

## Amoxicillin Is Effective against Penicillin-Resistant *Streptococcus pneumoniae* Strains in a Mouse Pneumonia Model Simulating Human Pharmacokinetics<sup>∇</sup>

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**High-dose oral amoxicillin (3 g/day) is the recommended empirical outpatient treatment of community-acquired pneumonia (CAP) in many European guidelines. To investigate the clinical efficacy of this treatment in CAP caused by *Streptococcus pneumoniae* strains with MICs of amoxicillin  $\geq 2$   $\mu\text{g/ml}$ , we used a lethal bacteremic pneumonia model in leukopenic female Swiss mice with induced renal failure to replicate amoxicillin kinetics in humans given 1 g/8 h orally. Amoxicillin (15 mg/kg of body weight/8 h subcutaneously) was given for 3 days. We used four *S. pneumoniae* strains with differing amoxicillin susceptibility and tolerance profiles. Rapid bacterial killing occurred with an amoxicillin-susceptible nontolerant strain: after 4 h, blood cultures were negative and lung homogenate counts under the  $2 \log_{10}$  CFU/ml detection threshold ( $6.5 \log_{10}$  CFU/ml in controls,  $P < 0.01$ ). With an amoxicillin-intermediate nontolerant strain, significant pulmonary bacterial clearance was observed after 24 h ( $4.3$  versus  $7.9 \log_{10}$  CFU/ml,  $P < 0.01$ ), and counts were undetectable 12 h after treatment completion. With an amoxicillin-intermediate tolerant strain, 24-h bacterial clearance was similar ( $5.4$  versus  $8.3 \log_{10}$  CFU/ml,  $P < 0.05$ ), but 12 h after treatment completion, lung homogenates contained  $3.3 \log_{10}$  CFU/ml. Similar results were obtained with an amoxicillin-resistant and -tolerant strain. Day 10 survival rates were usually similar across strains. Amoxicillin with pharmacokinetics simulating 1 g/8 h orally in humans is bactericidal in mice with pneumonia due to *S. pneumoniae* for which MICs were 2 to 4  $\mu\text{g/ml}$ . The killing rate depends not only on resistance but also on tolerance of the *S. pneumoniae* strains.**

*Streptococcus pneumoniae* remains the leading cause of community-acquired pneumonia (CAP) (22, 36, 39, 42). Bacteremic *S. pneumoniae* pneumonia accounts for 30% to 50% of admissions for CAP (36, 42, 47). Over the last two decades, *S. pneumoniae* strains with reduced susceptibility (MIC, 0.1 to 1  $\mu\text{g/ml}$ ) or resistance (MIC  $> 1$   $\mu\text{g/ml}$ ) to penicillin (4, 30) have emerged rapidly throughout the world (55). These strains contribute 50% to 60% of clinical *S. pneumoniae* isolates in some countries (23, 27, 40). According to data collected by the National Pneumococcus Reference Center in France (63) in 2002, strains with decreased susceptibility to penicillin (MIC  $> 0.1$   $\mu\text{g/ml}$ ) accounted for 53% of invasive pneumococcal infections, whereas only 8% of strains from adults with bacteremic pneumonia were resistant to penicillin (MIC  $> 1$   $\mu\text{g/ml}$ ). Penicillin-resistant strains exhibit variable patterns of resistance to other beta-lactams and are usually resistant to other classes of antibiotics that are active against pneumococci (12, 34, 53, 54, 55). The overall mortality rate in patients with *S. pneumoniae* pneumonia is about 10% (25). The overall mortality in patients admitted for bacteremic *S. pneumoniae* pneumonia has remained stable and high at about 25% over the last four decades (7, 31, 50). In patients with pneumonia, the clinical relevance of penicillin resistance is unclear because little is known about outcomes in patients infected with *S. pneumoniae* exhibiting high levels of penicillin resistance. Most studies suggest that

antibiotic resistance in *S. pneumoniae* may be devoid of clinical relevance (18, 21, 29, 32, 33, 50, 51), although a few found higher mortality rates in the patient subgroup with highly penicillin-resistant *S. pneumoniae* (22, 41, 65).

