. When corrected for the washout rate, the true growth rate of the control is 0.748 $\log_{10}$/h, corresponding to a doubling time of 56 min. For strain 2 those values were 0.329 (standard error of the mean, 0.005) $\log_{10}$/h and 55 min, respectively. Residuals were evenly distributed, although the graphs suggest that at the highest concentrations the growth rate of the surviving fraction of the inoculum is lower than that of the original inoculum. The pharmacodynamic parameters calculated on the basis of these results are given in Table 1. For each of the four combinations of antibiotic and strain the regression analysis was performed independently. The EC_{50} of MPM was considerably lower than that of CLC, but for each antibiotic the EC_{50}s for the two strains were very similar; the maximum value of E_R (i.e., S in equation 4) for MPM was only slightly higher. Net killing rates (R) can be calculated by subtracting E_R from the true growth rate for the controls. The minimum values for t_{max} (i.e., T in equation 5) for the two antibiotics and both strains were also very similar. The values for the correlation coefficients and for the residual errors again indicate that the model explains the greatest part of the total variation in the numbers of CFU. The analysis did not allow for calculation of the asymptotic confidence limits of the individual parameters.

Antibacterial effect at decreasing concentrations in vitro. The predictive value of the model for exponentially declining concentrations of the antibiotic obtained by using the parameter values from Table 1 at initial concentrations ranging between 0.063 and 8 mg/liter is illustrated in Fig. 3, which shows the time course of the numbers of *S. aureus* 1 organisms in the presence of MPM. Since in three different experiments the numbers of CFU for the controls were slightly different, the predicted numbers of CFU were also different. The correlation coefficients for the correlation between predicted and observed values for strain 1 were 0.885 and 0.885 for MPM and CLC, respectively, and for strain 2 they were 0.894 and 0.862, respectively. These values are not much less than those for the set of data from which the parameter values were derived, thus validating the model for its use under those conditions. Nevertheless, residuals were not evenly distributed. Figure 3 indicates that

TABLE 1. Pharmacodynamic parameters of the effects of MPM and CLC on growth rates of two *S. aureus* strains in vitro[a]

Drug and strain	EC_{50} (mg/liter)	S ($\log_{10}$/h)	T (h)	s_1	s_2	Mean residual error ($\log_{10}$)	R^2
MPM							
Strain 1	0.047	2.28	0.79	2.48	1.60	0.55	0.889
Strain 2	0.040	2.18	0.92	2.00	1.60	0.51	0.907
CLC							
Strain 1	0.105	1.94	1.07	1.24	1.60	0.57	0.881
Strain 2	0.121	2.00	1.04	2.00	2.60	0.39	0.945

[a] For the model equations (equations 4 and 5), see Materials and Methods.

