Pharmacokinetics and Efficacy of Linezolid in a Gerbil Model of Streptococcus pneumoniae-Induced Acute Otitis Media

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The oxazolidinone linezolid represents a new antibacterial class of potential benefit in managing multidrug-resistant gram-positive infections, including those caused by Streptococcus pneumoniae. In a gerbil model of acute otitis media (AOM) induced by either penicillin-resistant S. pneumoniae (PRSP; amoxicillin MIC = 8 \( \mu g/ml \)) or penicillin-susceptible S. pneumoniae (PSSP; amoxicillin MIC = 0.015 \( \mu g/ml \)), we explored the plasma and ear fluid levels of linezolid required to demonstrate efficacy. Threshold pathogen doses required to induce bilateral AOM (1,500 CFU/ear with PRSP; 30 CFU/ear with PSSP) were administered to gerbils by intrabullar injection on day 0. At peak infection (10^9 to 10^7 CFU/ear flush; day 2 for PRSP-AOM and day 3 for PSSP-AOM), twice-a-day oral doses of linezolid, amoxicillin, or vehicle were administered over 4.5 days prior to collection and assay of middle ear effluents for S. pneumoniae content. Linezolid doses of ≥10 mg/kg of body weight induced significant cure rates of ≥72% versus both PRSP and PSSP infections, whereas amoxicillin at ≤100 mg/kg was consistently effective only versus PSSP-AOM. Plasma and ear fluid levels of linezolid necessary to elicit pneumococcal eradication from the middle ear were measured by high-performance liquid chromatography-tandem mass spectrometry and found to be similar both within and between each infection protocol. The plasma-ear fluid pharmacodynamic profile associated with linezolid efficacy was a \( T >\text{MIC} \) of ≥42%, a \( C_{\text{max}}/\text{MIC} \) ratio of ≥31, and a (24-h area under the curve)/MIC ratio of ≥30 h. Application of this model will be useful in defining preclinical pharmacodynamic relationships of novel antibiotics necessary to cure S. pneumoniae-induced AOM.

Streptococcus pneumoniae is an obligate parasite in humans that often becomes the causative organism of upper respiratory tract infections, including pneumonia, sinusitis, and acute otitis media (AOM) (2, 3). Of particular concern has been the increase in the frequency of antibiotic-resistant isolates of this ubiquitous pathogen. Since the late 1980s, a steady rise in the incidence of beta-lactam- and macrolide-azalide-resistant pneumococcal strains has been well documented in epidemiological surveillance studies (13, 21, 22). Currently, 40 to 50% of S. pneumoniae clinical isolates have demonstrated reduced susceptibility to penicillin while exhibiting increased cross-resistance to other antibiotic classes, such as macrolides, trimethoprim-sulfamethoxazole, and tetracyclines (13, 21). In terms of treating AOM, this resistance development has been associated with clinical failures of drug therapy (10, 11, 29).

AOM is diagnosed over 25 million times annually in the United States, and in approximately 40% of cases the infection is caused by S. pneumoniae (4). One approach to overcoming the difficulties inherent in managing AOM induced by multidrug-resistant S. pneumoniae is the discovery of new antibiotics with improved antipneumococcal activity (23, 29). The oxazolidinone linezolid (Zyvox) represents a novel class of antibiotic with proven efficacy in treating drug-resistant, gram-positive infections in both preclinical models (12, 15) and human subjects (30, 33). Linezolid acts against such pathogens by inhibiting bacterial protein synthesis via prevention of the formation of the initiation complex (24, 34). With a MIC for 90% of strains tested for S. pneumoniae of 1.0 \( \mu g/ml \) (25, 32, 38), linezolid has been evaluated for efficacy in treating AOM in both the experimental and clinical settings. In chinchillas (27), an oral twice-a-day (b.i.d.) linezolid dose of 25 mg/kg of body weight affected cured in 100% of middle ear infections caused by penicillin-resistant S. pneumoniae (PRSP). In pediatric patients, a twice-daily oral linezolid dose of 10 mg/kg was successful in curing 84% of AOM cases known to be caused by S. pneumoniae (D. L. Fleishaker, D. C. Anderson, J. B. Brass, W. H. Chang, W. M. Todd, and B. Hafkin, abstract, Clin. Infect. Dis. 31:224, 2000).

