Streptococcus pneumoniae Pneumonia in Mice: Optimal Amoxicillin Dosing Predicted from a Pharmacokinetic-Pharmacodynamic Model

PIERRE MOINE and JEAN XAVIER MAZOIT

Service et Laboratoire d’Anesthésie, Centre Hospitalier Universitaire de Bicêtre, Université Paris-Sud, Faculté de Médecine du Kremlin-Bicêtre. Le Kremlin-Bicêtre, France (P.M., J.X.M.); and Contrat de Recherche Institut National de la Santé et de la Recherche Médicale CRI 4U 002 D Pharmacologie de la résistance aux anti-infectieux, Centre Hospitalier Universitaire Bichat-Claude Bernard, Paris, France (P.M.)

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ABSTRACT

In an attempt to better understand the interaction of amoxicillin with Streptococcus pneumoniae in the lung, and to determine the parameters of therapeutic efficacy of the antimicrobial agent amoxicillin, we used a pharmacokinetic-pharmacodynamic model to describe the overall dose-effect relationship of amoxicillin against 12 strains of S. pneumoniae with penicillin minimum inhibitory concentrations ranging from 0.00131 to 16 g/ml in a neutropenic murine pneumonia model. We were able to correlate amoxicillin dosing, pharmacokinetics, and the temporal changes in bacterial count in lung. Moreover, survival rates measured in one strain at different dosing were significantly related to the number of bacteria in lung calculated from the pharmacokinetic-pharmacodynamic model. Disappearance of amoxicillin from the effect compartment appeared to be very slow and the rate constant ($k_{e0}$) governing this process was significantly different between strains, ranging from 0.00131 to 0.03945 h$^{-1}$. These findings have two major implications: 1) after a single dose of amoxicillin, bacterial counts in lung rapidly decreased and the bacterial growth remained suppressed during a long period of time after cessation of exposure of microorganisms to amoxicillin; and 2) the duration of bacterial growth suppression was related to the intrinsic properties of S. pneumoniae strains rather than to host environment because $k_{e0}$ was significantly different between strains. These two premises clearly demonstrate that bacterial growth suppression is related to an in vivo postantibiotic effect. Furthermore, we have shown that the major determinant of amoxicillin in vivo bactericidal activity and therapeutic efficacy appeared to be the dose of amoxicillin because amoxicillin exhibits a rapid dose-dependent killing regardless of the S. pneumoniae strain. Our findings may have implications for the clinical use of amoxicillin. In view of our results, the guidance to increase the amoxicillin-loading dose in pneumococcal pneumonia appears to be immediately clinically relevant.

Infections caused by Streptococcus pneumoniae resistant to β-lactam antimicrobials are an increasingly frequent problem in clinical practice but the optimal antibiotic therapy for penicillin-resistant S. pneumoniae pneumonia is not clear (Austrian, 1994; Boswell et al., 1994; Finch, 1995). In a murine S. pneumoniae pneumonia model, with an $E_{\text{max}}$ model, we have recently demonstrated that the standard in vitro minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of amoxicillin were excellent predictors of the relative in vivo potency of amoxicillin against pneumococcal species, including highly penicillin-resistant strains with penicillin MICs ranging from <0.01 to 16 μg/ml (Moine et al., 1997a). Nevertheless, we do not know which pharmacokinetic (PK) and pharmacodynamic (PD) parameters correlated with efficacy in this S. pneumoniae pneumonia model.

Numerous PK parameters have been proposed for various classes of antibiotics to predict bactericidal activity and therapeutic efficacy in different animal models: the area under the inhibitory serum concentration-time curve, the time that the serum concentration exceeds MIC, and the ratio between peak serum concentration and MIC (Hyatt et al., 1995; Craig, 1998). The duration of time that serum levels exceed MIC has been shown to be the PK parameter most frequently correlated with β-lactam efficacy in different animal models (Hyatt et al., 1995; Craig, 1998). Frimodt-Møller et al. (1986), with a mouse model with i.p. inoculation of S. pneumoniae type 3 to compare in vivo effects of 14 cephalosporins, demonstrated a significant correlation between the 50% effective dose and the time the serum concentration remained above the MIC for each drug. Moreover, in a neutropenic murine S. pneumoniae thigh infection model, Vogelman et al. (1988a) demonstrated that maximum bactericidal activity was achieved when serum amoxicillin levels were constantly maintained above the MIC.