Most patients with pneumonia are treated as outpatients. In France, oral amoxicillin at 1 g every 8 h is the recommended first-line empirical treatment for apparently healthy adults who are treated on an outpatient basis for CAP possibly due to *S. pneumoniae* and who have no adverse prognostic factors (59).

Amoxicillin was effective in vivo in several studies of experimental pneumonia due to *S. pneumoniae* strains with varying degrees of resistance. However, most of these models failed to replicate amoxicillin pharmacokinetics in humans and therefore bear little relevance to clinical effectiveness (8, 28, 43, 57, 60). In studies by Woodnutt and Berry (67, 68) of a rat pneumonia model with amoxicillin-clavulanate doses simulating plasma concentrations achieved in humans, the treatment was effective against *S. pneumoniae* strains with amoxicillin MICs of 2 or 4  $\mu\text{g/ml}$ . In a mouse pneumonia model without simulation of human pharmacokinetics, we found a relationship between reduced in vivo pulmonary *S. pneumoniae* killing by amoxicillin and in vitro beta-lactam tolerance (8).

We used a reproducible model of lethal bacteremic *S. pneumoniae* pneumonia in mice to evaluate the effectiveness of amoxicillin with simulation of the pharmacokinetics seen in humans given the recommended dose of 1 g/8 h orally (24). Our objectives were to measure bacterial killing in serum and lung tissue over time according to the degree of penicillin susceptibility of the bacterial strain. In particular, we evaluated whether the amoxicillin regimen was effective against strains

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with amoxicillin MICs of 2 to 4  $\mu\text{g/ml}$ , and we assessed the impact of bacterial tolerance to amoxicillin on the treatment response.

## MATERIALS AND METHODS

**Challenge organisms and in vitro tests.** Four clinical *S. pneumoniae* isolates were used: a serotype 19 strain from a blood culture (strain P-52181), two serotype 23 strains from a tracheal aspirate (strain P-12698) and sinus fluid (strain P-54988), and a serotype 19 strain from middle ear fluid (strain P-15986). MICs and minimal bactericidal concentrations (MBCs) of the four strains were determined in Mueller-Hinton infusion broth (Diagnostic Pasteur, Marnes-la Coquette, France) supplemented with 5% sterile horse serum, using the tube dilution method (48). The tubes contained twofold dilutions of antibiotics and a final bacterial density of  $10^6$  CFU/ml. The tubes were incubated for 18 h at  $37^\circ\text{C}$  in 10%  $\text{CO}_2$ -air. MIC was defined as the lowest concentration of antibiotic at which no turbidity was visible to the naked eye. MBC was determined by plating 0.01-ml aliquots from tubes with no visible growth onto Columbia agar supplemented with 5% sheep blood (Bio-Mérieux, Lyon, France). The plates were incubated overnight at  $37^\circ\text{C}$  in 10%  $\text{CO}_2$ -air. The MBC was defined as the lowest concentration of antibiotic that killed  $\geq 99.9\%$  of the original inoculum.

To assess tolerance of the strains, we diluted samples in brain-heart infusion broth containing 5% horse serum. When the culture (at  $37^\circ\text{C}$ ) reached an optical density at 400 nm of about 0.1 to 0.2 (corresponding to  $10^8 \log_{10}$  CFU/ml), penicillin or amoxicillin was added in a concentration of 10 to 50 times the MIC for the strain (8). Counts of viable bacteria were determined by plating appropriately diluted cultures on Columbia agar supplemented with 5% sterile sheep blood 6 h after initiation of the cultures.