As new oxazolidinones become available, a thorough understanding of the pharmacodynamic relationships necessary for effecting cures of S. pneumoniae infections becomes critically important in setting appropriate susceptibility breakpoints and optimizing applied dosing regimens (8, 17). In this study, we investigated the pharmacokinetic and pharmacodynamic relationships of linezolid necessary to effect cures in a newly characterized gerbil model of AOM, in which infections were induced with either a penicillin-susceptible or penicillin-resistant strain of S. pneumoniae.

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MATERIALS AND METHODS

Animal considerations. Female Mongolian gerbils (35 to 50 g; Charles River Laboratories, Kingston, N.C.) were used in this study. All animal procedures...
were performed in compliance with the Animal Welfare Act regulations, 9 CFR Parts 1, 2, and 3 and with the 1996 Guide for the Care and Use of Laboratory Animals, issued by the ILAR Commission of Life Sciences, National Academy Press (Washington, D.C.), as well as with all internal policies set forth by Pharmacal. Infection
organisms, inoculum preparation, and MIC determinations. S. pneumoniae strains UC15087 (clinical isolate; serotype 19F) and ATCC 6305 (serotype 5 strain; American Type Culture Collection, Rockville, Md.) were chosen for use in this study based on prior susceptibility and virulence profiling. Strain UC15087 was designated as PRSP, as it has been shown to be resistant to penicillin and clindamycin. Gerbils were anesthetized in prior testing (14), while strain ATCC 6305 was designated as penicillin-susceptible S. pneumoniae (PSSP), as it is known to be a penicillin-susceptible strain of S. pneumoniae (37). Cultures of each strain were grown overnight at 35°C on blood agar plates (brain heart infusion agar [BHI agar]; Difco Laboratories, Sparks, Md.) supplemented with 5% (vol/vol) digested sheep’s blood (Becton Dickinson Microbiology Systems, Sparks, Md.). Aliquots of these cultures, suspended in BHI broth (Difco Laboratories) containing a 20% (vol/vol) glycerol suspension, were maintained in the vapor phase of a liquid nitrogen freezer until needed for testing. PRSP and PSSP inocula suspensions used for in vivo testing were prepared by diluting thawed aliquots directly with BHI broth supplemented with 5% (vol/vol) horse serum (Gibco BRL, Paisley, United Kingdom). The MIC susceptibility profiles for these strains to linezolid and amoxicillin were determined as described previously (38) that followed guidelines set forth by the National Committee for Clinical Laboratory Standards (26).

Induction and measurement of AOM. The techniques used to induce bilateral S. pneumoniae-induced AOM in gerbils were similar to those described previ-
ously by Barry and colleagues (3) with some modifications. Animals anesthetized with Ketamine (40 mg/kg intramuscular) and Xylazine (13 mg/kg intramuscular) received intrabular injections of bacteria in both ears by using 28-gauge insulin syringes. The inoculum volume was standardized at 30 μl/ear. Inoculations were deemed successful if the investigator heard a popping sound, indicative of a sudden increase in middle ear air pressure. With this technique, >99% of inoculated ears had intact tympanic membranes upon visual inspection with an operating microscope. Gerbils were allowed to recover in a dorsal recumbent position after the inoculation procedure and monitored for up to 14 days postinfection. Middle ear infection development was monitored periodically as follows. Gerbils were reanesthetized as above and euthanized by cervical dislocation, and the external ears were removed for visual inspection of the tympanic membranes. Middle ear S. pneumoniae levels were then monitored by microbiological assay. Each middle ear chamber was flushed with 30 μl of BHI broth that was delivered and immediately withdrawn via direct tympanic membrane puncture with a 26-gauge needle. In pharmacokinetic experiments (described below) and in early model development, direct removal of middle ear fluid from each infected bullae revealed pooled volumes ranging from immeasurable to 30 μl. The subsequent injection and withdrawal of BHI fluid most likely represented a n2-fold dilution of ear fluid relative to its volume, with the needle dead-space volume of BHI (approximately 40 μl). Final effluent volumes assayed for bacterial content usually approximated 60 μl/ear. Hence, with this flushing technique, it was estimated that collected ear fluid samples represented a ≥3-fold dilution of constitutive ear fluid. Once collected, the samples were serially diluted with BHI broth, and 20-μl aliquots of each dilution were subsequently plated on blood agar. Plates were incubated at 35°C for 18 to 20 h prior to CFU determination. Bacterial counts for each ear were reported as the CFU per ear fluid. The lowest detectable bacterial count was 1 CFU/20-μl drop of each ear fluid sample collected (ca. 3 CFU/ear flush). Ears were considered uninfected or cured if S. pneumoniae counts in their respective effluents were below this detection limit.

