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Rationale behind High-Dose Amoxicillin Therapy for Acute Otitis Media Due to Penicillin-Nonsusceptible Pneumococci: Support from In Vitro Pharmacodynamic Studies

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To evaluate whether increased doses of amoxicillin should be used to treat acute pneumococcal otitis media, an in vitro pharmacokinetic model was used to evaluate the killing of pneumococci by amoxicillin when middle ear pharmacokinetics were simulated. Logarithmic-phase cultures were exposed to peak concentrations of 3, 6, and 9 μg of amoxicillin per ml every 12 h, and an elimination half-life of 1.6 h was simulated. Changes in viable bacterial counts were measured over 36 h. All three doses rapidly decreased the viable bacterial counts of penicillin-susceptible strains below the 10-CFU/ml limit of detection by 6 to 10 h and maintained counts below this limit through 36 h. The 3-μg/ml peak dose was much less effective against two of three strains with intermediate penicillin resistance and all three penicillin-resistant strains, with bacterial counts approaching those in drug-free control cultures by 12 h. The 6-μg/ml peak dose completely eliminated two of three strains with intermediate penicillin resistance and maintained viable counts of the other nonsusceptible strains at 1.5 to 2 logs below the initial inoculum through 36 h. The 9-μg/ml peak dose was most effective, completely eliminating all three strains with intermediate penicillin resistance and maintaining the viable counts of the resistant strains at 3 to 4 logs below the original inoculum. The pharmacodynamics observed in this study suggest that peak concentrations of amoxicillin of 6 to 9 μg/ml may be sufficient for the elimination of penicillin-nonsusceptible pneumococcal strains causing otitis media, especially those with intermediate resistance to amoxicillin. In vivo pharmacokinetic studies are needed to determine if these levels can be achieved in middle ear fluid with amoxicillin at 70 to 90 mg/kg/day divided into two daily doses. If these levels are reliably achieved, then clinical studies are warranted.

Amoxicillin was often prescribed for treatment of acute otitis media in children, as it provided coverage for *Streptococcus pneumoniae*, the most common cause of this infection in children, as well as many strains of *Haemophilus influenzae* and *Moraxella catarrhalis*, the second and third leading pathogens in this disease (16). However, with the increased prevalence of β-lactamase among *H. influenzae* and *M. catarrhalis* strains (28) and the appearance of penicillin resistance in *S. pneumoniae* (4, 12, 18), therapy of otitis media has become more problematic. To provide adequate coverage for these more resistant pathogens, some investigators have recommended a combination of amoxicillin plus amoxicillin-clavulanate for either initial therapy of acute otitis media or second-line treatment of amoxicillin failures (3). The rationale behind this recommendation is that the amoxicillin-clavulanate combination is active against β-lactamase-producing *H. influenzae* and *M. catarrhalis*, while the additional amoxicillin may provide middle ear drug levels sufficient to treat infections with *S. pneumoniae* with intermediate or full resistance to penicillin. However, there are no clinical or in vitro data to support such a high-dose amoxicillin regimen for the treatment of penicillin-nonsusceptible strains causing acute otitis media. Therefore, an in vitro pharmacokinetic model (IVPM) of infection was used to simulate the middle ear pharmacokinetics of amoxicillin and to evaluate the pharmacodynamic activity of amoxicillin against susceptible and nonsusceptible *S. pneumoniae* strains over three 12-h dosing intervals.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. The test panel included nine clinical isolates of *S. pneumoniae*. The serotypes and susceptibility profiles of the test panel are shown in Table 1. Logarithmic-phase cultures were prepared by suspending 10 colonies from a 14-h culture on Trypticase soy agar supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) into 6 ml of Todd-Hewitt broth (Unipath/Oxoid, Ogdensburg, N.Y.) supplemented with 0.5% yeast extract (THY) (20). Cultures were incubated at 37°C in 5% CO_2. Preliminary experiments demonstrated that cultures of all of the strains first became turbid 6 to 8 h after inoculation and that viable bacterial counts continued to increase exponentially through at least 12 h after inoculation. The number of viable bacteria in cultures at 10 h consistently ranged from 1×10^8 to 5×10^8 CFU/ml. Therefore, 10 h was selected as the time point when the logarithmic-phase cultures were introduced into the pharmacokinetic model for pharmacodynamic studies.

Antibiotic preparations. Amoxicillin powder (Sigma Chemical Co., St. Louis, Mo.) was reconstituted in phosphate buffer (KH_2PO_4 at 4 g/liter and K_2HPO_4 at 13.6 g/liter) and sterilized by passage through a 0.20-μm-pore-size Acrodisc syringe filter membrane (Gelman Sciences, Ann Arbor, Mich.). Desired concentrations of amoxicillin were obtained through dilution in sterile phosphate buffer.

Antimicrobial susceptibility testing. Susceptibility of the test panel to penicillin, amoxicillin, cefpodoxime, cefprozil, ceftriaxone, trimethoprim-sulfamethoxazole, and erythromycin was measured by broth microdilution assay in accordance with the procedures recommended by the National Committee for Clinical Laboratory Standards (24).