**Experimental pneumonia model.** Female Swiss albino mice aged 6 to 7 weeks (body weight, 25 to 27 g) were obtained from Iffa-Credo Laboratories, Les Oncins, France. Sustained leukopenia was induced by three intraperitoneal injections (150 mg/kg of body weight) of cyclophosphamide (Endoxan; Sarget Laboratories, Mérignac, France) per day starting 4 days before the bacterial challenge. Circulating leukocyte counts dropped from about  $7,000/\text{mm}^3$  initially to about  $1,200/\text{mm}^3$  on the day of infection, with neutrophil counts at about  $100/\text{mm}^3$ . The average leukocyte count reached  $1,000/\text{mm}^3$  of blood 1 day after infection and then increased progressively to  $4,700/\text{mm}^3$  of blood 3 days after infection (neutrophil count around  $500/\text{mm}^3$ ) to return to a normal count around  $7,100/\text{mm}^3$  of blood 5 days after infection (10, 11). The organisms were introduced by intratracheal instillation as described in detail elsewhere (9). Briefly, the animals were anesthetized by intraperitoneal injection of 0.2 to 0.25 ml of 0.65% sodium pentobarbital and were suspended by the upper incisors. The trachea was cannulated via the mouth using a blunt needle, and 40  $\mu\text{l}$  of bacterial suspension containing approximately  $10^7$  logarithmic-phase CFU was administered. Acute pneumonia developed consistently, and bacteremia occurred 1 to 4 h after the challenge. Untreated control mice died within 2 to 3 days. Bacterial counts exceeded  $10^8$  CFU/ml of lung homogenate at the time of death.

We used amoxicillin sodium salt (Smith Kline Beecham Laboratories, Nanterre, France) reconstituted as recommended in the package insert and diluted in sterile water to the desired concentrations. To simulate the pharmacokinetic profile in humans, we induced renal failure by intraperitoneal injection of a single dose of 6-mg/kg uranyl nitrate 4 days before the bacterial challenge. We chose this protocol because it induces less early mortality than the protocol described by Craig (10 mg/kg 3 days before infection) (1). Uranyl nitrate induces reversible acute tubular necrosis and has long been used to induce experimental renal failure (3, 37). We dissolved the uranyl nitrate crystals, and we diluted the solution to the desired concentration. Serial serum creatinine assays showed worsening renal failure over the 4 days following the injection, followed by a plateau phase during the three treatment days and, finally, by recovery. Thus, this model of simulated human pharmacokinetics can be used for simulations of up to 3 days. Because cyclophosphamide is excreted chiefly via the kidneys, we looked for an effect of renal failure on the duration and severity of the leukopenia. Circulating leukocyte counts were not significantly different in cyclophosphamide-treated mice with and without renal failure by the unpaired *t* test.

**Bactericidal activity in vivo.** We evaluated the effectiveness of amoxicillin in eradicating bacteria from the lungs in our model. Amoxicillin was started 3 h after the bacterial challenge. Nine subcutaneous injections were given at 8-h intervals in 0.25 ml of sterile water. Three doses were used: 7, 15, and 25 mg/kg of body weight. Controls received the same volume of isotonic saline. Mice were killed 4, 8, 24, and 84 h after the first amoxicillin injection by intraperitoneal injection of sodium pentobarbital. The mice were exsanguinated by cardiac puncture, and the blood was used for cultures. The lungs were removed and

homogenized in 1 ml of saline. The bacterial load was assessed as the number of CFU in whole-lung homogenates, determined by serial 10-fold dilutions plated onto Columbia agar. Results are expressed as the mean number ( $\log_{10}$ ) of CFU per lung  $\pm$  the standard deviation for groups of three mice. The lower detection limit was  $2 \log_{10}$  CFU per lung, which corresponded to the weakest dilution of tissue homogenate ( $10^{-1}$ ) that avoided significant drug carryover with control inocula.

**Survival studies.** Detailed treatment schedules are presented in Fig. 1. Each treatment group comprised 12 to 15 animals, and all the animals in the same experiment were infected simultaneously. The observation period was 10 days. Deaths were recorded daily, and cumulative mortality rates were compared as described in "Statistical analysis" below. Controls received injections of isotonic saline.

**Pharmacokinetic studies.** Concentrations in serum were determined after administration of a single subcutaneous dose of amoxicillin in noninfected mice. Serums were collected from groups of six mice 0.5 h, 1 h, 2 h, 4 h, 6 h, and 8 h after the amoxicillin injection. Animals were killed by intraperitoneal injection of sodium pentobarbital and exsanguinated by cardiac puncture. Blood samples were centrifuged, and sera were collected and stored at  $-80^\circ\text{C}$  until assay.