AOM model development and in vivo antibiotic efficacy studies. Virulence testing of PRSP and PSSP were performed by inoculating gerbils via intrabular doses ranging from 3 to 1.5 × 105 CFU/ear and evaluating the frequency and intensity at which AOM was established 48 h later. For each strain, the lowest inoculum inducing AOM in 100% of treated ears was then used to define the time course of infection and applied during in vivo testing of antibiotics. Gerbils receiving this inoculum were examined between 6 h and 14 days later to monitor progression or regression of the established infection in terms of middle ear bacterial content and tympanic membrane appearance (subjectively determined if opaque or clear). For each specific AOM model, the postinfection period where middle ear bacterial levels peaked was determined and used to establish a standardized, multiday dosing interval in subsequent antibiotic efficacy trials. Antibiotic efficacy testing was performed in each AOM model using a nine-dose regimen that included at least the minimal dose (MID) calculated parameters included peak drug level (Cmax), time to peak (Tmax), apparent terminal disposition half-life (t1/2), and the area under the concentration-time curve from 0 to 12 h (AUC0-12). Pharmacodynamic relationships such as percentage of time drug concentrations met or exceeded the MIC threshold (T>MIC), the Cmax/MIC ratio, and the AUC0-24/MIC ratio ([AUC0-24/MIC] × 2 MIC) were determined by directly relating the pharmacokinetic data to the MIC for each drug concentration.
and vancomycin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Cefotaxime (sodium salt of acetate ester) was obtained from Calbiochem (La Jolla, Calif.).

For in vivo studies, stock suspensions of linezolid were formulated at 10 mg/ml in an Avicel vehicle containing 1% Avicel RC-591 (FMC Corp., Philadelphia, Pa.), 5% polysorbate 80, and 50 mM acetate buffer (pH 4.5). Stock suspensions of amoxicillin were prepared using 0.1 M NaH₂PO₄–0.1 M NaHPO₄ phosphate buffer (pH 6) as vehicle. For each experiment performed, a mean body weight was determined from 12 to 18 gerbils randomly selected at the time of pathogen inoculation. Drug concentrations in the final dosing suspensions were adjusted by vehicle dilution to provide a final dosing volume of 0.2 ml, which was given by oral gavage. After preparation, drug and vehicle suspensions were used throughout each protocol and kept at 4°C when not in use.

**Data presentation and statistical analysis.** All data represent means ± standard deviations of means unless otherwise noted. Treatment group geometric means of middle ear bacterial levels included those ear flush samples that were culture negative. In such instances, a value of 3 CFU/ear (detection limit of assay) was given to each sample. Differences between drug- and time-matched vehicle groups in terms of AOM frequency and intensity were analyzed using Fisher’s exact test and the Kruskal-Wallis one-way analysis of variance with post hoc application of a pair-wise Dunnett’s test, respectively. Group differences were considered statistically significant at a P level of <0.05.

**RESULTS**

**In vitro susceptibility tests.** The in vitro antibiotic susceptibility profiles of the two infecting strains of *S. pneumoniae* are summarized in Table 1. The divergence in MICs of the beta-lactams versus PRSP and PSSP was consistent with the penicillin-resistant and penicillin-susceptible designations of these strains, with PRSP demonstrating a high level of amoxicillin resistance (amoxicillin MIC = 8 µg/ml). These tests confirmed the lack of cross-resistance to linezolid, which had an MIC of 1 µg/ml for both pneumococcal isolates.