In vitro pharmacokinetic model. The IVPM used in these studies was a modification of the original model described by Blaser and colleagues (2). A schematic representation of the model is presented in Fig. 1. A hollow-fiber cartridge (HFC; Unisyn Fibertech, San Diego, Calif.) was connected to a central reservoir by a continuous loop of silicone tubing. Each HFC consisted of 2,250 hollow cellulose acetate fibers contained within a polycarbonate housing. At the start of each experiment, peak antibiotic concentrations in THY in the central reservoir were pumped through the hollow fibers of the cartridge and back into the central reservoir. As drug-containing THY passed through the hollow fibers, pores in the fiber walls allowed antibiotic and nutrients to freely diffuse from the lumen of the fibers into the space surrounding the hollow fibers within the cartridge (peripheral compartment) and back into the lumen of the hollow fibers.

TABLE 1. Characteristics of test panel

Strain	Serotype	MIC (μg/ml)[a]						
		Penicillin	Amoxicillin	Cefpodoxime	Cefprozil	Ceftriaxone	TMP/SMX[b]	Erythromycin
212	6	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	>2/38	≤0.06
256	4	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.12/2.4	≤0.06
9209	19	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.25/4.8	≤0.06
7622	19	0.5	0.25	1	2	0.5	>2/38	>1
7624	19	0.25	0.25	1	1	0.5	>2/38	≤0.06
BT41	6	0.5	0.5	0.5	1	0.25	>2/38	>1
3933	23	4	4	4	8	1	>2/38	≤0.06
3934	23	4	2	4	8	1	>2/38	≤0.06
3935	23	4	2	4	16	2	>2/38	≤0.06

[a] MICs were measured by microbroth dilution assay in accordance with the procedures recommended by the National Committee for Clinical Laboratory Standards (24).
[b] TMP/SMX, trimethoprim-sulfamethoxazole.

The exclusion size of the pores in the fiber walls (a molecular weight of 30,000) prohibited bacteria introduced into the peripheral compartment from entering the lumen of the hollow fibers. Thus, the drug concentration within the peripheral compartment could be altered without disrupting bacterial growth. The total surface area of exchange between the lumen of the hollow fibers and the peripheral compartment was 1.5 ft^2. The bacterial culture within the peripheral compartment was continuously circulated through a loop of silicone tubing attached to two ports entering and exiting the peripheral compartment, and samples were removed from the peripheral compartment through a three-way stopcock connected within the loop of silicone tubing. The initial culture volume circulated through the peripheral compartment and silicone tubing was 30 to 35 ml.

A comparison of the amoxicillin concentrations in the central reservoir and the peripheral compartment every 15 min after dosing demonstrated that equilibrium was established between the compartments at approximately 0.5 h. After peak concentrations were achieved within the peripheral compartment, human elimination pharmacokinetics of amoxicillin were simulated by a process of dilution and elimination of the drug in the central reservoir. Drug concentrations in the central reservoir (and the peripheral compartment as equilibrium was maintained) were decreased by addition of drug-free THY from a dilution reservoir (Fig. 1). To maintain a constant volume in the central reservoir, drug-containing THY was pumped from the central reservoir into an elimination reservoir. The rate at which drug concentrations in the central reservoir and peripheral compartment were decreased by this method was determined by the flow rate of the peristaltic pumps. This rate was calculated from an equation for clearance by monoexponential decline, based on the middle ear elimination half-life of amoxicillin and the volume of medium in the central reservoir.

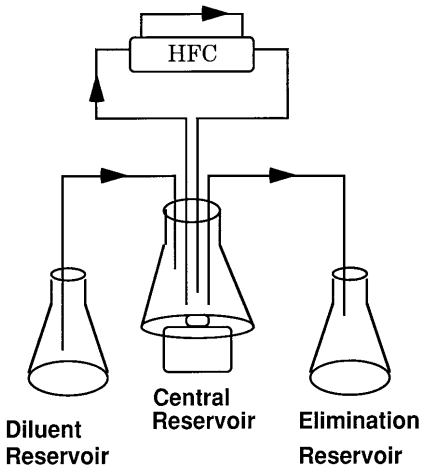

FIG. 1. Schematic representation of the two-compartment IVPM. Each arrow represents a peristaltic pump within the system. Peak concentrations of amoxicillin were injected into the central reservoir and pumped through the lumens of hollow fibers in an HFC. Pores (exclusion size, molecular weight of 30,000) in the fiber walls allowed the antibiotic to diffuse freely from the lumen of the hollow fibers into the peripheral compartment of the HFC, where bacteria were inoculated. The antibiotic was eliminated from the central reservoir by addition of drug-free broth from a diluent reservoir and elimination of drug-containing broth into the elimination reservoir. As antibiotic concentrations in the central reservoir decreased, antibiotic concentrations within the peripheral compartment also decreased as the drug diffused into the lumen of the hollow fibers to maintain an equilibrium between the two compartments.

No data have been published on the elimination pharmacokinetics of amoxicillin from the middle ear in humans. Therefore, an elimination half-life of 1.6 h was chosen based on middle ear pharmacokinetic data on chinchillas (6). In those studies, Canafax et al. observed an elimination half-life of 0.9 h from plasma, which is similar to the reported 1-h plasma elimination half-life in humans (23). Although it is difficult to predict pharmacokinetic parameters in humans from those observed in animal models, the similar plasma elimination half-life observed in chinchillas suggested that 1.6 h would be a reasonable estimation of the middle ear elimination half-life in humans.