Amoxicillin was assayed by high-performance liquid chromatography using the technique of ion-pair reversed-phase chromatography with a LiChrosorb RP-18 column and a hydrophilic mobile phase. Plasma was deproteinized with ethanol, and the supernatant was directly injected onto a column of  $7\text{-}\mu\text{m}$  particle size. Chromatography was performed with the mobile phase at a 1.5-ml/min flow rate and a detection wavelength of 274 nm, with a sensitivity of 0.01 or 0.02 absorbance units at full scale. Plasma calibration curves showed good linearity between concentration and peak height over the concentration range of 5 to 400  $\mu\text{g/ml}$ . Results are expressed as  $\mu\text{g/ml}$  of serum. The lower limit of detection was 0.5  $\mu\text{g/ml}$  for blood samples. The relative standard deviation in duplicate plasma analysis was below  $\pm 3\%$  above 10  $\mu\text{g/ml}$  (62).

Concentration data were modeled: the best fit with experimental data was obtained using a single-compartment open model with zero-order absorption and first-order elimination. Parameters were estimated using standard methods.  $C_{\text{max}}$  was the maximal concentration measured;  $t_{1/2}$  was the terminal half-life calculated as  $\ln 2/k_{\text{el}}$ , where  $k_{\text{el}}$  was the elimination rate constant derived from the slope obtained by least-squares regression analysis for apparently linear portions of the log concentration-time curve;  $T_{>\text{MIC}}$  was the time during which concentrations exceeded the MIC for the test pathogen; and  $\text{AUC}_{0-24}$  was the area under the concentration-time curve from 0 to 24 h, computed using the trapezoid method (Siphar/Win version 1.1 software).

**Statistical analysis.** Survival rates were compared between treatment groups using the Mantel-Haenzel method. Data on bacterial clearance were compared between groups by analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons. *P* values of 0.05 or less were considered statistically significant.

## RESULTS

**Susceptibility and tolerance profiles of *S. pneumoniae* strains.** According to the criteria developed by the French Committee for Antibiotic Susceptibility Testing, P-52181 was susceptible to penicillin (MIC = 0.01  $\mu\text{g/ml}$ ) and amoxicillin (MIC = 0.03  $\mu\text{g/ml}$ ). P-54988 and P-12698 showed resistance to penicillin (MIC = 4  $\mu\text{g/ml}$ ) and intermediate susceptibility to amoxicillin (MIC = 2  $\mu\text{g/ml}$ ). P-15986 was highly resistant to penicillin (MIC = 8  $\mu\text{g/ml}$ ) and resistant to amoxicillin (MIC = 4  $\mu\text{g/ml}$ ). Both P-12698 and P-15986 were tolerant to penicillin, whereas P-52181 and P-54988 were not tolerant. Thus, the profiles were PsAsT<sup>-</sup> for P-52181, PrAiT<sup>-</sup> for P-54988, PrAiT<sup>+</sup> for P-12698, and PrArT<sup>+</sup> for P-15986.

**Serum pharmacokinetics of amoxicillin.** Table 1 shows serum amoxicillin levels measured after injection of 7, 15, or 25 mg/kg to uninfected mice with renal failure. After administration of 15 mg/kg of amoxicillin,  $C_{\text{max}}$  was  $28 \pm 11 \mu\text{g/ml}$ ,  $t_{1/2}$  was 1.6 h, and AUC was  $80 \text{ h} \cdot \mu\text{g/ml}$ . This dose produced the best simulation of pharmacokinetic values in humans after 1 g of oral amoxicillin ( $C_{\text{max}}$ ,  $19 \pm 7 \mu\text{g/ml}$ ;  $t_{1/2}$ , 1.5 h, and AUC,  $68 \text{ h} \cdot \mu\text{g/ml}$ ) (15, 61). This is the  $C_{\text{max}}$  concentration measured at 0.5 h, but the actual  $C_{\text{max}}$  could be higher.