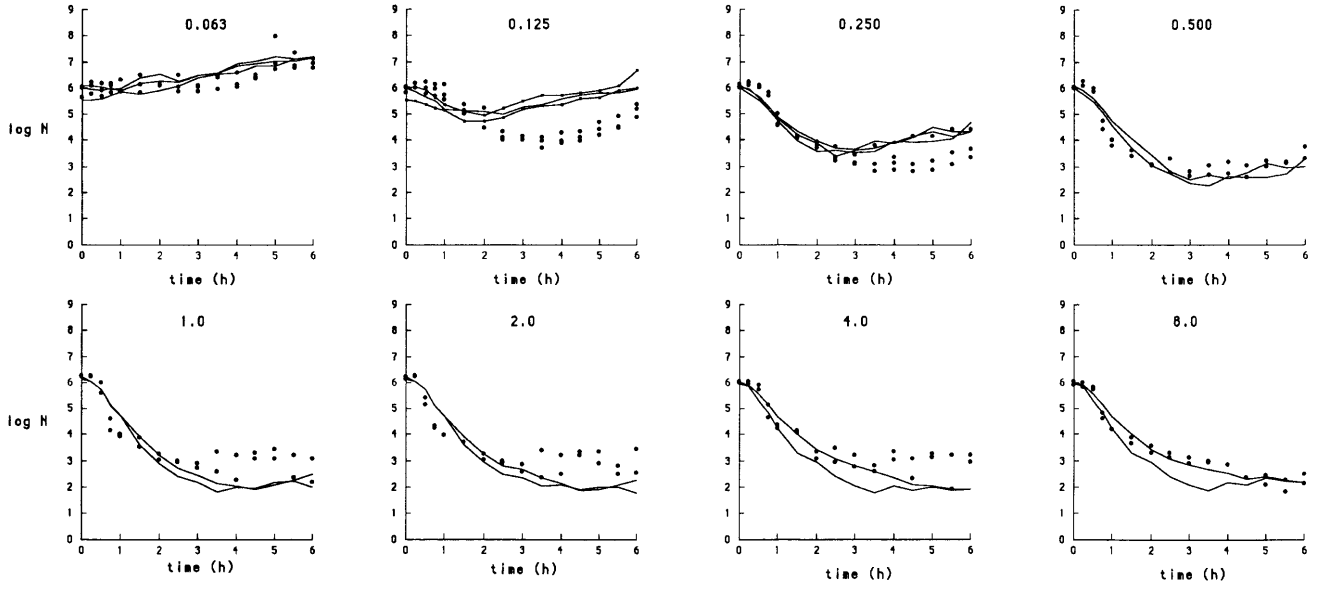

FIG. 3. Numbers of *S. aureus* 1 organisms in the presence of decreasing concentrations of MPM in vitro (the concentrations of MPM are indicated at the top of each panel). The concentration of MPM decreased according to the equation $C = C_0 e^{-0.955t}$. Values of C_0 are indicated. Continuous lines connect estimates calculated according to equation 2.

at an intermediately effective concentration (C_0 = 0.125 mg/liter) the effect was more prolonged than was predicted. The same observation was made for strain 2 at that concentration and for both strains at a CLC concentration of 0.5 mg/liter.

Experiments in vivo. (i) Pharmacokinetics. The level of binding of CLC to murine plasma was about 70%; there was no relationship between the binding percentage and the concentration at concentrations ranging between 5 and 50 mg/liter. About 10% of MPM was bound. For each antibiotic three experiments were performed by simultaneous infection of one animal with the two strains. At a dose of 80 mg/kg at 15 min, peak concentrations of MPM in plasma were about 55 mg/liter and those of CLC were about 255 mg/liter, corresponding to free concentrations of about 50 and 75 mg/liter, respectively. The apparent half-life of MPM was 2 h and 5 min, and that of CLC was 2 h and 15 min. Concentrations became undetectable after 5 h.

(ii) Experimental infection. The results for MPM are presented in Fig. 4. For the six experiments, correlation coefficients ranged between 0.731 and 0.973 (mean, 0.889). The mean values of p (i.e., the ratio of efficacy in vivo to that in vitro) are as follows: for MPM, 0.36 ± 0.05 and 0.28 ± 0.08 (standard deviation; n = 3) for strains 1 and 2, respectively, and for CLC, 0.42 ± 0.06 and 0.23 ± 0.02 (standard deviation; n = 3) for strains 1 and 2, respectively. Values of p for strain 1 are higher than those for strain 2: However, as can be seen from Fig. 5, they are correlated with the growth rate for the controls. Analysis of variance, taking into account the growth rate for the controls as a covariable, indicates that there are no significant differences between the antibiotics (P = 0.93) or strains (P = 0.76), with the covariation with R_0' being very significant (P = 0.00006); the residual error was 0.043 for the log number, which is about 10% of the actual value. Results from additional unpaired experiments (data not shown) gave a similar strong correlation.

DISCUSSION

In the present study the predictive value of a new mathematical model describing the relationship between the concentration of an antibiotic and killing in a time-related model was evaluated for two β-lactam antibiotics and two strains of *S. aureus*. In the pharmacodynamic model used in previous studies (2, 4, 7, 8) E_R was regarded as a single parameter of the efficacy of a given concentration. This model made it possible to express the efficacy of the antibiotic according to an E_{max} model in terms of EC_{50} and E_{max}. These parameters were sufficiently precise to allow for predictions of the relative efficacies of related antibiotics in an experimental infection. The present model was developed to improve on this by describing the effect of the antibiotic on the numbers of bacteria in terms of killing rate as a function of time of exposure at any concentration. For constant concentrations in vitro the model gave a very good fit, and no systematic deviations from the model were seen.