Pharmacokinetics of amoxicillin in the IVPM. Middle ear concentrations of amoxicillin vary widely in humans, with mean peak values 1 to 3 h after administration of a single 15-mg/kg dose ranging from 3 to 6 μg/ml (8, 17, 25). No reliable data have been published for middle ear drug levels following higher doses in humans, but they may be estimated at 5 to 10 μg/ml for a single dose of 35 to 45 mg/kg (5, 21). Therefore, bacteria were exposed to peak concentrations of 3, 6, and 9 μg of amoxicillin per ml in this study to most accurately reflect the range of peak middle ear concentrations expected with single 15- and 35- to 45-mg/kg doses in humans. To evaluate the pharmacokinetics of amoxicillin in uninfected chambers, samples were removed from the peripheral compartment at 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 h after drug injection into the central compartment and drug concentrations were measured by bioassay with a susceptible strain of *Staphylococcus epidermidis.*

Pharmacodynamics of amoxicillin against *S. pneumoniae.* Logarithmic-phase *S. pneumoniae* cultures were diluted into fresh 37°C THY for a final concentration of 10^6 to 10^7 CFU/ml, introduced into the peripheral compartment of the IVPM, and exposed to amoxicillin as described above. Peak concentrations of amoxicillin were closed at 0, 12, and 24 h, and pharmacodynamic interactions were evaluated under ambient-air conditions at 37°C over three 12-h dosing intervals. At 0, 1, 2, 4, 6, 8, 10, 12, 14, 24, and 36 h, 400-μl samples taken from the peripheral compartment were treated for 15 min at 37°C with 100 μl of penicillinase from culture supernatants of *Bacillus cereus* (BBL) to inactivate amoxicillin. Viable bacterial counts were then determined by plating serial 10-fold dilutions of each sample into Todd-Hewitt agar (BBL) and incubating the plates overnight at 37°C in 5% CO_2. To evaluate the selection of mutants with decreased susceptibility, samples taken at 36 h were also plated into Todd-Hewitt agar containing amoxicillin at a concentration four times the MIC. The lowest dilution plated was 0.1 ml of an undiluted sample from the peripheral compartment. Since 30 colonies is the lower limit of accurate quantitation by pour plate methodology, the lowest concentration of bacteria that could be accurately determined was 300 CFU/ml. The lowest level of detection, although actual counts were inaccurate, was 10 CFU/ml.

To ensure that peak levels were within the desired range, samples were removed from the peripheral compartment at 0.5 h, filtered sterilized to remove bacteria, and assayed for amoxicillin. Preliminary experiments indicated that the pharmacokinetics of amoxicillin were unaffected by the introduction of *S. pneumoniae* into the IVPM (data not shown). Therefore, drug levels were not measured at other points during pharmacodynamic experiments.

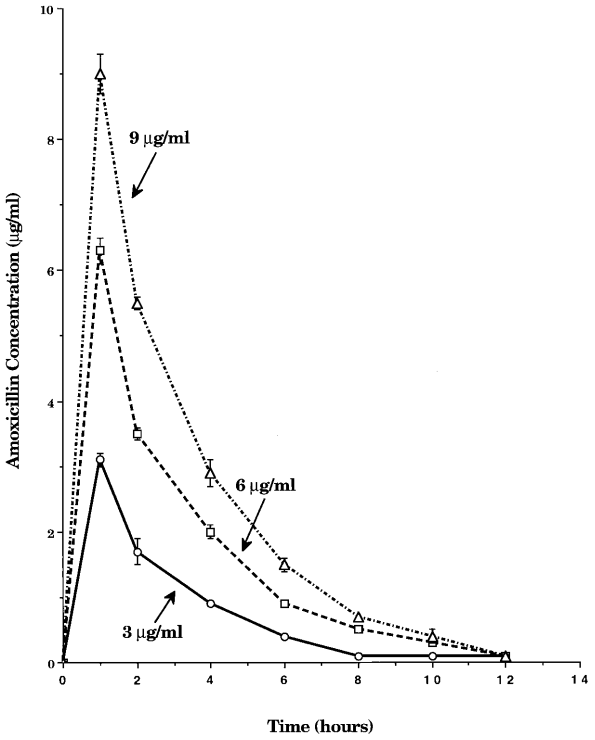

FIG. 2. Single-dose pharmacokinetic profiles of 3-, 6-, and 9-μg/ml peak amoxicillin doses in the peripheral compartment of the IVPM after introduction into the central reservoir. Drug levels were measured by bioassay. Each datum point represents the mean drug level in the peripheral compartment (in micrograms per milliliter) for three experimental runs. Error bars show the SEM.