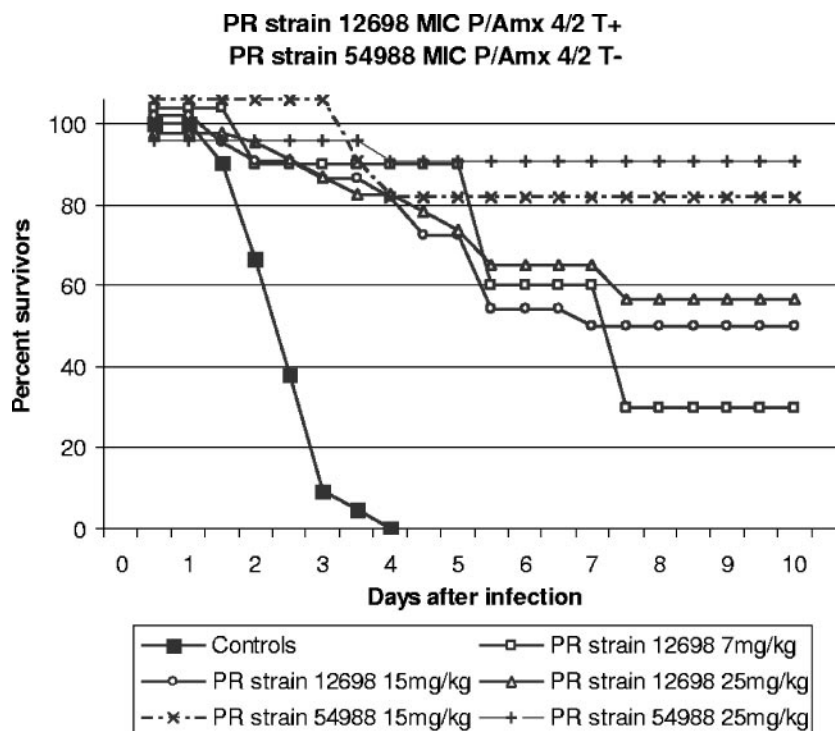


FIG. 1. Survival of leukopenic mice challenged with penicillin-resistant nontolerant strain P-54988 and penicillin-resistant tolerant strain P-12698. Mice received subcutaneous amoxicillin dosages of 7, 15, or 25 mg/kg every 8 h for 3 days.

**Bacterial clearance from blood and lungs.** Results for bacterial clearance are reported in Table 2. With the PsAsT- strain P-52181, blood cultures were consistently negative 4 h after the first amoxicillin injection. Bacterial killing in the lungs was significant as early as 4 h after the first amoxicillin injection. Bacterial killing was extremely rapid, and 24 h after the first injection, pulmonary bacterial killing was greater than  $5 \log_{10}$  CFU/lung with the 7-mg/kg and 15-mg/kg doses.

With the PrAiT- strain (P-54988), blood cultures were consistently negative 8 h after the first amoxicillin injection. Bacterial killing in the lungs was slower than with the PsAsT- strain P-52181 and was not significant until 24 h after the first amoxicillin injection ( $-3 \log_{10}$  CFU/lung with 7 mg/kg or 15 mg/kg). Bacterial regrowth was not present 12 h after completion of the 3-day treatment period.

TABLE 1. Serum pharmacokinetic parameters of amoxicillin in leukopenic mice with renal failure compared with results for humans

Subject group	Dose	Result for parameter <sup>a</sup> :			
		$C_{\max}^b$ ( $\mu\text{g/ml}$ )	$t_{1/2}$ (h)	AUC (h $\cdot$ $\mu\text{g/ml}$ )	$T_{>\text{MIC}}$ (h)
Mice <sup>c</sup>	7 mg/kg s.c. <sup>e</sup>	13 $\pm$ 3.5	1.6	46	4-6
	15 mg/kg s.c.	28 $\pm$ 11	1.6	80	4-8
	25 mg/kg s.c.	85 $\pm$ 30	1.7	227	8
Humans <sup>d</sup>	1 g orally	19 $\pm$ 7	1.5	68	

<sup>a</sup> Results are after 1 injection in mice (compared with 1 g orally in humans).