**AOM model development.** In initial dose-response testing, the threshold inocula required by the differing serotype strains to induce a reproducible AOM response—an opaque tympanic membrane with confirmed middle ear presence of pneumococci in 100% of ears (n = 8 to 10 ears/dose) within 2 days of infection—were 1,500 and 30 CFU/ear for PRSP (serotype 19F) and PSSP (serotype 5), respectively. These inoculum levels became the standard doses used in all subsequent studies. Differential leukocyte counts performed on fixed samples of ear effluent collected on or after day 5 postinfection revealed a high percentage of neutrophils (>80%) intermingled among ubiquitous amounts of cellular debris indicative of an active inflammatory response.

The infection time courses of AOM induced by PRSP (serotype 19F) and PSSP (serotype 5) are illustrated in Fig. 1. Middle ear *S. pneumoniae* levels rose ≥2 logs/ear flush within 48 h of infection in both models, peaking to approximately 10⁶ to 10⁷ CFU/ear flush between days 2 and 5 of PRSP infection (Fig. 1A) and between days 3 and 7 of PSSP infection (Fig. 1B). Evidence of resolution of middle ear infections appeared between days 10 to 14 in both models, but more so in PRSP-infected animals (Fig. 1). From these data, we chose to administer 4.5-day antibiotic regimens between days 2 and 6 of PRSP infection and between days 3 and 7 of PSSP infection. This

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**TABLE 1. In vitro antibiotic susceptibility profiles of infecting *S. pneumoniae* strains used to develop gerbil models of AOM**

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Test agent</th>
<th>MIC (µg/ml)*</th>
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<tbody>
<tr>
<td>PRSP (serotype 19F)</td>
<td>Penicillin G</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Cefotaxime</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>1</td>
</tr>
<tr>
<td>PSSP (serotype 5)</td>
<td>Penicillin G</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Cefotaxime</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>0.5</td>
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<tr>
<td></td>
<td>Linezolid</td>
<td>1</td>
</tr>
</tbody>
</table>

* PRSP, penicillin-resistant *S. pneumoniae* strain UC15087 (15); PSSP, penicillin-susceptible *S. pneumoniae* strain ATCC 6305 (38).

* Values represent modes of MIC assays performed in triplicate.
slight shift in treatment schedule was applied to more closely match the plateau phase of each respective infection time course.

The divergent serotypes of the two *S. pneumoniae* strains were accompanied by divergent pathogenicities of the two *S. pneumoniae* strains following intrabullar inoculation. During AOM induced by PSSP (serotype 5), the mortality rate of untreated animals was approximately 50% (9 of 17), with the majority of deaths occurring between days 2 and 6 of infection. Of those animals surviving the protocol, approximately 50% (4 of 8) showed signs of systemic infection (ruffled fur, reduced body weight) which was confirmed by the detection of pneumococci in the brain. In contrast, no infection-related deaths were observed after AOM induction with PRSP (serotype 19F), and only 6 of 28 infected animals (21%) sacrificed between days 1 and 14 had detectable brain levels of *S. pneumoniae*.

**Antibiotic efficacy testing with PRSP-AOM.** Figure 2 summarizes the antibacterial effects of amoxicillin and linezolid as well as the antibacterial time course of selected oxazolidinone treatments in a gerbil model of AOM induced by PRSP. Two days following bilateral intrabullar inoculation of PRSP (1,500 CFU/ear), subgroups of gerbils were given either no treatment, 4.5-day oral antibiotic therapy, or corresponding antibiotic vehicle. (A and B) In the standard dose-response protocol, middle ear *S. pneumoniae* levels in terminal ear flush samples (solid circles) were measured by microbiological assay on day 6 postinfection, 4 h after the last drug dose. (C) In the time course experiment, middle ear bacterial levels (solid circles) were determined on the third day of linezolid therapy (day 4 postinfection) as well as at the end of the drug treatment period (day 6 postinfection). Open bars and open circles represent group geometric mean CFU data and culture-negative ear flush samples, respectively. The *S. pneumoniae* detection limit (D.L.) was 3 CFU/ear flush. *, *P* < 0.05 versus the respective vehicle group.