Quantitative comparison of the outcomes of the four independent nonlinear regression analyses for the two antibiotics and the two strains showed a remarkable consistency. The most important quantitative difference between MPM and CLC, according to this model, was the difference in EC_{50}s; all other parameters were very similar. This already indicates that, notwithstanding the structural differences between the two β-lactam antibiotics, their efficacies are not essentially different if only the difference in potency is taken into account.

The differences between the EC_{50}s of the two antibiotics showed some discrepancy with the differences in MICs. This may be explained by the fact that the MIC was determined in a twofold dilution series and therefore is not very precise compared to the estimate of the EC_{50}. Nevertheless, the MIC was higher than the EC_{50} for both antibiotics. Since the EC_{50} represents the concentration at which the killing rate is 50% of its maximum value, it may be concluded that the MIC does not

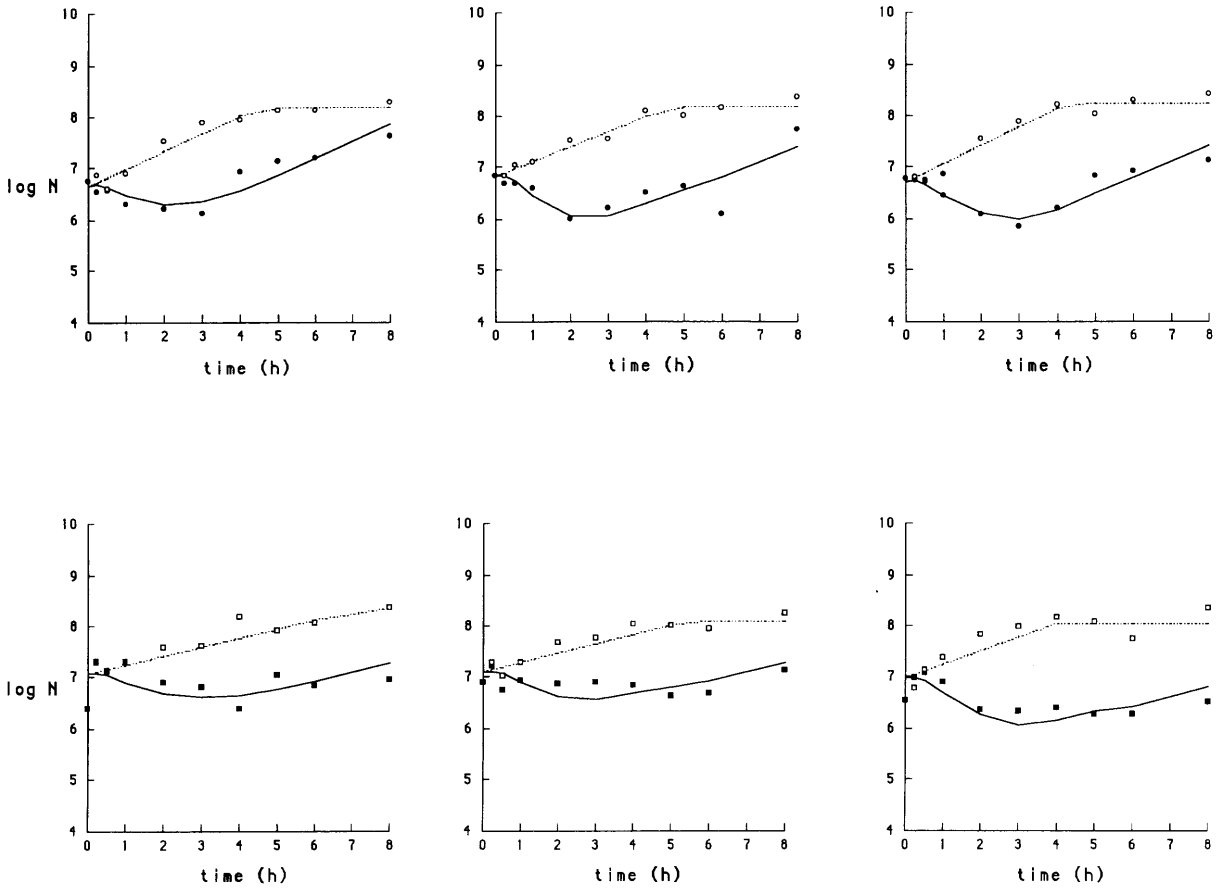

FIG. 4. Numbers of *S. aureus* 1 (upper panels) and 2 (lower panels) organisms in an experimental thigh infection in untreated animals (open symbols, broken lines) and after the subcutaneous administration of a dose of 80 mg of MPM per kg (closed symbols, continuous lines). Continuous lines connect estimates calculated according to equations 6 and 7.