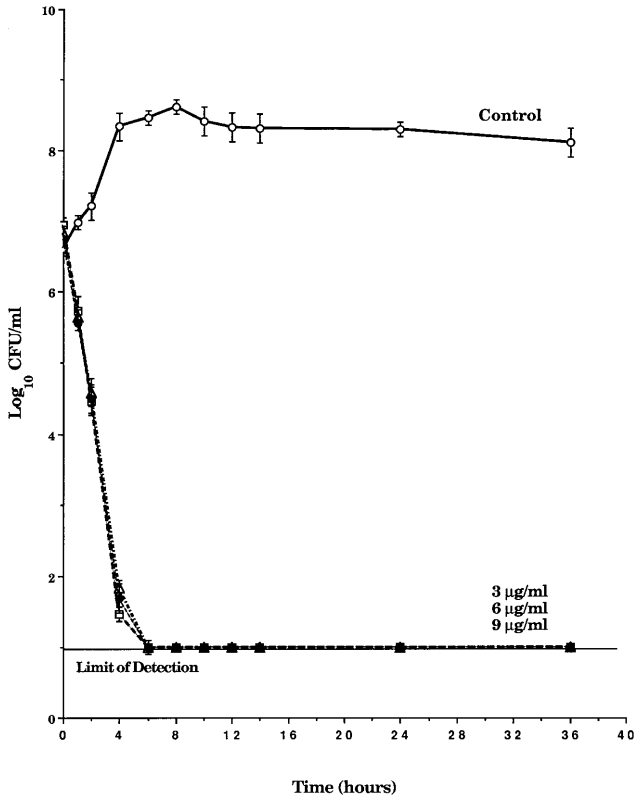

FIG. 3. Time-kill pharmacodynamics of amoxicillin against penicillin-susceptible *S. pneumoniae* 212 (amoxicillin MIC, ≤0.06 μg/ml). Amoxicillin doses were administered at 0, 12, and 24 h. Each datum point represents the mean number of CFU per milliliter of THY from the peripheral compartment for duplicate experiments. Error bars show the SEM.

RESULTS

Pharmacokinetics of amoxicillin in the IVPM. The pharmacokinetic profiles of the three amoxicillin doses over one dosing interval are shown in Fig. 2. Peak levels (mean ± the standard error of the mean [SEM]) of amoxicillin observed 0.5 h after dosing were 3.1 ± 0.3, 6.3 ± 0.2, and 9.0 ± 0.3 μg/ml for the 3-, 6-, and 9-μg/ml doses, respectively. The elimination half-life stimulated by the IVPM was 1.7 ± 0.2 h.

Pharmacodynamics of amoxicillin against penicillin-susceptible *S. pneumoniae*. Since the pharmacodynamics of the 3-, 6-, and 9-μg/ml peak doses of amoxicillin were almost identical against all three penicillin-susceptible *S. pneumoniae* strains, only the pharmacodynamics against strain 212 are shown in Fig. 3. All three doses of amoxicillin were rapidly bactericidal against the penicillin-susceptible strains, and the rates of bacterial killing were almost identical among the doses. Viable bacterial counts with all three doses decreased below the 10-CFU/ml limit of detection between 6 and 10 h after dosing, and bacterial counts remained below 10 CFU/ml throughout the remainder of the first and subsequent dosing intervals.

Pharmacodynamics of amoxicillin against intermediately penicillin-resistant *S. pneumoniae*. The pharmacodynamics of the 3-, 6-, and 9-μg/ml peak doses of amoxicillin against the strains with intermediate penicillin resistance are shown in Fig. 4. When killing of all three strains was observed, the three doses produced almost identical rates of killing. When inoculum regrowth was observed, initiation of bacterial growth coincided closely with the time when amoxicillin fell below the MIC. No mutants were selected from any of the intermediately resistant strains over the 36-h experimental period.

In studies with *S. pneumoniae* 7624 (Fig. 4B), all three doses were rapidly bactericidal, with bacterial counts falling 6 logs, to just above the 10-CFU/ml limit of detection by 12 h. Viable bacterial counts fell below detectable levels within 2 h after administration of the second dose and remained below 10 CFU/ml throughout the remainder of the 36-h experimental period. Similar bactericidal activity was observed with the 6- and 9-μg/ml peak regimens against *S. pneumoniae* 7622 (Fig. 4A). Although there was a slight increase in viable bacterial counts of this strain between 8 and 12 h, bacterial counts fell below detectable levels within 2 h after administration of the second dose and remained below 10 CFU/ml throughout the second and third dosing intervals. In contrast to the 6- and 9-μg/ml peak doses, rapid inoculum regrowth was observed with the 3-μg/ml peak dose between 8 and 12 h, with bacterial counts increasing 2.5 logs by administration of the second dose at 12 h (Fig. 4A). In addition, the antibacterial activities of the second and third doses were diminished compared to that of the first dose, with slight net increases in viable bacterial counts being observed at 24 and 36 h, compared to the viable counts at 12 h.