**ABBREVIATIONS:** MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; PK, pharmacokinetic; PD, pharmacodynamic; CFU, colony forming units; S, survival; MTD, minimal therapeutic dosage; PAE, postantibiotic effect.
Materials and Methods

With the exception of serum and lung amoxicillin concentration-time data, all other data used were obtained from previously published studies (Azoulay-Dupuis et al., 1991; Moine et al., 1994, 1997a,b).

Challenge Organisms and Experimental Pneumococcal Pneumonia in Mice

Twelve S. pneumoniae clinical strains were used (Moine et al., 1997a): two strains were penicillin-susceptible (penicillin MIC < 0.012 \( \mu \)g/ml) \( P^R \) (P52181 and P30923); four were penicillin intermediate resistant (0.012 < penicillin MIC = 1 \( \mu \)g/ml) \( P^I \) (P31192, P30189, P40225, and P54b); and six were penicillin resistant (penicillin MIC > 1 \( \mu \)g/ml) \( P^S \) (P45988, P12698, P19596, P40422, P41375, and P53651). Pneumonia was induced in 20- to 24-g b.wt. female Swiss mice rendered neutropenic by injecting cyclophosphamide (150 mg/kg i.p. daily), starting 3 days before infection. Animals were infected by direct intratracheal bacterial suspension instillation (50 \( \mu \)l of bacterial suspension, e.g., 10\(^7\) colony forming units (CFU) per mouse) via the mouth as described elsewhere (Azoulay-Dupuis et al., 1991; Moine et al., 1994, 1997a,b). Leukopenic mice developed acute bacteremic pneumonia and died within 2 to 3 days. Bacterial counts ranging from <0.01 to 16 \( \mu \)g/ml in a neutropenic murine pneumonia model.

PKs and Assay

In neutropenic Swiss mice, amoxicillin (Beecham Paris, France) was determined in serum and lung after single s.c. injection of 2.5, 5, 10, 25, 50, 100, 200, 300, and 400 mg/kg. At 0.5, 1, 2, 4, 6, 8, 12, and 24 h following drug administration, three to five animals per dose group were sacrificed with CO\(_2\), and exsanguinated by cardiac puncture. Blood samples were centrifuged to isolate serum, which was frozen at −80°C until assay. Lungs were harvested from exsanguinated mice, washed in sterile saline solution, and frozen. On the day of assay, organs were weighed, pooled, and homogenized in phosphate buffer (pH 6.8). Homogenates were centrifuged and supernatants were used for assay. Amoxicillin concentrations were determined by the agar well diffusion method with Sarcina lutea ATCC 9341 (American Type Culture Collection, Manassas, VA) as the bioassay organism and Antibiotic Medium 1 (Difco Laboratories, Detroit, MI) as the growth medium. Standard curves for serum and tissue concentrations were determined with solutions of amoxicillin in phosphate buffer. Correlations between standard curves in buffer and in serum or in lung homogenates were found to follow the line of identity with a correlation coefficient between 0.96 and 0.94 for serum and lung, respectively. Standard curves were linear from 0.125 to 32 \( \mu \)g/ml. The lower limit of detection was 0.1 \( \mu \)g/ml, with a between- and within-day coefficient of variation of ±7.5% at 0.5, 1, 7.5, and 20 \( \mu \)g/ml.

Antibiotic Susceptibility Tests: Bactericidal Activity In Vivo

As stated above, we used published data for 12 different S. pneumoniae strains (Moine et al., 1997a). MICs and MBCs were determined for each strain in Mueller-Hinton infusion broth supplemented with 5% lysed horse blood by means of the tube dilution method (National Committee for Clinical Laboratory Standards, 1995). Bactericidal activity in vivo was determined in neutropenic mice receiving various doses of amoxicillin according to the infective strain as described in detail elsewhere (Moine et al., 1997a). Briefly, the total CFU recovered from whole-lung homogenates was determined 1, 3, 6, and 9 h after amoxicillin injection. The lower limit of detection was 4.6 ln CFU/lung (2 log\(_{10}\) CFU/lung), which corresponded to the weakest dilution tissue homogenates (10\(^{-1}\)) that avoided significant drug carryover with control inocula. In control animals mean bacterial count increased 2-fold (from 16.8 to 17.5 ln CFU/lung) during the time of observation (1–9 h after amoxicillin injection).