discriminate between effective and ineffective concentrations. All quantitative parameters were very similar for the two *S. aureus* strains.

The model equation makes it mathematically possible to predict killing rates as a function of time and concentration and therefore to predict numbers of bacteria as a function of a concentration that changes with time. Any deviation between predicted and observed values may thus serve to reject a null hypothesis. The numbers of bacteria in the presence of an exponentially declining concentration in vitro were predicted. On the whole, the correspondence between the predicted and the observed numbers was good for both drugs and both strains. However, there was a consistent discrepancy for both antibiotics and both strains at a small range of intermediately effective concentrations. At those concentrations the duration of the effect was consistently longer than was predicted; since postantibiotic effects of both drugs were previously shown in the classical way (7), it seems logical to explain the observed discrepancy as a postantibiotic effect at declining concentrations. Interestingly, this effect was not seen at higher concentrations.

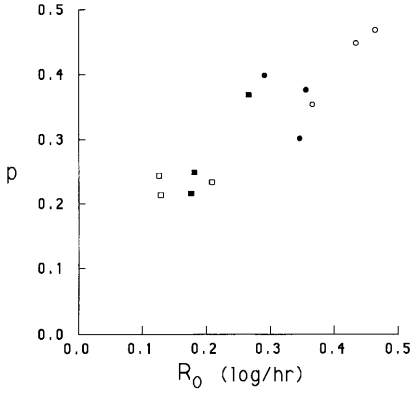

FIG. 5. Ratio *p* of efficacy in vivo to that in vitro in relation to growth rate in vivo. Data for MPM (closed symbols), CLC (open symbols), *S. aureus* 1 (circles), and *S. aureus* 2 (squares) are presented.

Now that the predictive value of the model and its possible limitations for changing concentrations in vitro have been demonstrated, it is possible to base on the same model a prediction for the relation between a time course of concentrations in vivo and the efficacy in an experimental infection. The prediction allows for determination of a quantitative difference between the in vitro and in vivo killing rates, if only because of the difference in growth rates, and therefore susceptibility to β-lactam antibiotics, in vitro and in vivo. The model could thus be used to quantify the difference in susceptibility in vitro and in vivo by estimating a proportionality factor, indicating the ratio between efficacy in vitro and in vivo. This ratio was determined for each experiment independently. It was expected that the efficacy in vivo would be less than that in vitro because of the slower growth rate (5). The strong

correlation between the ratio *p* and the control growth rate in vivo even indicates that the growth rate in vivo is by far the most important determinant of the killing of *S. aureus* by β-lactam antibiotics. The discrepancy in vitro that was ascribed to a possible postantibiotic effect was not seen in vivo, but initial concentrations in vivo were indeed much higher than the concentrations at which this effect was seen in vitro.

The values of the ratio *p* were slightly higher than those found under comparable experimental conditions in vivo for cephalosporins against gram-negative microorganisms (2) and for vancomycin and teicoplanin against *S. aureus* (4), but admittedly, the model used in those previous studies did not take into account the time-related killing rate.

The model must still be shown to be valid for repeated dosages, but preliminary experiments in vitro indicate that this is the case. Also, from in vivo studies by others (6) it may be assumed that repeated administration does not invalidate a model like ours. Although this model apparently cannot be directly verified by clinical studies, it may well serve as a basis for optimization of dosage schedules for β-lactam antibiotics or for the creation of rational initial dosage schedules for newly developed β-lactam antibiotics.

ACKNOWLEDGMENTS

We thank A. A. Firsov, P. J. van den Broek, and M. L. van Ogtrop for critical remarks.

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