![Graphs showing antibacterial effects of amoxicillin and linezolid](http://aac.asm.org/)

**FIG. 2.** Antibacterial effects of amoxicillin (A) and linezolid (B) as well as the antibacterial time course of selected oxazolidinone treatments (C) in a gerbil model of AOM induced by PRSP. Two days following bilateral intrabullar inoculation of PRSP (1,500 CFU/ear), subgroups of gerbils were given either no treatment, 4.5-day oral antibiotic therapy, or corresponding antibiotic vehicle. (A and B) In the standard dose-response protocol, middle ear *S. pneumoniae* levels in terminal ear flush samples (solid circles) were measured by microbiological assay on day 6 postinfection, 4 h after the last drug dose. (C) In the time course experiment, middle ear bacterial levels (solid circles) were determined on the third day of linezolid therapy (day 4 postinfection) as well as at the end of the drug treatment period (day 6 postinfection). Open bars and open circles represent group geometric mean CFU data and culture-negative ear flush samples, respectively. The *S. pneumoniae* detection limit (D.L.) was 3 CFU/ear flush. *, *P* < 0.05 versus the respective vehicle group.
colonies collected from those ears expressing infection in the test-of-cure period were reassayed for linezolid susceptibility. In each case, there was no evidence of development of resistance to the oxazolidinone.

Antibiotic efficacy testing with PSSP-AOM. The antibacterial effects of amoxicillin and linezolid in gerbils with PSSP-AOM are summarized in Fig. 3. As predicted from the in vitro susceptibility profile of PSSP, the beta-lactam effectively cured the middle ear infections. The oral b.i.d. ED\textsubscript{50} and ED\textsubscript{100} of amoxicillin in this protocol were 0.2 and 0.3 mg/kg, respectively (Fig. 3A). In a separate trial, the dose response and antimicrobial time course of linezolid were examined in the same experiment (Fig. 3B). Significant antibacterial effects were observed within 2.5 days at linezolid doses of $\geq$10 mg/kg (>5-log reduction in CFU/ear flush compared to the vehicle group; $P < 0.05$), with a 100% cure rate being achieved by the 30-mg/kg b.i.d. regimen. A test-of-cures analysis performed 4 days after completion of linezolid therapy revealed cure rates consistent with those observed within 4 h of treatment cessation (seven of eight ears cured in the 10-mg/kg group, eight of eight ears cured in the 30-mg/kg group; $P < 0.05$ versus vehicle group infection rate of 86%). In all cases, the microbiological cures achieved with these antibiotic regimens were again accompanied by tympanic membrane clearing.

The mortality rates of vehicle-treated animals with PSSP-AOM during the 4.5-day dosing period ranged between 33% (4 of 12 in the linezolid experiment) and 40% (6 of 15 in the amoxicillin experiment). These rates were significantly lower in animals placed on antibiotic therapy. Overall mortality rates in those groups receiving amoxicillin doses of $\geq$0.3 mg/kg or linezolid doses of $\geq$3 mg/kg were reduced to 8% (2 of 26; $P < 0.05$) and 3% (1 of 31; $P < 0.05$), respectively.

Pharmacokinetics of oral antibiotic treatments. Studies of the pharmacokinetics associated with selected doses of linezolid and amoxicillin in gerbils with *S. pneumoniae* AOM focused primarily on the plasma and/or ear fluid drug levels achieved after the third of nine scheduled b.i.d. doses (between 24 and 36 h of drug treatment). This protocol was applied in part to allow time for drug levels to reach steady state and in part because measurable ear fluid samples ($\geq$10 l/ear) were readily collectable during this period of the infection time course.

Plasma and concomitant ear fluid drug concentration-time curves generated from infected gerbils receiving either 10- or 30-mg/kg repeat oral doses of linezolid are illustrated in Fig. 4. The patterns of drug exposure achieved with these doses were similar regardless of whether PRSP (Fig. 4A and B) or PSSP (Fig. 4C and D) was the infectious agent. Additionally, there was little evidence of drug accumulation with either dosing regimen. Specifically, mean trough levels of linezolid, which were equivalent in plasma and ear fluid, remained relatively steady over the course of the 4.5-day treatment period used in this study (Fig. 4).