Survival Studies

We have previously determined for each tested strain the minimal therapeutic dosage [MTD (mg/kg)] of amoxicillin (treatment schedule consisting of s.c. injections at 12-h intervals over 3 days) as the dose required to achieve 75 to 85% survival (Moine et al., 1994, 1997a). For each strain, the survival rate was not significantly improved with larger doses of amoxicillin than the respective MTD (Moine et al., 1997a).

Modeling Procedure

We separately fitted the PK, PD, and survival (S) data with a linear, stationary parametric approach (Fig. 1).

Structural Model. PK. We made two assumptions. First, amoxicillin bioavailability was complete after s.c. injection because no extrahepatic metabolism and/or excretion of amoxicillin has been described. Second, no flip-flop effect occurred after this injection. Serum concentration-time and lung tissue concentration-time data were simultaneously fitted to the following parametric models: one-, two-, and three-compartment open models (with the lung included in the central compartment or constituting a peripheral compartment) with first-order absorption and linear first-order or Michaelis-Menten elimination from the central compartment. In the models with the lung considered as part of the central compartment, lung tissue concentration was related to the serum concentration by a partition coefficient (\( K \)). Fitting was done with standard equations (Gibaldi and Perrier, 1982) with the procedures ADVAN 5 and ADVAN 6 from the program NONMEM (Sheiner et al., 1979–1984). The following parameters were calculated: the volume of the central compartment (\( V_c \)), the absorption rate constant (\( k_a \)), and the individual compartment rate constants from compartment i to compartment j (\( k_{ij} \)). For the two- and three-compartment models, we also calculated the volume of distribution at steady state (\( V_{ss} \)) and the total body clearance (\( CL \)).

PD. The relationship between effect (E) (decrease in the natural logarithm of bacterial count in lung [ln CFU·g\(^{-1}\)]) and drug concentration at the (virtual) site of action (\( Ce \)) was modeled with the well known Hill equation (Holford and Sheiner, 1981; Verotta and Sheiner, 1991):

\[
P(s) = \frac{E}{1 + \left(\frac{Ce}{EC50}\right)^{nHill}}
\]

where \( E \) is the observed effect (decrease in bacterial count in lung), \( Ce \) is the concentration at the site of action, \( EC50 \) is the concentration at which half the effect is observed, and \( nHill \) is the Hill coefficient. This equation is linearized as:

\[
\ln E = \ln \left(\frac{E}{E_{max}}\right) = \ln \left(\frac{1}{1 + \left(\frac{Ce}{EC50}\right)^{nHill}}\right) = \ln \left(\frac{1}{1 + \left(\frac{Ce}{EC50}\right)^{nHill}}\right)
\]

PK and PD model. Conversion of dosing to plasma concentration (\( C_p \)) and to virtual effect compartment concentration (\( Ce \)) is governed by PKs. \( L \) represents the link between \( C_p \) and \( Ce \). The observed effect (decrease in bacterial count in lung) (\( E \)) is related to \( Ce \) by the classical Hill equation and the resulting survival rate is related to \( E \) by a simple exponential survival function (\( EXP \)).

