Pharmacokinetic-Pharmacodynamic Modeling of the Antibiotic Effect of Piperacillin in Vitro

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Purpose. It was the aim of the present study to investigate the in vitro antimicrobial effects of the β-lactam antibiotic piperacillin on *Escherichia coli* using concentration-time profiles similar to those encountered in vivo.

Methods. An in vitro dilution model was used to expose *E. coli* to various piperacillin concentration profiles. The antimicrobial effect was evaluated by determination of the number of bacteria over time.

Results. A modified E_{max}-model was found appropriate to describe the pharmacodynamic effect. This model was linked with the respective piperacillin concentrations to provide a suitable pharmacokinetic-pharmacodynamic (PK-PD) model. The average growth half-life in absence of piperacillin was 28 min and the maximum kill half-life was 25 min. The EC_{50} for the various dosing regimens averaged 5.2 μg/mL and was independent of dose. These parameters were used the simulate the bactericidal effects of commonly administered doses or dosing regimens in humans.

Conclusions. Based on the in vitro data a more frequent administration of piperacillin will be more efficacious. The proposed PK-PD-model allows a more detailed evaluation of dosing regimens than the use of minimum inhibitory concentrations.

KEY WORDS: piperacillin; *Escherichia coli;* E_{max}-model; PK-PD modeling.

INTRODUCTION

Antibiotic dosing regimens are often based on empirical experience rather than on rational design. Although, there has been considerable progress to characterize pharmacokinetic-pharmacodynamic relationships for many drugs, in the field of anti-infectives, the established pharmacodynamic parameter used in most cases still is the in vitro minimum inhibitory concentration (MIC). This approach has several disadvantages: a constant drug level as it is present during MIC determinations does not reflect the in vivo situation where the drug undergoes metabolism and elimination; also, MIC has an innate inaccuracy up to a factor of two due to the twofold dilution steps used during its determination. The MIC is frequently erroneously considered as an effect/no effect-threshold value. This is not appropriate, since concentration just below MIC can show some activity, and concentrations just above MIC do not have maximum effect (1). However, the most commonly used PK-PD-approaches for anti-infectives today imply this MIC-threshold concept, e.g. area under the curve divided by MIC (AUC/MIC), maximum concentration divided by MIC (C_{max}/MIC) and time above MIC (T>MIC) (1,2). These concepts all utilize MIC as the only pharmacodynamic parameter. Another approach is the pharmacodynamic E_{max}-model which has been successfully applied in many other drug classes and is able to account for changing drug concentrations. There have been some attempts to quantitate anti-infective activity with an E_{max}-model (2,3). Particularly, one study by Zhi et al. stands out, where the in vivo pharmacodynamics of piperacillin was studied in neutropenic mice with a systemic infection with *Pseudomonas aeruginosa* (3). The pharmacodynamic activity of anti-infective drugs can also be studied in vitro using the concentration profiles of interest simulated either by dilution (4-7) or diffusion and dialysis (8-10).

It was the goal of the present study to investigate the pharmacodynamics of piperacillin in an in vitro model of infection by expoing E. coli to constant and fluctuating concentrations similar to those encountered in vivo; to describe the pharmacodynamic effect (change in number of bacteria as a function of time) with an E_{max}-model; and to derive a set of parameters that would allow to simulate the anti-infective effect for any given dose. Ultimately, these pharmacodynamic simulations may contribute to a rational recommendation for an optimal dosing regimen of piperacillin.

MATERIALS AND METHODS

In Vitro Concentration Profiles

Two different sets of experiments were conducted in which *E. coli* was exposed to constant and fluctuating piperacillin concentrations. In the first set of experiments, constant piperacillin concentrations of 1, 2, 4, 8, 16 and 32 μg/mL were used for up to twelve hours. In the second set of experiments, initial piperacillin concentrations of 50, 100, 150 and 200 μg/mL were diluted in a step-wise fashion to simulate an in vitro half-life of one hour. Multiple dosing was simulated by adding appropriate amounts of drug after intervals of 4, 6, 8, 12, or 24 hours. The in vitro piperacillin concentration were validated by HPLC-analysis.