In studies with *S. pneumoniae* BT41 (Fig. 4C), the 3- and 6-μg/ml amoxicillin doses were least active. With the 3-μg/ml peak dose, approximately 3 logs of bacterial killing was observed over 6 h, followed by rapid inoculum regrowth until bacterial counts were similar to those in the drug-free control culture at 12 h. No antibacterial activity was observed with the second and third 3-μg/ml doses. With the 6-μg/ml peak dose, approximately 5 logs of bacterial killing was observed over 8 h, after which bacterial counts increased 2 logs. Although the second 6-μg/ml peak dose exhibited some bactericidal activity,

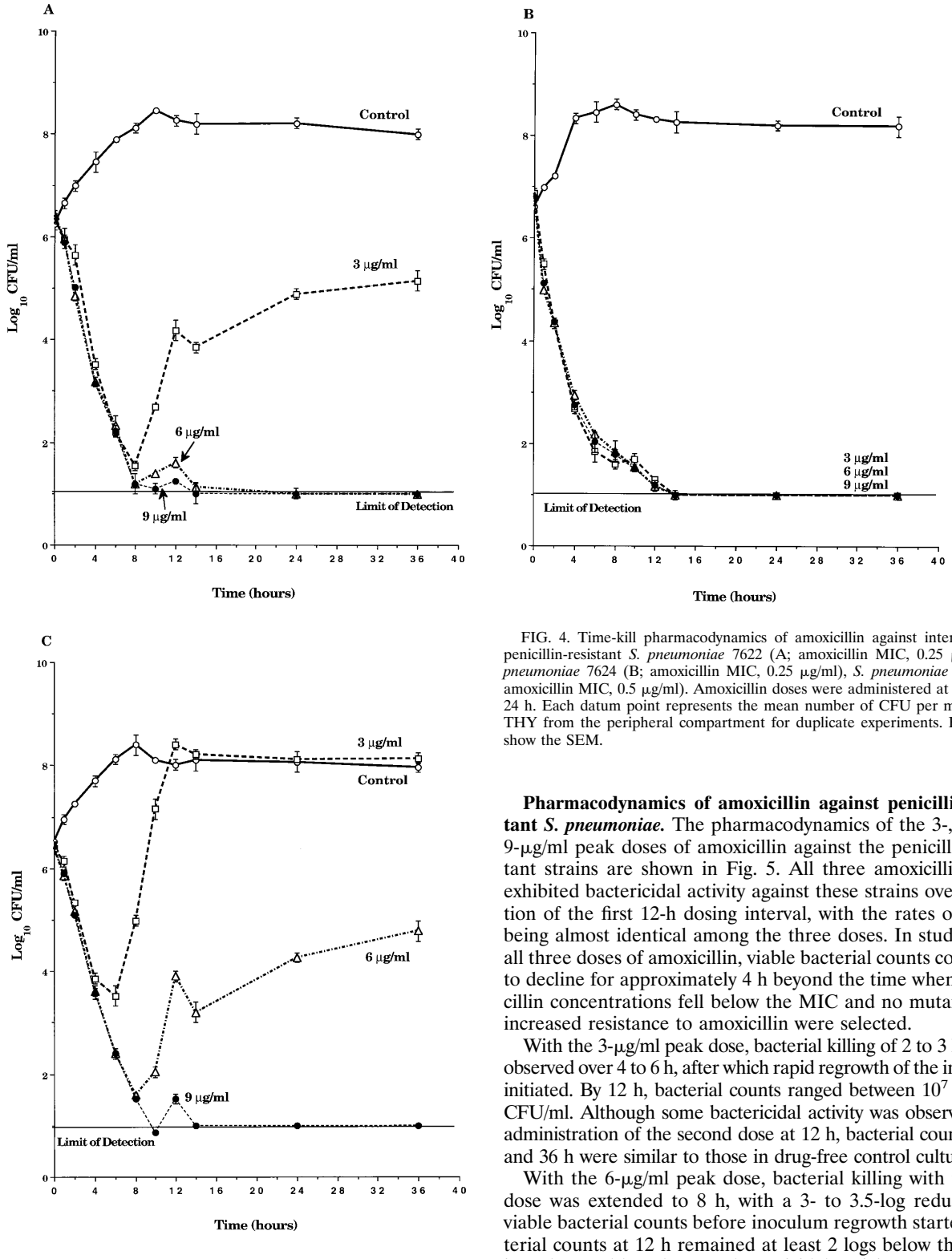

FIG. 4. Time-kill pharmacodynamics of amoxicillin against intermediately penicillin-resistant *S. pneumoniae* 7622 (A; amoxicillin MIC, 0.25 μg/ml), *S. pneumoniae* 7624 (B; amoxicillin MIC, 0.25 μg/ml), *S. pneumoniae* BT41 (C; amoxicillin MIC, 0.5 μg/ml). Amoxicillin doses were administered at 0, 12, and 24 h. Each datum point represents the mean number of CFU per milliliter of THY from the peripheral compartment for duplicate experiments. Error bars show the SEM.

the antibacterial activities of the second and third doses were diminished compared to that of the first, with viable counts at 36 h being approximately 1.5 logs higher than those observed at 12 h. The pharmacodynamics of the 9-μg/ml peak dose against *S. pneumoniae* BT41 (Fig. 4C) were similar to the dynamics of this dose against *S. pneumoniae* 7622 (Fig. 4A).

Pharmacodynamics of amoxicillin against penicillin-resistant *S. pneumoniae*. The pharmacodynamics of the 3-, 6-, and 9-μg/ml peak doses of amoxicillin against the penicillin-resistant strains are shown in Fig. 5. All three amoxicillin doses exhibited bactericidal activity against these strains over a portion of the first 12-h dosing interval, with the rates of killing being almost identical among the three doses. In studies with all three doses of amoxicillin, viable bacterial counts continued to decline for approximately 4 h beyond the time when amoxicillin concentrations fell below the MIC and no mutants with increased resistance to amoxicillin were selected.