Fig. 1. PK and PD model. Conversion of dosing to plasma concentration (\( C_p \)) and to virtual effect compartment concentration (\( Ce \)) is governed by PKs. \( L \) represents the link between \( C_p \) and \( Ce \). The observed effect (decrease in bacterial count in lung) (\( E \)) is related to \( Ce \) by the classical Hill equation and the resulting survival rate is related to \( E \) by a simple exponential survival function (\( EXP \)).
where $E_0$ is the initial bacterial count in lung, and $C_{e50}$ is the effect-site concentration producing half $E_{max}$, which is the maximum effect attainable. We did not include any factor of sigmoidicity. The link between PKs and PDs was considered a linear first-order process: $Ce = Cp \otimes L$, where $Cp$ is serum concentration, $\otimes$ is the convolution operator, and $L$ is a nonnegative, continuous, integrable function (Verotta and Sheiner, 1991) (Fig. 1). $L$ has been taken to be $k_{e} \cdot \exp(-k_{e} \cdot t)$. It is then possible to express $E$ as function of $k_{e}$, the rate constant of drug exit from effect compartment and $Cp_{ss50}$, the serum drug concentration corresponding to $C_{e50}$ at steady state (Sheiner et al., 1979). The PD values were comparatively fitted to models with $Ce$ in the central compartment, in the peripheral compartment, or in a special effect compartment. As usual, we considered negligible mass transfer between $Cp$ and $Ce$.

Survival analysis. Survival data with the same strain (P15986) were obtained from previous studies (Moine et al., 1994, 1997a,b). Briefly, amoxicillin 400, 300, 200, or 150 mg/kg was administered at 12-h intervals with a total of 6 injections, 100 mg/kg at 8-h intervals with a total of 9 injections, and 150 mg/kg at 6-h intervals with a total of 12 injections. In each experiment the animals were infected simultaneously. In two experiments, 15 animals per group were used, and in a third experiment, 30 animals per group were used. The observation period was 14 days. Death rates were recorded daily and cumulative survival rates were compared. Control animals received identical treatment with saline.

Fifteen to 30 animals were used per experiment and the fractional number of surviving animals ($S$) was recorded every day during 2 weeks. We fitted the raw survival data with the simplest exponential model: $S(t) = \exp(-\theta)$, $\theta$ was first considered as a constant parameter, thus allowing statistical comparisons between doses (see infra), and in a second step as a function of bacterial count in lung: $S(t) = \alpha \cdot E(t)$, where $\alpha$ is a constant parameter (fixed effect) and $E$ is the predicted effect (ln CFU·g$^{-1}$) obtained at the PD step.

Statistical Model and Fitting Procedure. We used the program NONMEM (version IV, level 2.1, Sheiner et al., 1979–1994). It uses extended least-squares as measure of goodness-of-fit (Sheiner and Beal, 1985) and allows the fitting of mixed effects models by using two levels of random errors (intra- and interindividual variability). The choice between the different PK and PD models was made with the Akaike criterion (Yamaoka et al., 1978). The choice between full models (i.e., with all interindividual variability parameters considered as relevant) and reduced models was made with the log-likelihood ratio test (Eadie et al., 1982). Intraindividual variability (assay error, model misspecification) was modeled with a combined constant coefficient of variation and additive error. Interindividual variability was modeled as $\theta \cdot \exp(\eta)$ (assuming a log-normal distribution), where $\theta$ is the fixed effect parameter and $\eta$ is the vector of interindividual variability with mean zero and variance $\sigma^2$. We assumed no covariance between the elements of $\eta$ and between the elements of $\epsilon$, the vector of residual error due to intraindividual and measurement variability.

In addition to Michaelis-Menten analysis, we modeled linear PKs with each dose considered as an “individual” with random interindividual variability. For PD analysis we modeled the entire data set as a whole, with the kinetic parameters calculated at the PK step. Each strain was treated as an individual subject. The different parameters for each strain were obtained by post hoc procedure. Goodness-of-fit was assessed for each strains with the root mean square error (Sheiner and Beal, 1981). $S$ was fitted with $\theta$ considered as a constant parameter (fixed effect) (model S1), with intraindividual variability $\eta$ with mean zero and variance $\sigma^2$ (model S2). The effect of dose was tested with the log-likelihood ratio test. In a second step, survival data were modeled as $S(t) = \exp(\theta \cdot E(t) \cdot t)$ (model S3), where $E$ is as previously defined, i.e., the effect calculated at the PD step and considered equal to $E_{max}$ after 72 h. A random interdose variability effect $\eta$ also was added like in model S2.

Results

Fitting was adequate with the combined constant CV and additive error model (Fig. 2). The best PK model was found to be the two compartment open model with linear first-order elimination from the central compartment and the lung included in the central compartment with a lung serum partition ratio $K = 0.447$ (Table 1). Neither Michaelis-Menten elimination nor interdose variability significantly improved the quality of fitting. Therefore, the kinetics of amoxicillin may be considered linear in mice in the range of concentrations studied. PK parameters are displayed in Table 1.