Bacteria

Escherichia coli ATCC 25922 was used as the test strain. This strain is known to be sensitive to piperacillin. The MIC for piperacillin against this strain was determined to be 2-4 μg/mL by broth dilution method for an inoculum of $5 \cdot 10^5$ colony forming units (CFU)/mL in MHB supplemented with 25 μg/mL Mg^{2+} and 50 μg/mL Ca^{2+} (11). This MIC is in good agreement with results of other investigators using the same strain (12).

In Vitro Model of Infection

Bacteria from previously frozen inocula of approximately $4 \cdot 10^7$ CFU/mL were thawed, plated and incubated over night at 37°C. Colonies were selected from the plates

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and suspended in sterile water. The turbidity of these suspensions was compared with McFarland Equivalence Turbidity Standard (Remel Microbiology Products, Lenexa, Kansas, USA) to determine the approximate number of bacteria per mL. A sample (0.1-0.3 mL) of this suspension was injected into the in vitro model containing 20 mL of supplemented MHB to obtain a final inoculum of approximately $5 \cdot 10^5$ CFU/mL.

A one-compartment in vitro model was used to simulate piperacillin concentration-time profiles. The total volume of this compartment was 20 mL of supplemented MHB. To simulate drug elimination, broth solution containing piperacillin was withdrawn through sterile filters (0.2 μm, Sterile Accords, Gelman Sciences, Ann Arbor, Michigan, USA) and replaced by fresh sterile broth. These filters prevent the passage of bacteria. In order to simulate the half-life of piperacillin in humans (1 hour), 3.2 mL of broth solution was withdrawn every fifteen minutes from the in vitro model and replaced by the same volume of piperacillin-free sterile broth. During each of these 15-minute time intervals the piperacillin concentration in the in vitro model remained constant. For the entire duration of the experiment the in vitro model was housed in an incubator to maintain a temperature of 37°C. Broth solution for the dilution was also kept at 37°C.

Bacterial Quantification

Samples (50 μL) were collected from the in vitro model and bacterial counts (CFU/mL) were determined by plating serial 10-fold dilutions of the sample on agar plates (Blood Agar Plates TSA with 5% sheep blood, Remel Microbiology Products, Lenexa, Kansas, USA). The dilutions were made with sterile 0.9% NaCl solution. 100 μL of each dilution were plated in duplicate and incubated for 18 h at 37°C.

Each experiment was repeated on a different day. Furthermore, for each experiment a positive and a negative control was included. For the positive control the number of bacteria was determined for a constant piperacillin concentration of 100 μg/mL to ensure maximum effect for up to 12 hours. For the negative control, the number of bacteria was determined in absence of drug for up to 8 hours. The average coefficient of variation for replicates done on different days was approximately 40%. This variability is acceptable since the range of measured CFU values covered up to six orders of magnitude.

PK-PD Modeling

The pharmacodynamic data was analyzed with a modified E_{max}-model. Using the non-linear least-squares regression program SCIENTIST (Micromath, Salt Lake City, Utah, USA) the experimental data was fitted to the following differential equation:

$$\frac{dN}{dt} = \left(k - \frac{k_{max} \cdot C_t}{EC_{50} + C_t}\right) \cdot (1 - e^{-z \cdot t}) \cdot N \qquad (1)$$

where dN/dt is the change in number of bacteria as a function of time (pharmacodynamic effect), k_{max} (h^{-1}) the maximum killing effect, C_t the concentration of piperacillin at time t, EC_{50} (μg/mL) the concentration required for 50% of the maximum effect, z (h^{-1}) a constant to model the lag time during approximately the first two hours of each experiment, and k (h^{-1}) is the generation rate constant in absence of any drug. The drug effect is measured in inhibition of growth or killing (reduction of number of bacteria). In absence of any drug, bacteria grow at their normal growth rate. In this case, the term $(k_{max} \cdot C_t)/(EC_{50} + C_t)$ equals zero. The resultant growth rate constant is k. For high drug concentrations, actual killing of bacteria is induced. The maximum resultant kill rate constant is $(k\text{-}k_{max})$. The inclusion of the constant z was necessitated by the fact that at the beginning of most experiments not all bacteria were in the logarithmic growth phase. During this period of time the growth rate is not constant but gradually increasing. The exponential expression $1\text{-}e^{-z \cdot t}$ approaches 1 with increasing time. The impact of z on k and, thus, on the shape of the curve is greatest for the first two hours. After this time period, the expression $e^{-z \cdot t}$ disappears since it approaches zero.