<sup>b</sup>  $C_{\max}$  is the concentration measured at 0.5 h; the actual  $C_{\max}$  could be higher.

<sup>c</sup> Values were calculated from six serum samples taken 0.5, 1, 2, 4, 6, and 8 h postdose.

<sup>d</sup> References 13 and 31.

<sup>e</sup> s.c., subcutaneously.

For results with the PrAiT+ strain (P-12698), bacterial killing in serum was slower, so that some blood cultures were still positive 24 h after the first amoxicillin injection. Although bacterial killing in the lungs was significant after 24 h ( $-3 \log_{10}$  CFU/lung with 7 mg/kg or 15 mg/kg), the lungs were positive ( $3.3 \log_{10}$  CFU/lung) 12 h after the end of the 3-day treatment period. Increasing the amoxicillin dose to 25 mg/kg failed to improve bacterial killing.

With the PrArT+ strain (P-15986), results were similar to those obtained with the PrAiT+ strain P-12698. Blood cultures did not become negative until after the first 24 h ( $-3 \log_{10}$  CFU/lung with 7 mg/kg or 15 mg/kg). Bacterial killing was slow, and the lungs were positive 12 h after the 3-day treatment period ( $3.1 \log_{10}$  CFU/lung).

**Therapeutic effect of amoxicillin.** Principal results are presented in Fig. 1. With the PsAsT- strain (P-52181), amoxicillin therapy in a dose of 15 mg/kg was associated with 83% survival (versus 0% in untreated controls,  $P < 0.001$ ). With the PrAiT- (P-54988) strain, survival (81%) after 15 mg/kg amoxicillin was not significantly different from that seen with the PsAsT- strain (P-52181). Survival was only 50% with the PrAiT+ (P-12698) strain, and 15 mg/kg amoxicillin and was not significantly improved by increasing the amoxicillin dosage to 25 mg/kg (57%). In contrast, survival with the PrArT+ strain (P-15986) (82%) was not significantly different from that seen with the PsAsT- strain after administration of 15 mg/kg amoxicillin.

## DISCUSSION

In France and other European countries, amoxicillin in a dosage of 1 g every 8 h by the oral route is the recommended

TABLE 2. Clearance of *S. pneumoniae* from lungs and blood of Swiss mice infected with different strains and treated with subcutaneous injections of amoxicillin every 8 h for 3 days

Strain and treatment group <sup>a</sup>	Result at time (h) after treatment:							
	4		8		24		84 <sup>b</sup>	
	Blood <sup>c</sup>	Lungs <sup>d</sup>	Blood	Lungs	Blood	Lungs	Blood	Lungs
P-52181								
Control	3/3	5.4 ± 0.4	3/3	6.5 ± 0.1	3/3	7.9 ± 1.0		
AMX, 7 mg/kg	0/3	3.9 ± 1.1	0/3	2.8 ± 0.9	0/3	2.3 ± 0.2		
AMX, 15 mg/kg	0/3	3.0 ± 0.1 <sup>e</sup>	0/3	<2.0 <sup>e,f</sup>	0/3	<2.0 <sup>e,f</sup>		
P-54988								
Control			3/3	6.2 ± 0.4	3/3	7.9 ± 0.1		
AMX, 7 mg/kg			0/3	5.6 ± 1.1	0/3	4.8 ± 0.9	0/3	<2.0
AMX, 15 mg/kg			0/3	5.0 ± 0.1	0/3	4.3 ± 0.1 <sup>g</sup>	0/3	<2.0 <sup>e,f</sup>
P-12698								
Control			3/3	7.6 ± 0.6	3/3	8.3 ± 0.1		
AMX, 7 mg/kg			1/3	7.2 ± 0.1	1/3	5.6 ± 0.4	0/3	4.2 ± 0.1
AMX, 15 mg/kg			1/3	6.3 ± 0.1	1/3	5.4 ± 0.4 <sup>g</sup>	0/3	3.3 ± 0.1 <sup>e</sup>
AMX, 25 mg/kg			1/3	6.0 ± 0.1	1/3	4.4 ± 0.1	0/3	3.3 ± 0.1
P-15986								
Control					3/3	8.1 ± 1.0		
AMX, 7 mg/kg					1/3	4.8 ± 0.4	0/3	3.7 ± 0.6
AMX, 15 mg/kg					0/3	3.5 ± 0.1 <sup>e</sup>	0/3	3.1 ± 0.2 <sup>e</sup>