Third-dose pharmacokinetic parameters determined from the linezolid exposure experiments are listed in Table 2. In each of four pharmacokinetic trials, linezolid readily and rapidly distributed between the plasma and middle ear fluid compartments. Overall, mean ear fluid $C_{\text{max}}$ levels (102% ± 35% of mean plasma levels; $n = 4$) and AUC\textsubscript{0-12} levels (113% ± 16% of mean plasma levels; $n = 4$) were equivalent to those observed in host plasma, while the delay in $T_{\text{max}}$ observed in ear fluid was $\leq$0.5 h (Table 2). Under both experimental conditions (PRSP or PSSP infection), the threshold linezolid antibacterial regimen of 10 mg/kg was associated with mean plasma and ear fluid drug $C_{\text{max}}$ levels of 3.1 to 5.1 l/ear and mean AUC\textsubscript{0-12} levels of 14.9 to 20.4 l/ear $\cdot$ h/ml. Increasing the b.i.d. dose to 30 mg/kg resulted in a linear increase in linezolid exposure, as mean plasma and ear fluid drug $C_{\text{max}}$ and AUC\textsubscript{0-12} levels rose to between 10.9 and 16.7 l/ear and 53.7 to
75.6 μg · h/ml, respectively. This half-log dose increase was also associated with a modest 1.3- to 2.1-fold increase in the t1/2 of linezolid in these two fluid compartments (Table 2).

Plasma pharmacokinetic parameters associated with amoxicillin treatment are also summarized in Table 2. In PRSP-infected gerbils, the 100-mg/kg b.i.d. therapy was associated with high plasma drug levels (Cmax = 30.2 ± 0.8 mg/ml; Tmax = 0.25 h) that cleared rapidly (t1/2 = 2.2 h). In PSSP-infected gerbils, the narrow detection window that existed between the relatively low plasma drug levels and the detection limit of the HPLC-MS/MS assay complicated pharmacokinetic analysis of the maximally effective 0.3-mg/kg dose of amoxicillin. From the limited data thus collected, only mean plasma Cmax (0.042 μg/ml; n = 2) and Tmax (0.25 h) could be estimated for this dosing regimen (Table 2). With both treatment regimens, there was no indication of drug accumulation with each successive dose. The pharmacokinetics of amoxicillin, observed here, are in line with those reported in humans receiving multiple-dose oral therapy (5).

**Pharmacodynamics of oral antibiotic treatments.** Table 3 summarizes the estimated plasma and ear fluid drug pharmacodynamics as well as the antibacterial effects that accompanied doses of linezolid and amoxicillin in this model.

Linezolid antibacterial potency, efficacy, and concomitant pharmacodynamic profiles were similar regardless of the infecting *S. pneumoniae* strain. Additionally, ear fluid pharmacodynamic parameters estimated for this agent usually met or exceeded those determined in the plasma compartment. In the combined AOM experiments, the threshold antibacterial regimen of 10 mg/kg was accompanied by plasma and ear fluid drug Cmax/MIC ratios ranging from 3.1 to 5.1, T>MICs ranging from 42 to 63%, and AUC0-24/MIC ratios ranging from 30 to 41 h. Increasing the linezolid dose threefold caused a similar increase in most of the parameters that were monitored (Table 3). In PRSP-infected animals, 100-mg/kg b.i.d. amoxicillin therapy was associated with a Cmax/MIC ratio of 3.8 and a T>MIC of 22%. Stringent analysis of the pharmacodynamics of the 0.3-mg/kg regimen was not attempted due to the limited pharmacokinetic data (noted above) and the lack of separation between the amoxicillin MIC level (for PSSP) and the detection limit of the HPLC-MS/MS assay (Table 3).

**DISCUSSION**

Mongolian gerbils are often used to establish antibiotic screening models of AOM due to their enlarged middle ear...
chambers or bullae and the fact that common human pathogens normally do not reside in either the bullae or the interconnected nasopharynx (16, 36). In this study, we have characterized a gerbil model of *S. pneumoniae*-induced AOM that should prove useful in evaluating the antimicrobial efficacy of multiday antibiotic dosing regimens. Once experimental conditions were established to elicit reproducible infections with either a PRSP or PSSP strain, the model was validated by the success (or lack thereof) of amoxicillin and linezolid therapies in eradicating pneumococci from the gerbil middle ear. Variations in virulence and infection invasiveness between PRSP- and PSSP-AOM were not unexpected, considering their respective serotype differences (2). These variations were accounted for (during the creation of a standardized protocol) by adjusting the intrabullar inoculum doses of each strain as well as the time frame after which antibiotic treatments were initiated. During the design phase, a concerted effort was made to implement a protocol that not only matched the clinical situation, i.e., antibiotic therapy initiated at peak infection, but also one that allowed simultaneous measurement of drug exposures achieved in plasma and ear fluid during efficacious drug treatment.