The goodness of fit was evaluated using the model selection criterion (MSC) provided by SCIENTIST, as well as the coefficient of determinations (r^2). MSC is a modified Akaike information criterion that allows comparison of various data sets fitted to a selected model. Increased MSC values indicate more appropriate fits (13).

Pharmacokinetic Simulations

The PK-PD model was applied to predict the antimicrobial effect of piperacillin in humans for commonly administered dosing regimens. Plasma concentrations of piperacillin (Cp) after i.v. bolus injection can be described by an open two-compartment body model (Eq. 2).

$$Cp = a \cdot e^{-\alpha \cdot t} + b \cdot e^{-\beta \cdot t} \qquad (2)$$

where t is time and a, b, α and β are hybrid constants. For the calculation of concentration-time profiles of free, unbound piperacillin in human tissue Eqs. 3 and 4 were used (14-16):

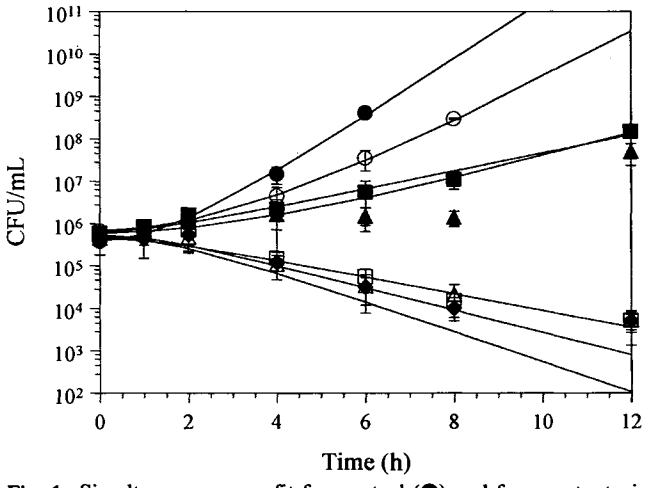

Fig. 1. Simultaneous curve fit for control (●) and for constant piperacillin concentrations of 1 μg/mL (○), 2 μg/mL (■), 4 μg/mL (▲), 8 μg/mL (□), 16 μg/mL (△) and 32 μg/mL (◆). Error bars indicate SD.