<sup>a</sup> AMX, amoxicillin. MICs of penicillin and AMX and tolerance status for strains are as follows: P-52181, 0.01 µg/ml penicillin, 0.03 µg/ml AMX, not tolerant; P-54988, 4 µg/ml penicillin, 2 µg/ml AMX, not tolerant; P-12698, 4 µg/ml penicillin, 2 µg/ml AMX, tolerant; P-15986, 8 µg/ml penicillin, 4 µg/ml AMX, tolerant.

<sup>b</sup> Twelve hours after the end of treatment.

<sup>c</sup> Number of animals with positive blood cultures/total number of animals.

<sup>d</sup> Log<sub>10</sub> CFU/ml of lung homogenate; values are means ± standard deviations (*n* = 3).

<sup>e</sup> Lower than values for controls (*P* < 0.01).

<sup>f</sup> The lower limit of detection was 2.0 log<sub>10</sub> CFU/ml.

<sup>g</sup> Lower than values for controls (*P* < 0.05).

first-line empirical treatment for suspected pneumococcal CAP in apparently healthy adults who have no poor prognostic factors and are treated on an outpatient basis (14, 19, 20, 24, 26, 52, 59). Overall, the many studies conducted with adults found no correlation between resistance levels of the causative organism and outcomes when patients were stratified according to disease severity and treated with high-dose intravenous penicillin, ampicillin, amoxicillin, cefotaxime, or ceftriaxone (5, 41, 46, 50, 51, 69). Only two studies showed an association between resistance and mortality with strains exhibiting high-level resistance to penicillin G (MIC ≥ 4 µg/ml), and both have methodological weaknesses (22, 65). In a recent multicenter study of 465 patients admitted to French hospitals for pneumococcal pneumonia, including 47.5% with bacteremia, only 30 strains had amoxicillin MICs of ≥ 2 µg/ml (6.4%) (32). Similarly, of 638 patients with pneumococcal pneumonia studied by Aspa et al., only 3 patients had strains with amoxicillin MICs of 8 µg/ml (5, 6). This low rate of strains having amoxicillin MICs of ≥ 2 µg/ml is a major obstacle to studies of the clinical effectiveness of amoxicillin against such strains in patients with bacteremic pneumococcal pneumonia. The discriminating experimental approach therefore emerges as a valuable means of obtaining relevant preclinical information that supplements clinical data.

In earlier experiments using the same model, we showed that standard in vitro efficacy parameters, namely, MIC and MBC, predicted in vivo amoxicillin activity and that the MIC breakpoint for in vivo amoxicillin resistance was probably around 4 µg/ml (43). These results prompted the present study

with the same model with added renal failure to simulate amoxicillin pharmacokinetics in humans given 1 g every 8 h for 72 h. In addition, the amoxicillin protein-binding rate is low and very similar in humans and mice (about 15% to 20%) (2). Furthermore, we investigated several strains covering a range of amoxicillin susceptibility and tolerance profiles. The fast killing rate with the amoxicillin-susceptible strain suggests that reducing the duration of antibiotic therapy to 3 to 5 days may be as effective as the standard 7 to 10 days in patients with no other foci of infection (13, 38, 59). With the three strains having MICs of ≥ 2 µg/ml, in contrast, lung bacterial killing was slower and varied across strains. Differences in tolerance (44, 45, 49) characteristics may explain the observed differences in killing kinetics. It has been reported that after exposure to 20 times the MIC for 6 h, a nontolerant strain loses 4 to 5 log units of its viable counts, whereas a tolerant strain loses only 1 log unit (45). That tolerance affects treatment efficacy has been reported in other models, including streptococcal endocarditis models (64). In an earlier study using the same mouse *S. pneumoniae* pneumonia model without renal failure, tolerant and highly resistant strains were associated with failure of amoxicillin treatment in vivo (8). Few data are available on the prevalence of *S. pneumoniae* strains tolerant to beta-lactams. In a 2003 study of 73 strains recovered in France in blood or cerebrospinal fluid samples from children younger than 16 years of age, none of the strains were tolerant to penicillin or vancomycin (17).