With the 3-μg/ml peak dose, bacterial killing of 2 to 3 logs was observed over 4 to 6 h, after which rapid regrowth of the inoculum initiated. By 12 h, bacterial counts ranged between 10^7 and 10^8 CFU/ml. Although some bactericidal activity was observed with administration of the second dose at 12 h, bacterial counts at 24 and 36 h were similar to those in drug-free control cultures.

With the 6-μg/ml peak dose, bacterial killing with the first dose was extended to 8 h, with a 3- to 3.5-log reduction in viable bacterial counts before inoculum regrowth started. Bacterial counts at 12 h remained at least 2 logs below the initial inoculum. Although some bactericidal activity was observed with administration of the second dose at 12 h, antibacterial activity was diminished with the second and third doses compared to the first dose. However, even with net increases in viable bacterial counts over the second and third dosing intervals, bacterial counts remained 1.5 to 2 logs below the initial inoculum.

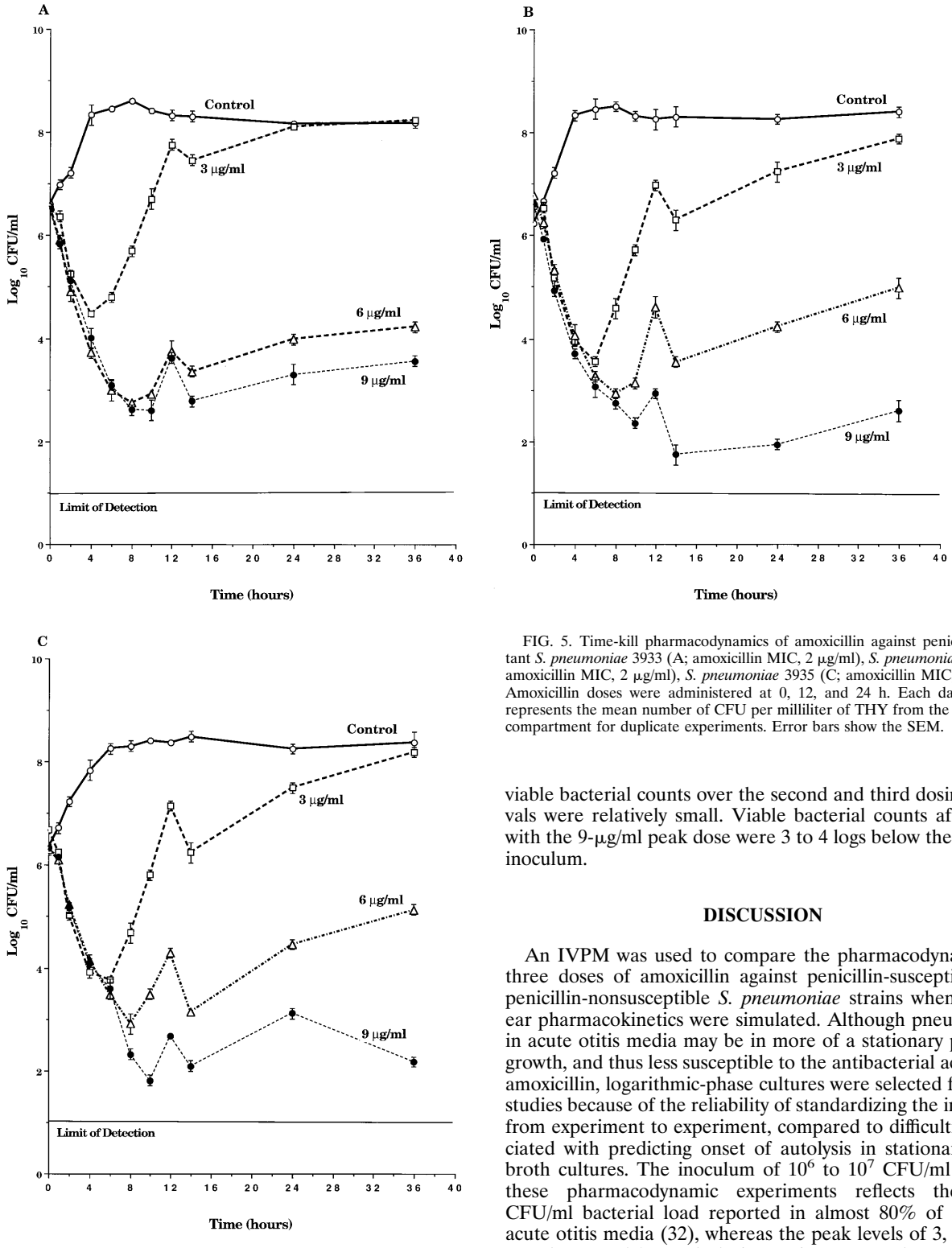

FIG. 5. Time-kill pharmacodynamics of amoxicillin against penicillin-resistant *S. pneumoniae* 3933 (A; amoxicillin MIC, 2 μg/ml), *S. pneumoniae* 3934 (B; amoxicillin MIC, 2 μg/ml), *S. pneumoniae* 3935 (C; amoxicillin MIC, 2 μg/ml). Amoxicillin doses were administered at 0, 12, and 24 h. Each datum point represents the mean number of CFU per milliliter of THY from the peripheral compartment for duplicate experiments. Error bars show the SEM.