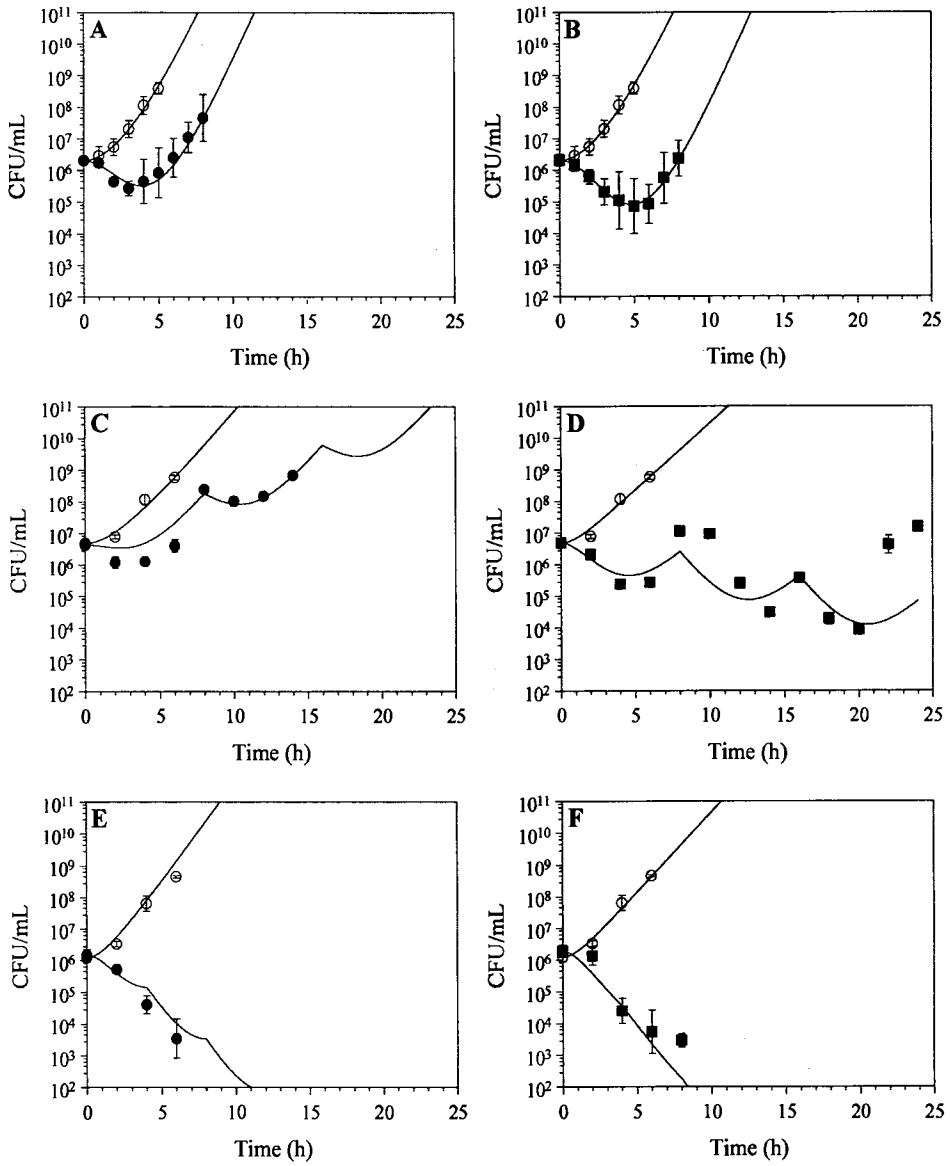

Fig. 2. Simultaneous curve fit for control (○) and the following concentration regimens: A. 50 μg/mL q24h. B. 100 μg/mL q24h. C. 50 μg/mL q8h. D. 100 μg/mL q8h. E. 50 μg/mL q4h. F. 100 μg/mL q4h. Error bars indicate SD.

$$C_{tissue,free} = \frac{f_u \cdot D \cdot k_{21}}{V_c \cdot (\alpha - \beta)} \cdot (e^{-\beta \cdot t} - e^{-\alpha \cdot t}) \quad (3)$$

$$\text{with} \quad \frac{D \cdot k_{21}}{V_c} = a \cdot \beta + b \cdot \alpha \quad (4)$$

where k_{21} is the first-order rate constant from the peripheral to the central compartment, D is the dose administered as an i.v. bolus injection, f_u is the free fraction of piperacillin in plasma, and V_c is the volume of distribution of the central compartment. Parameters were taken from previously published pharmacokinetic studies (17). Doses of 15, 30 and 60 mg/kg equivalent to 1, 2 and 4 g of piperacillin in a 67 kg patient were studied. Since the pharmacokinetics of piperacillin have been reported to be nonlinear, different pharmacokinetic parameters were used for each dose (17). The protein binding of piperacillin has been found to be very low at approximately 21% (18). Maximum tissue concentrations of free, unbound piperacillin are reached after 23, 33 and 25 minutes and were 25, 54 and 156 μg/mL for a 1, 2 and 4 g dose, respectively, and are of comparable magnitude to the in vitro concentrations studied.

RESULTS

Examples of the pharmacodynamic results for the various concentration profiles are displayed in Fig. 1 and 2.

Fig. 1 displays the pharmacodynamic effects for various constant piperacillin concentrations as they would be observed after continuous i.v. infusion of the drug. For lower concentrations (1, 2 and 4 μg/mL) the bacterial growth is slowed down. Actual killing of bacteria (reduction in number