PDs were best modeled with the effect in a special compartment linked to the central PK compartment rather than directly in the central compartment or in the peripheral compartment. The best PD model was the full model with $k_{e}$, $E_{max}$, and $Cp_{ss50}$ relevant for each strain ($P < .0001$). Parameters for each strain obtained by post hoc procedure are displayed in Table 2. Figure 3 displays experimental data and fitted lines for three representative strains. The $k_{e}$, which represents the temporal distance between central compartment and effect compartment, was very low. $T_{1/2}k_{e}$ the elimination half-life of amoxicillin from the effect compartment (in fact after total disappearance of the drug from the central compartment) ranged from 17.5 h ($P = .0422$) to 22 days ($P = .02698$) (Fig. 4). For the 12 strains of $S. pneumoniae$, $Cp_{ss50}$ highly correlated with MIC (Spearman $r = 0.93$, $P < .0001$) and MBC (Spearman $r = 0.90$, $P < .0001$). In contrast, there was no significant correlation between $k_{e}$ and MIC or MBC nor between $E_{max}$ and MIC or MBC. The MTD highly corre-

Fig. 2. Adequacy of fitting. The logarithm of the measured/predicted value ratio is displayed for PKs (top) and for PDs (bottom).
Fig. 5). In a previous study (Moine et al., 1997a), we have shown that amoxicillin was significantly related to the number of bacteria in lung. In a previous study (Moine et al., 1997a), we have shown that amoxicillin was significantly related to the number of bacteria in lung. Moreover, we were able to show that the animal survival rate was significantly different between strains rather than to host environment because survival suppression was related to the intrinsic properties of 

**Discussion**

The methodology of our study differs markedly from those published previously in infectious disease. We used a PK-PD model to describe the overall dose-effect relationship of amoxicillin against 12 strains of *S. pneumoniae* with penicillin MICs between <0.01 and 16 μg/ml in a neutropenic murine pneumonia model. We were able to accurately describe the complex relationship between antibiotic dosing, PKs, and the temporal changes in bacterial count in lung. In a previous study (Moine et al., 1997a), we have shown that amoxicillin dose producing half-maximal decrease in bacterial count in lung (ED₅₀) closely correlated with the reciprocal of MICs measured in vitro. The present study permits to predict the decrease in bacterial count in lung from the dosing scheme. Moreover, we were able to show that the animal survival rate was significantly related to the number of bacteria in lung calculated from the PK-PD model (Table 3 and Fig. 5). The latter results fully confirmed the reliability of the model.

PK analysis showed that amoxicillin distributes in mice in a central compartment probably including blood and the rich vascularized organs (lung was included in this central compartment with a partition coefficient), and in a deeper peripheral compartment. However, effect (decrease in ln CFU/ml in lung) was best modeled in a separate effect compartment linked to the central compartment. Under these conditions, the peripheral compartment acts only as a reservoir buffering drug input and output.

Disappearance of amoxicillin from the effect compartment appeared to be very slow and the rate constant (kₑ₀) governing this process was significantly different between strains, ranging from 0.00131 to 0.03945 h⁻¹ (Table 2). These findings have two major implications: 1) after a single dose of amoxicillin, bacterial counts in lung rapidly decreased and the bacterial growth remained suppressed during a long period of time after cessation of exposure of microorganisms to amoxicillin (Fig. 4), and 2) the duration of bacterial growth suppression was related to the intrinsic properties of *S. pneumoniae* strains rather than to host environment because kₑ₀ was significantly different between strains.