With the 9-μg/ml peak dose, viable bacterial counts continued to decrease for 10 h after the first dose, with 4 to 5 logs of killing before inoculum regrowth initiated. Viable bacterial counts at 12 h remained 2.5 to 3.5 logs below the original inoculum. Although some bactericidal activity was observed with administration of the second dose at 12 h, net changes in viable bacterial counts over the second and third dosing intervals were relatively small. Viable bacterial counts after 36 h with the 9-μg/ml peak dose were 3 to 4 logs below the original inoculum.

DISCUSSION

An IVPM was used to compare the pharmacodynamics of three doses of amoxicillin against penicillin-susceptible and penicillin-nonsusceptible *S. pneumoniae* strains when middle ear pharmacokinetics were simulated. Although pneumococci in acute otitis media may be in more of a stationary phase of growth, and thus less susceptible to the antibacterial activity of amoxicillin, logarithmic-phase cultures were selected for these studies because of the reliability of standardizing the inoculum from experiment to experiment, compared to difficulties associated with predicting onset of autolysis in stationary-phase broth cultures. The inoculum of 10^6 to 10^7 CFU/ml used in these pharmacodynamic experiments reflects the $\geq 10^4$ CFU/ml bacterial load reported in almost 80% of cases of acute otitis media (32), whereas the peak levels of 3, 6, and 9 μg/ml targeted for each dosing regimen were chosen to most accurately reflect the range of peak concentrations of biologically active amoxicillin observed in the middle ear fluid of humans treated with single doses of 15 mg/kg (8, 17, 25) and 35 to 45 mg/kg (5, 21). Pharmacodynamic experiments were performed within a pH range of 7.0 to 7.2 without addition of serum proteins to the growth medium. Although variations in pH from 6.0 to 8.0 and addition of 50% human serum to the

growth medium have little effect on the antibacterial activity of amoxicillin (29), Erdmann et al. have reported that amoxicillin penetration of the middle ear fluid decreases when the pH falls below 7.0 or when binding of amoxicillin to serum proteins increases (13). The establishment of pharmacokinetic parameters in this model based on levels of biologically active amoxicillin in human middle ear fluid has already controlled for any factors influencing penetration. There are no middle ear elimination data for humans, and therefore a middle ear elimination half-life of 1.6 h was selected for this study based on middle ear pharmacokinetic data on chinchillas (6). Since antibiotics are typically eliminated at a faster rate from laboratory animals than from humans, it is possible that the pharmacokinetic parameters established in this study eliminated amoxicillin faster than would be observed in human middle ears.

The pharmacodynamics of all three doses against the penicillin-susceptible strains were similar, with almost identical rates of bacterial killing. The similar rates and levels of killing among the three doses support the conclusions of other investigators that the bactericidal activity of β-lactam antibiotics is not dose dependent at concentrations above the MIC (9, 31). Even the 3-μg/ml peak dose maintained bacterial counts below detectable levels throughout the 36-h experimental period. These data suggest that it may be possible to extend the interval between doses of amoxicillin in the treatment of susceptible pneumococcal infections from 8 to 12 h without compromising efficacy. Clinical studies are warranted to corroborate these in vitro observations.

In contrast to the penicillin-susceptible strains, the pharmacodynamics of amoxicillin against the penicillin-nonsusceptible isolates were much more variable in strain-to-strain comparisons and in comparisons between the different doses. Against only one intermediately resistant strain was the 3-μg/ml peak dose shown to be effective in clearing the bacterial inoculum. Against all of the other nonsusceptible strains, the 3-μg/ml peak dose was unable to maintain viable bacterial counts below the original inoculum over the first dosing interval. Although the antibacterial activities of the 6- and 9-μg/ml peak doses against the majority of the nonsusceptible strains were also diminished, these two doses were able to eliminate two of the three intermediately resistant strains and maintain viable bacterial counts of the other strains at least 1.5 logs below the initial inoculum through 36 h. With most of the strains, viable bacterial counts after three dosing intervals were at least 2 to 4 logs below the initial inoculum. These data suggest that if amoxicillin doses of 35 to 45 mg/kg can reliably provide middle ear levels within the range of 6 to 9 μg/ml, these higher doses may be sufficient for the treatment of acute otitis media with penicillin-nonsusceptible strains of *S. pneumoniae*, especially those exhibiting intermediate resistance to amoxicillin. Clinical studies are needed to corroborate these in vitro observations.