Table I. Results of Curve Fits for the Investigated Concentration Profiles

Dosing regimen	k (h^{-1})	z (h^{-1})	k_{max} (h^{-1})	EC_{50} (μg/mL)	r^2	MSC
1 μg/mL (constant)	1.70	0.50	4.6	5.6	0.92	1.9
2 μg/mL (constant)	1.07	0.66	2.2	6.0	0.96	1.8
4 μg/mL (constant)	1.60	0.80	3.1	7.2	0.95	2.6
8 μg/mL (constant)	1.44	0.78	3.3	5.0	0.94	2.2
16 μg/mL (constant)	1.05	0.71	2.2	7.2	0.92	2.3
32 μg/mL (constant)	1.57	0.59	2.8	5.5	0.91	2.4
50 μg/mL q24h	2.80	0.23	6.4	5.0	0.99	4.3
100 μg/mL q24h	2.06	0.35	4.6	5.2	0.96	2.9
200 μg/mL q24h	2.63	0.24	5.6	7.2	0.94	2.5
100 μg/mL q12h	1.56	0.38	2.3	6.4	0.94	2
150 μg/mL q12h	1.20	0.66	1.4	3.9	0.94	2
200 μg/mL q12h	1.27	0.56	1.7	5.0	0.87	1.6
50 μg/mL q8h	1.23	0.42	2.1	6.4	0.97	2.7
100 μg/mL q8h	0.98	0.95	2.4	5.4	0.75	0.8
150 μg/mL q8h	0.97	0.93	2.7	5.0	0.80	0.9
50 μg/mL q6h	1.41	0.32	4.2	8.2	0.85	0.9
100 μg/mL q6h	2.49	0.14	5.1	4.7	0.96	0.9
150 μg/mL q6h	0.94	0.88	2.0	4.2	0.70	0.6
50 μg/mL q4h	1.45	0.86	3.2	3.7	0.99	3.2
100 μg/mL q4h	1.06	1.50	2.2	1.0	0.85	2.6
150 μg/mL q4h	1.07	1.50	2.1	1.0	0.96	2.6
Mean	1.50	0.66	3.19	5.17		
SD	0.56	0.37	1.39	1.81		

of bacteria) is induced for higher piperacillin concentrations (8 μg/mL and above).

For Fig. 2, the situation is different, since the piperacillin concentrations fluctuate. In cases where piperacillin concentrations decrease to low levels such as in the q24h regimens (Fig. 2A-B), bacterial regrowth occurs at the same rate as the control. In case of the q8h regimens the observed effect depends on the initial concentration. For concentra-

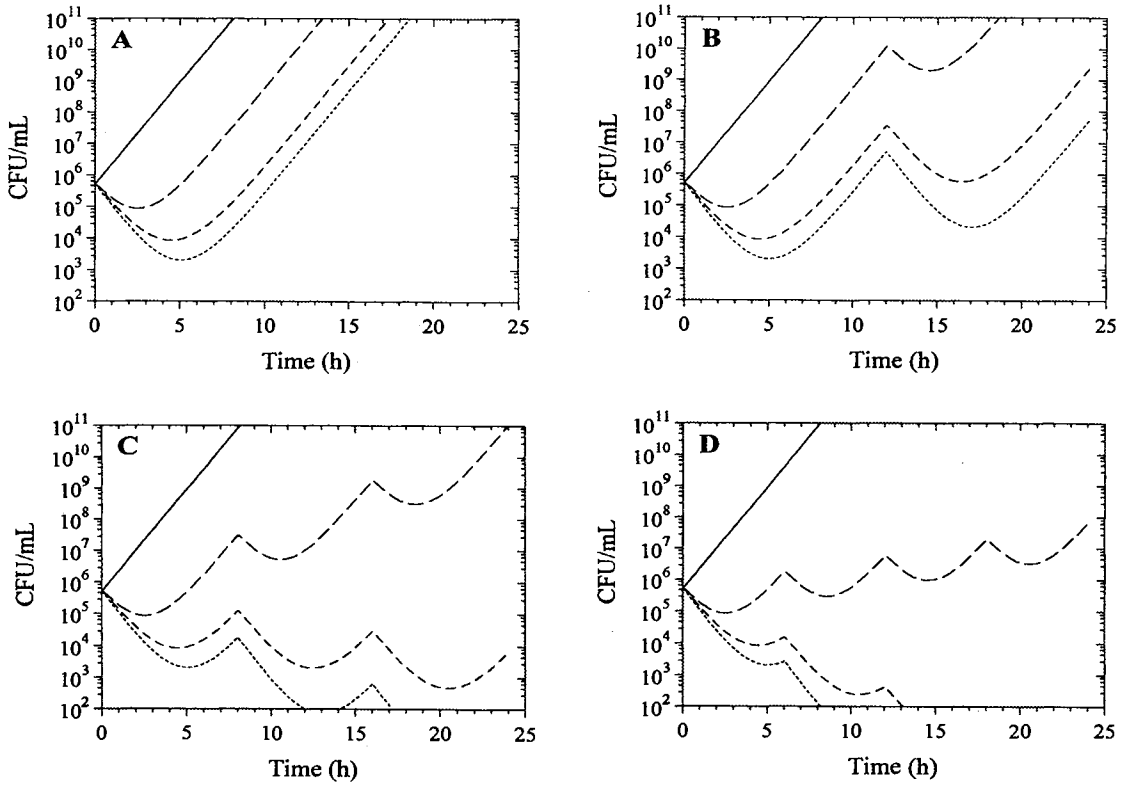

Fig. 3. Simulated effect for control (solid line) and for 1g (long-dashed line), 2g (medium-dashed line) and 4g (short-dashed line) of piperacillin given q24h (A), q12h (B), q8h (C) and q6h (D).