The objective of this study was to define the overall pharmacodynamic profiles of linezolid associated with efficacy in a clinically relevant model of *S. pneumoniae* infection. Using a chinchilla model of AOM, Pelton and colleagues (27) were the first to demonstrate that orally administered linezolid readily distributes into ear fluid at concentrations sufficient to cure *S. pneumoniae* infections of the middle ear. In their study, a twice-daily linezolid dose of 25 mg/kg cured 100% of infected ears within 2 days of treatment while maintaining an ear fluid drug *T > MIC* of 100% (27). In this gerbil study, we have confirmed and extended this observation through a more extensive pharmacodynamic evaluation. Significant middle ear eradication of *S. pneumoniae* was achieved with linezolid therapy when the ear fluid drug *T > MIC*, *Cmax/MIC*, and *AUC0-24/MIC* ratios reached thresholds of ~60%, 4 to 5, and 40 h, respectively. The in vitro and in vivo antibacterial potency of linezolid was similar for the PRSP and PSSP strains, as was the ear fluid drug pharmacodynamic profile that accompanied pneumococcal eradication from the gerbil middle ear.

Pharmacodynamic profiling of linezolid has been performed in other *S. pneumoniae* infection models. In a rat model of

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<th>Table 2. Third-dose plasma and ear fluid drug pharmacokinetics associated with selected regimens of linezolid and amoxicillin in a gerbil model of <em>S. pneumoniae</em>-induced AOM</th>
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<tr>
<td><strong>Drug and oral b.i.d. dose</strong>&lt;sup&gt;a&lt;/sup&gt; (mg/kg)</td>
</tr>
<tr>
<td>PRSP</td>
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<sup>a</sup> Nine-dose regimen given over 4.5 days; drug exposures measured after third scheduled dose.

<sup>b</sup> Mean ± standard deviation (*n* = 3 for plasma; *n* = 4 to 6 for ear fluid) or actual levels when *n* < 3.

<sup>c</sup> Apparent terminal disposition half-life.

<sup>d</sup> Two-hour time point missed.

<sup>e</sup> ND, not determined due to a lack of time points with detectable drug levels.

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<th>Table 3. Estimated pharmacodynamic profiles associated with selected linezolid and amoxicillin regimens in a gerbil model of <em>S. pneumoniae</em>-induced AOM</th>
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<td><strong>Infectious strain</strong></td>
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<td>PSSP</td>
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<sup>a</sup> Nine-dose oral b.i.d. regimen given over 4.5 days.

<sup>b</sup> Geometric mean reduction in ear flush CFU levels versus respective vehicle group measured 4 h after the last drug dose.

<sup>c</sup> Frequency of ear flush samples within each treatment group that were culture negative.

<sup>d</sup> *P* < 0.05 versus respective vehicle group.

<sup>e</sup> ND, not determined due to lack of time points with detectable drug levels in pharmacokinetic experiments and insufficient separation between amoxicillin MIC (0.015 μg/ml for PSSP) and the lower limit of quantitation of the HPLC-MS/MS assay (0.013 μg/ml).
lethal pneumonia, significant reductions in mortality (42% reduction) and concomitant bacteria levels (>5-log reduction after five doses) were observed with an oral b.i.d. regimen of 25 mg/kg that maintained the total serum drug T>MIC, mean Cmax/MIC, and mean AUC0-24/MIC at 36%, 16.9, and 119 h, respectively (18). In a neutropenic thigh infection model in mice, Andes and colleagues (1) demonstrated that the serum drug AUC0-24/MIC ratio was the best predictor of antibacterial efficacy of linezolid and that, for eight different S. pneumoniae strains, bacteriostatic doses of linezolid maintained mean AUC0-24/MIC ratios between 22 and 97 h. In this AOM study, antibacterial efficacy was associated with a plasma drug T>MIC of 42%, a Cmax/MIC of 3, and an AUC0-24/MIC of 30 h. Determination of the specific pharmacodynamic parameter most predictive of efficacy in this model is a topic of future study. Such a determination will require a comparison of activities of similar daily doses applied at various dosing intervals.