The inoculum regrowth observed in these experiments was not due to the growth of mutants with decreased penicillin susceptibility. When inoculum regrowth was observed with intermediately resistant strains 7622 and BT41, loss of antibacterial activity and initiation of regrowth coincided closely with the time when amoxicillin levels fell below the MIC. In studies with the resistant strains, however, loss of antibacterial activity was not observed for at least 4 h after amoxicillin concentrations had fallen below the MIC. This observation suggests that a substantial postantibiotic sub-MIC (PA-SME) (7) interaction was occurring between amoxicillin and these strains. The continued suppression of *S. pneumoniae* growth through PA-SME interactions with penicillins both in vitro and in vivo has been described by other investigators. Odenholt-Tornqvist et al. observed a similar 3- to 6-h PA-SME suppression of *S. pneumoniae* growth in vitro by penicillin when cultures were exposed to concentrations 0.1 to 0.3 times the MIC during the postantibiotic phase (26). In a rabbit model of meningitis, Sande et al. reported a postantibiotic effect of 6 to 12 h for amoxicillin against *S. pneumoniae* (27). However, when β-lactamase was injected at the site of infection, no postantibiotic effect was observed, suggesting that continued suppression of bacterial growth was the result of subinhibitory levels of amoxicillin or PA-SME interactions. In a recent study by Andes et al., 1-log or greater net reductions in *S. pneumoniae* bacterial counts were reported in a neutropenic mouse thigh model after three consecutive 8-h amoxicillin dosing intervals (1). These net reductions in bacterial counts were observed despite amoxicillin levels remaining above the MICs for the challenge strains for only 25 to 30% of each dosing interval. As a comparison, in the current study, a 1.5- to 2-log net reduction in bacterial counts of the resistant strains was observed at 36 h with the 6-μg/ml dose, despite amoxicillin levels remaining above the MICs for the resistant strains for only 2.5 to 4 h (20 to 35% of the dosing interval). These data suggest that PA-SME interactions play an important role in the pharmacodynamics of amoxicillin against some, but not all, *S. pneumoniae* isolates.

In experiments in which substantial inoculum regrowth was observed during the first dosing interval, the second and third doses of amoxicillin exhibited diminished antibacterial activity compared to the first. Viable counts at 24 and 36 h gradually increased above those at 12 h, despite the fact that no stable mutants were selected. The diminished antibacterial activity of the second dose was not due to loss of bactericidal activity, as decreases in viable counts were consistently observed between 12 and 14 h. Since viable counts were not measured at any time points between 24 and 36 h, the bactericidal activity of the third dose could not be assessed in these studies. Multiple-dose pharmacodynamic experiments with amoxicillin-sulbactam against TEM-1-producing *Escherichia coli* have demonstrated that similar net increases in viable counts observed with the second and subsequent doses, without any apparent change in susceptibility, are a reflection of decreased levels of killing coupled with increased inoculum regrowth (19). This phenomenon has been described as adaptive resistance or a reversible decrease in susceptibility upon exposure of bacteria to an antibiotic and has been observed with β-lactams (22), as well as aminoglycosides (10, 11, 15, 30).

The mechanism(s) responsible for the differing pharmacodynamic interactions between intermediately and fully penicillin-resistant strains and amoxicillin during the sub-MIC phase after dosing is unknown. However, it is possible that the observed differences are related to the degree of penicillin-binding protein (PBP) alterations between intermediately and fully penicillin-resistant pneumococci. Development of penicillin resistance in *S. pneumoniae* has been shown to be a gradual, stepwise process in which sequential alterations in essential PBPs result in decreased affinity for penicillin and progressively decreased susceptibility to penicillin (33). As the affinity of PBPs for penicillin decreases in resistant strains, the affinity of PBPs for their preferred natural murein synthesis substrates also appears to decrease. In contrast to susceptible strains, which utilize linear stem peptides for cell wall synthesis, resistant strains have been shown to prefer branched peptides for cell wall synthesis (14). What role, if any, these alterations in PBP affinity and substrate preferences play in differences observed between intermediate and resistant strains in this study is unknown. However, it is possible that the extensive alterations in PBPs required to achieve full resistance to penicillin

may slow the recovery of penicillin-resistant strains after drug levels fall below the MIC. In contrast, intermediately penicillin-resistant strains, which have not altered their PBPs as extensively as fully resistant strains, may be able to recover much more rapidly during the postantibiotic period, and thus, regrowth is observed shortly after drug levels fall below the MIC.

In summary, the 6- and 9-μg/ml peak doses of amoxicillin used in this study were significantly bactericidal against all of the pneumococcal strains evaluated, with greater than 3 logs of bacterial killing before regrowth initiated, if regrowth was observed at all. Substantial PA-SME interactions between amoxicillin and the penicillin-resistant strains limited bacterial regrowth to a level 2 to 3.5 logs below the original inoculum after the first 12-h dosing interval, and subsequent doses continued to maintain bacterial counts 1.5 to 4 logs below the original inoculum through 36 h. Considering that these studies were performed in the absence of any host defense mechanisms, these data suggest that middle ear levels of 6 to 9 μg of amoxicillin per ml may be sufficient for the treatment of acute pneumococcal otitis media with penicillin-nonsusceptible strains, especially those exhibiting intermediate resistance to amoxicillin. Further in vivo pharmacokinetic studies are therefore warranted to determine if higher doses of amoxicillin (e.g., 70 to 90 mg/kg/day administered every 12 h) can reliably provide middle ear levels of amoxicillin within the range of 6 to 9 μg/ml. If these levels are achievable, then clinical studies with higher doses of amoxicillin are warranted.

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