These two premises clearly demonstrate that bacterial growth suppression is related to an in vivo postantibiotic...
effect (PAE). The persisting suppression of bacterial growth after a short exposure to antimicrobial agents is known as the PAE (Zhanel and Craig, 1994). In vitro experiments with Gram-positive cocci such as *Staphylococcus aureus*, *S. pneumoniae*, *Enterococcus faecalis*, and *Streptococcus* spp. consistently demonstrated a PAE with different β-lactams (Eagle et al., 1950a; Sande et al., 1981). An in vivo PAE of penicillins on *S. pneumoniae* also has been demonstrated (Eagle et al., 1950a,b; Sande et al., 1981). However, studies with neutropenic animals and infection models in animals with impaired host resistance provided sharply contrasting data and the in vivo pertinence of PAE has been questioned (Vogelman et al., 1988b). These authors among others suggested that the observed PAE was due to residual antibiotic concentrations that were below the limit of detectability of the microbiological assay (Tauber et al., 1984; Vogelman et al., 1988b), or to the postantibiotic leukocyte enhancement effect (McDonald et al., 1981; Vogelman et al., 1988b). Indeed, these two factors have been clearly demonstrated to be involved in the duration and intensity of bacterial suppression in various animal models (Tauber et al., 1984; Vogelman et al., 1988b).

Nevertheless, our results clearly demonstrate the presence of an in vivo PAE for amoxicillin with *S. pneumoniae* in this pneumonia model. Duration of this PAE ($T_{1/2}$) was significantly different between strains, thus demonstrating that PAE is function of strain intrinsic properties (Table 2 and Fig. 4). Then, both postantibiotic leukocyte enhancement and antimicrobial activity of residual amoxicillin concentrations cannot be incriminated in this neutropenic murine pneumonia model. These differences in strains remain to be characterized and do not have something to do with inherent virulence not captured in the PK-PD model because all these strains belonged to serotypes 6, 19, and 23 (Moine et al., 1997a), which are naturally avirulent for mice independently of their isolation sites in humans (Bédos et al., 1991; Briles et al., 1992).

Duration of PAE ($k_{e0}$) was not correlated with in vitro
TABLE 3
Survival data fitted with increasingly complex models
Parameters are given with their asymptotic coefficient of variation (CV%). \( \omega^2 \) is the variance of \( \eta \), the interdose variability and LL is the log likelihood of the data with the particular model.

<table>
<thead>
<tr>
<th>Model</th>
</tr>
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<tbody>
<tr>
<td>S1 = \exp(-\theta t)</td>
</tr>
<tr>
<td>S2 = \exp(-\theta t) \cdot \exp(\alpha \cdot \ln CFU(t))</td>
</tr>
<tr>
<td>S3 = \exp(-\theta \cdot \ln CFU(t) \cdot t) \cdot \exp(\alpha \cdot \ln CFU)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CV%</th>
<th>( \omega^2 )</th>
<th>-2 LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>( \theta = 0.00491 \text{ h}^{-1} )</td>
<td>20</td>
<td>-190.5</td>
</tr>
<tr>
<td>S2</td>
<td>( \theta = 0.00273 \text{ h}^{-1} )</td>
<td>40</td>
<td>-280.3*</td>
</tr>
<tr>
<td>S3</td>
<td>( \theta = 0.00112 \text{ h}^{-1} \cdot \ln CFU )</td>
<td>22</td>
<td>-308.4**</td>
</tr>
</tbody>
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* \( P < 0.0001 \) versus S1; ** \( P < 0.0001 \) versus S2.

Fig. 5. Survival rate of mice infected with strain P15986 and having received different dosing protocols. Raw data are represented by symbols and fitted curves by lines. Fitting has been performed only for the first 9 days. The top graph shows that the same daily dose (600 mg/kg/24 h, i.e., \( 2 \times \text{MTD} \)) led to better survival rate when injected twice a day than when divided in four injections. Increasing the dose from 300 mg/kg/12 h to 400 mg/kg/12 h did not improve the survival rate. The bottom graph displays all various dosing patterns used showing that a simple exponential model incorporating the result of PD modeling (\( \text{ln CFU g}^{-1} \)) correctly predicts survival rate.

amoxicillin susceptibility tests, i.e., MIC and MBC. In fact, \( k_{e0} \) is related to duration of effect, not to the magnitude of this effect. The intrinsic activity of amoxicillin against bacterial growth in lung (\( E_{\text{max}} \)) was significantly different between strains. We did not show any relationship between \( E_{\text{max}} \) and MIC or MBC for amoxicillin against \( S. \ pneumoniae \) strains. However, there was a highly significant correlation between \( \text{Cpss}_{50} \), the serum drug concentration producing half-maximal effect at steady state and MIC and MBC. The latter result confirms our previous findings with the same bacterial data in lung (Moine et al., 1997a). Indeed, with a simpler dose-effect model, we showed that the dose producing half-maximal effect (\( \text{ED}_{50} \)) highly correlated with MIC and MBC, indicating that the doses leading to in vitro and in vivo bacterial growth inhibition (or killing) were correlated.