tion of 50 μg/mL, the growth rate is slowed down (Fig. 2C) but only when the initial concentration is increased to 100 μg/mL, killing is observed (Fig. 2D). For the q4h dosing regimens, maximum kill rates where found for all concentrations studied (Fig. 2E-F).

Table I shows the calculated pharmacodynamic parameters. The mean values for k, z, k_{max} and EC_{50} are 1.5 ± 0.56 h^{-1}, 0.66 ± 0.37 h^{-1}, 3.19 ± 1.39 h^{-1}, and 5.17 ± 1.81 μg/mL, respectively. This is equivalent to an average growth half-life in absence of piperacillin of 28 min and a maximum kill half-life of 25 min. The results indicate that the model chosen is appropriate for curve fitting of the data. The observed standard deviations in the pharmacodynamic parameters are relatively low, considering the large number and variety of concentration profiles studied.

DISCUSSION

In the present study, it was possible to describe the observed pharmacodynamic effects with the same E_{max}-model for a wide range of concentration regimens. The same model could be applied both for constant and fluctuating piperacillin concentrations. The parameters found (Table I) were consistent for all concentration profiles. This model may be more useful than traditional MIC approaches since it allows more detailed predictions. The MIC value only reflects the pharmacodynamic effect of an antibiotic at one constant concentration. It reveals nothing about the rate at which bacterial killing is induced. Furthermore, since most of the infections occur in the tissue and not in the blood, knowledge of the concentration profiles of free antibiotic in these compartments is important for the design of optimal dosing regimens (16).

The present study with piperacillin used a similar PK-PD model than that used in an in vivo animal model with *P. aeruginosa* infected neutropenic mice (3). The growth and kill rate constants reported in that study are similar to those found in the present in vitro model. However, the reported EC_{50} value of piperacillin for *P. aeruginosa* of 0.05 ± 0.11 μg/mL (3) is much lower than the EC_{50} of 5.2 ± 1.8 μg/mL found for *E. coli* in this in vitro study. This is surprising since the MIC-values found for *P. aeruginosa* were 10-20 μg/mL (3), hence higher than the MIC of 4 μg/mL for *E. coli* investigated here.

The pharmacokinetic-pharmacodynamic model derived can be useful to simulate and predict changes in the number of bacteria for common therapeutic dosing regimens in humans. Fig. 3 shows the simulated effects for three piperacillin doses (1, 2 and 4g) when given q24h, q12h, q8h and q6h. A once-daily regimen does not seem appropriate since regrowth can be expected (Fig. 3A). In case of the q12h regimens, the expected effect depends on the administered dose. Whereas for 1g piperacillin the bacterial growth rate is only slowed down minimally, the effect for 2 and 4g is more pronounced although the overall number of bacteria is still increasing (Fig. 3B). In order to expect a bactericidal effect of piperacillin, dosing regimens of 2 or 4g q8h are needed (Fig. 3C). The same conclusions can be drawn for the q6h treatment (Fig. 3D). A dose of 1 g given every eight hours does not induce overall killing. Even with a dosing regimen of six times a day, 1g of piperacillin does not seem to be sufficient to decrease bacterial count.

Applying these results to the clinical situation will be difficult, since the six-times-a-day administration of antibiotic is not practical. Administering piperacillin three times a day is feasible without significant loss of efficacy. However, based on these studies, a less frequent administration of piperacillin (once or twice per day) cannot be recommended.

These curves are based on the in vitro activity against *E. coli* as determined in this study. It is obvious that there may be many factors present in vivo that will modify the PK-PD parameters found in vitro. Growth rates and killing rates in vivo may be different and also depend on the bacteria and sites of infection. Furthermore, it is difficult to simulate the effects of the immune system in vitro. Therefore, the situation studied maybe of more predictive value for immunocompromised patients. In any case, clinical trials will be necessary to confirm the findings of this study.

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