The similarities of the plasma and serum drug pharmacokinetic profiles of linezolid associated with efficacy against preclinical pneumococcal infections, regardless of infection site, strongly reflects the relatively high volume of distribution of the drug, which approximates total body water (6, 31). The ability of linezolid to penetrate soft tissues and/or cure localized infections has been well demonstrated in both animals (15, 18) and humans (7, 30, 33). In terms of treating upper respiratory tract infections, Pelton and colleagues (27) showed that linezolid eradicated S. pneumoniae not only from the middle ear but also from the nasopharynx, which can become an important reservoir for resistant bacteria. The ability of an agent to distribute throughout the upper respiratory tract at antibacterial concentrations decreases the chances of rebound middle ear infection caused by resistant strains of targeted organisms, and it also decreases the potential spread of such infections via nasopharynx carriage and transmission (10, 27).

The test-of-cure data generated 4 days posttherapy in the gerbils infected with either PRSP or PSSP revealed only one case of potential AOM relapse (6% of all ears examined) in those animals given the ED100 of linezolid. In this single case, the persistent S. pneumoniae isolate had retained its susceptibility to the oxazolidinone. When interpreting relapse data in this model, it is important to realize inherent protocol limitations, such as an abbreviated dosing schedule, and the fact that time-dependent infection frequencies were monitored in separate populations of animals. Nonetheless, it is interesting that in various clinical trials, between 24 and 58% of bacterial isolates collected from persistent cases of AOM were shown to be susceptible to the previously prescribed antibiotics (20, 28, 35). The exact cause of this phenomenon is unknown but may include such factors as the brevity of the dosing regimen, poor compliance, poor accessibility of drug-containing ear fluid secretions to the site of infection origin, pathogen biofilm production, and/or variability in the host immune response necessary for proper infection clearance.

Amoxicillin, given alone or in combination with clavulanic acid, is prescribed often for the empirical treatment of AOM because of its effectiveness against both S. pneumoniae and the common gram-negative pathogens Haemophilus influenzae and Moraxella catarrhalis. It has been well characterized that a serum drug T>MIC approaching 40% is a key pharmacodynamic determinant of in vivo efficacy for this and other beta-lactams (8, 9). Amoxicillin regimens were applied in this study as a means of confirming the transference of the differential susceptibilities of the PRSP and PSSP strains from the in vitro to the in vivo setting. In PRSP-infected animals, administration of a 100-mg/kg b.i.d. dose of amoxicillin was associated with a T>MIC of only 22% despite achieving markedly elevated plasma drug levels, a pharmacodynamic insufficiency caused by the high level of pathogen resistance. In turn, the inconsistent antibacterial effects obtained with high-dose amoxicillin against PRSP-AOM were most likely due to the suboptimal T>MIC profile of the treatment regimen (Fig. 2; Table 3).

The continuing increase in the frequency of drug-resistant clinical isolates of S. pneumoniae poses concern towards achieving pharmacological cures of AOM. Complications associated with unresolved cases of S. pneumoniae-induced AOM can be severe and include meningitis, mastoiditis, and persistent effusion with hearing loss (2, 4, 29). In treating these infections, there has been much debate as to how best to minimize the development of resistant strains while improving clinical outcomes of antibiotic intervention. Besides the need to continue the ongoing search for drugs active against resistant bacteria (11, 29), it is apparent that breakpoint determinations guiding current and future antibiotic usage must take into account the respective pharmacokinetic and pharmacodynamic properties of such agents (8, 14, 21). In this study, we defined the pharmacodynamic relationships of linezolid necessary to affect preclinical cures of S. pneumoniae-induced AOM. Application of this methodology will prove beneficial in the determination of appropriate breakpoints for other novel antibiotics.

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