A limitation of using uranyl nitrate-induced acute tubular necrosis to simulate amoxicillin pharmacokinetics in humans is

that only 3 days at the most are available for the study, which may lead to underestimation of the efficacy of amoxicillin. With the PrAiT+ strain (P-12698), treatment longer than 3 days might improve overall survival, but 40% of the deaths occurred while on therapy. Nevertheless, our results are consonant with those of two earlier studies with animal models with simulation of human pharmacokinetics. In an *in vivo* model of nonlethal thigh infection in neutropenic mice injected with uranyl nitrate, the amoxicillin breakpoint was 4  $\mu\text{g/ml}$  with resistant *S. pneumoniae* strains and simulation of pharmacokinetics in humans given 500 mg of amoxicillin every 8 h (1). The other study used a model of nonlethal bacteremic pneumococcal pneumonia in immunocompetent rabbits given amoxicillin intravenously with a variable flow rate to simulate human pharmacokinetics following 1 g orally or intravenously every 8 h. The amoxicillin MIC was 2  $\mu\text{g/ml}$ . After 48 h of amoxicillin therapy, significant bacterial clearance was noted in the lungs and spleen, as was a significant decrease in mortality (56).

Our results are in agreement with the pharmacokinetic and pharmacodynamic principles that predict the maximum efficacy of beta-lactam therapy. With the dose simulated in our study, the effect of amoxicillin is entirely time dependent, and the time spent with concentrations greater than the MIC during the dosing interval ( $T_{>\text{MIC}}$ ) is the main determinant of *in vitro* activity (66). *In vivo*, the killing effect is greatest when  $T_{>\text{MIC}}$  exceeds 50% (35), which was the case with the strains and simulated amoxicillin dosage used in our study. With the PrAiT+ strain (P-12698), increasing the dosage from 15 to 25 mg/kg/8 h induced threefold increases in  $C_{\text{max}}$  and AUC and produced a  $T_{>\text{MIC}}$  of 100% but failed to significantly increase the killing kinetics or survival rate.

Another means of potentiating the effect of amoxicillin is concomitant administration of an aminoglycoside. With the PrArT+ strain (P-15986), amoxicillin and gentamicin have shown synergistic effects in terms of lung bacterial killing with a significant increase in survival in our mouse model of neutropenic pneumococcal pneumonia (16).

Our experimental results are consistent with findings from a 2002 clinical study conducted in France in 465 adults admitted for *S. pneumoniae* pneumonia (32). The MIC was  $\geq 2 \mu\text{g/ml}$  for 25 (5.3%) strains and  $\geq 4 \mu\text{g/ml}$  for 5 (1.1%) strains. Most patients were given either amoxicillin with or without clavulanate (3 g/day) or parenteral cephalosporin therapy. No significant difference in overall mortality was found between the penicillin-susceptible group and the non-penicillin-susceptible group. Of the 5 patients with strains having MICs of  $\geq 4 \mu\text{g/ml}$ , 3 received an aminopenicillin with or without clavulanate (3 g/day) and experienced a full recovery. In another study, 4 patients with pneumonia caused by *S. pneumoniae* strains having amoxicillin MICs of  $\geq 2 \mu\text{g/ml}$  were successfully treated with amoxicillin-clavulanate (58).

In conclusion, in a model of lethal bacteremic pneumococcal pneumonia in neutropenic mice with induced renal failure, 15 mg/kg amoxicillin every 8 h, to replicate human pharmacokinetics, after 1 g orally every 8 h was associated with rapid and powerful bacterial killing when an amoxicillin-susceptible strain was used. In contrast, with strains having MICs of 2 or 4  $\mu\text{g/ml}$ , bacterial killing in serum and the lungs was influenced not only by the level of resistance but also by tolerance to beta-lactams across *S. pneumoniae* strains.

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