In fact, in our model, the two major determinants of amoxicillin in vivo bactericidal activity and therapeutic efficacy appeared to be the dose of amoxicillin because amoxicillin exhibits a rapid dose-dependent killing regardless of the \( S. \ pneumoniae \) strain tested, and the PAE duration, which was an intrinsic bacterial property. Indeed, for all strains, even strains with short PAE (\( k_{e0} > 0.01 \text{ h}^{-1} \) in our model), the amoxicillin-loading dose is the primary factor governing bactericidal activity. This finding may have implications for the clinical use of amoxicillin. Thus, increasing the amoxicillin-loading dose appears to be immediately clinically relevant. Furthermore, the “clinical” significance of the PAE lies in its application to dosing regimens (Vogelman et al., 1988b; Zhanel and Craig, 1994). For the strains with moderate-to-long duration of PAE (i.e., with \( k_{e0} < 0.01 \text{ h}^{-1} \) in our model), a persisting suppression of bacterial growth after the initial bactericidal effect appears to be effective with only one or two daily injections, and the extent of amoxicillin efficacy on bacterial count is related to the dose (P12698 and P15986 strains (Fig. 4). We found no gain in bactericidal activity with more frequent administration of the same daily amount of amoxicillin against \( S. \ pneumoniae \) P15986 strain. In fact, dividing the same daily dose into multiple administrations led to a slower decrease in \( \text{ln CFU g}^{-1} \) in animals infected with P15986 or P12698 strain (Fig. 4) and to a lesser survival rate in animals infected with P15986 strain (Fig. 5). In animals infected with P15986 strain, at 600 mg/kg daily dose, amoxicillin 300 mg/kg administered at 12-h intervals resulted in a better survival rate that amoxicillin 150 mg/kg administered at 6-h intervals (Fig. 5). This better survival rate was mainly due to the fact that the initial loading dose (300 mg/kg) led more rapidly to an important bactericidal effect in the lungs (Fig. 4). However, the strains with the shorter duration of PAE (\( k_{e0} > 0.01 \text{ h}^{-1} \) in our model) need a more frequent dosing to achieve a constant decrease in \( \text{ln CFU g}^{-1} \) (P40422 strain) (Fig. 4). Nevertheless, because amoxicillin also exhibits a rapid dose-dependent killing with these strains, increasing the amoxicillin-loading dose appears to be relevant.

In conclusion, with a PK-PD model to describe the overall dose-effect relationship of amoxicillin against 12 strains of \( S. \ pneumoniae \) with penicillin MICs between <0.01 and 16 \( \mu g/ml \) in a neutropenic murine pneumonia model, we have shown that the major determinant of amoxicillin in vivo bactericidal activity and therapeutic efficacy appeared to be the dose of amoxicillin because amoxicillin exhibits a rapid dose-dependent killing whatever the \( S. \ pneumoniae \) strain.
We also clearly demonstrated an in vivo PAE for amoxicillin with *S. pneumoniae*, which was a nonpredictable intrinsic bacterial property. Our findings may have implications for the clinical use of amoxicillin and could provide a theoretical rationale in its application to dosing regimens. Determination of the PAE does offer important information on the interaction between antimicrobial agent and microorganism that simple susceptibility testing and PK studies do not provide. This study gives a new approach on the PD properties of antimicrobial agents and further studies, including search for rapid, predictive, and clinically accessible PAE quantification tests, are needed before direct application of these principles to patients. However, in view of our results, the guidance to increase the amoxicillin-loading dose appears to be immediately clinically relevant in pneumococcal pneumo-

**References**


Send reprint requests to: Pierre Moine, M.D., Ph.D., Département d’Anesthésie-Réanimation, CHU de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre cedex, France. E-mail: darkb@imaginet.fr