Efficacy of Linezolid in Experimental Otitis Media

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Therapy for otitis media (OM) due to resistant Streptococcus pneumoniae (MIC of penicillin, ≥2.0 μg/ml) is challenging. Linezolid, an oxazolidinone, represent a new class of antimicrobial agents with excellent in vitro activity against penicillin-resistant S. pneumoniae; however, in vitro activity against nontypeable Haemophilus influenzae (NTHI) is limited. We evaluated its efficacy against experimental acute OM due to a multidrug-resistant S. pneumoniae isolate and two isolates of NTHI. The chinchilla model was utilized to evaluate the efficacy of linezolid against experimental infection due to S. pneumoniae or NTHI. Serum and middle ear antibiotic concentrations were determined, and sterilization of experimental OM was evaluated. Chinchillas were inoculated directly with S. pneumoniae into the superior bulla. Twenty-four hours after inoculation, all animals had positive middle ear and nasopharyngeal cultures. Animals were given linezolid at 25 mg/kg/dose twice a day (b.i.d.) by orogastric feeding tube or amoxicillin at 40 mg/kg/dose b.i.d. intramuscularly for 5 days. By day 5, all animals in the linezolid group had sterile middle ear cultures and eradication of S. pneumoniae from the nasopharynx. In the amoxicillin group, all nine animals remained middle ear and nasopharynx positive (P < 0.01). In animals inoculated with NTHI, 25 and 37.5 mg/kg b.i.d. failed to sterilize middle ear infection or eradicate colonization. Mean levels in middle ear fluid measured during experimental infection were 12.8 μg/ml at 2 to 6 h and 4.1 μg/ml at 16 to 17 h after orogastric dosing at 25 mg/kg. Linezolid achieved a high concentration in the middle ear during experimental OM. Linezolid eradicated multidrug-resistant S. pneumoniae from the middle ear and nasopharynx. Experimental infection and nasopharyngeal colonization due to NTHI persisted despite achievement of concentrations in the middle ear that were above the MIC (for NTHI).

MATERIALS AND METHODS

Isolates of S. pneumoniae and H. influenzae. S. pneumoniae 645, a serotype 9 pneumococcus, was obtained from Michael Jacobs (Case Western Reserve, Cleveland, Ohio). The isolate was cultured from the respiratory tract of a pediatric patient and is characterized by its resistance to amoxicillin and erythromycin (MIC = 4.0 and 8.0 μg/ml, respectively) and susceptibility to linezolid (0.5 and 1.0 μg/ml) (Table 1). The MIC and minimal bactericidal concentration (MBC) were determined using an inoculum of 102 CFU incubated overnight in brain heart infusion plus NADH plus lysed horse blood with appropriate concentrations of antibiotic. MBCs were determined by streaking a standard loop from each tube following overnight incubation. Two isolates of NTHI cultured from the respiratory tracts of pediatric patients were used in the experimental studies. The amoxicillin and linezolid MICs for isolate BCH35 are 0.5 and 16 μg/ml, respectively, and those for isolate no. 2 are 0.125 and 8 μg/ml, respectively (Table 1).

Experimental infection. Prior to inoculation, the middle ears of adult chinchillas (8 to 48 months of age) were confirmed as normal using otomicroscopy and tympanometry. Isolates of S. pneumoniae and NTHI were grown for 16 h at 31 and 37°C, respectively, and cultures were diluted appropriately to a concentration of 100 to 1,000 CFU/ml in Gey’s balanced salt solution. Ten to 100 CFU was inoculated directly into the middle ear with a 25-gauge needle and tuberculin syringe in a final volume of 100 μl. Otoendoscopic and tympanometric examinations were performed at 24 to 48 h after inoculation. Middle ear contents were cultured through a 3-mm hole in the superior bulla by using a Calgi swab. Calgi swabs were streaked directly onto blood or chocolate agar plates. Nasopharyngeal cultures were performed by cannulating the nares with a 24-gauge angiocatheter, instilling 0.5 to 1.0 ml of Gey’s balanced salt solution, and collecting the effluent (approximately 100 ml) from the contralateral nares directly onto blood or chocolate agar plates.

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Animals were randomly assigned to either the linezolid or amoxicillin group, and therapy was initiated 24 to 48 h after inoculation (after culture of middle ear fluid to document infection). Linezolid was administered at 25 mg/kg/dose twice daily (b.i.d.) by orogastric feeding tube (p.o.) at 9:00 a.m. and 6:00 p.m. for 5 days. Amoxicillin was administered at 40 mg/kg/dose by intramuscular (i.m.) injection on the same schedule. Animals were reexamined at 2, 4 or 5, and 8 to 10 days after initiation of therapy. Otomicroscopic examination of the tympanic membrane and examination of the middle ear through a small hole in the superior bulleal for fluid were done at each time, and middle ear culture was performed. Nasopharyngeal cultures were performed on day 4 or 5 and day 8 or 10. Comparisons of antibiotic regimes were made using Fisher’s exact method.

Serum and/or middle ear fluid was obtained for measurement of linezolid concentration by cardiac puncture (serum) or aspiration of middle ear contents through a 20-gauge angiocatheter inserted through the hole created in the bullar membrane and examination of the middle ear through a small hole in the tympanic membrane. Samples were collected 2 to 6 or 16 to 17 h after the third dose of linezolid (25 mg/kg/dose per gavage). Serum specimens collected 2 1/2 to 10 days after initiation of therapy. The proportion of animals with middle ear fluid on the final day of therapy (10 of 15 treated with linezolid versus 9 of 9 treated with amoxicillin) and 3 days after completing therapy (6 of 12 versus 5 of 6) suggested a more rapid resolution in the linezolid-treated group, but the difference was not statistically significant. Nasopharyngeal carriage of *S. pneumoniae* was rapidly cleared in the linezolid-treated cohort but persisted in the cohort treated with amoxicillin.

**TABLE 1. Antimicrobial susceptibilities of isolates of *S. pneumoniae* and NTHI**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pneumoniae</em> 645</td>
<td>4.0</td>
<td>8.0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>NTHI BCH3</td>
<td>0.5</td>
<td>1.0</td>
<td>16.0</td>
<td>&gt;64</td>
</tr>
<tr>
<td>NTHI no. 2</td>
<td>0.125</td>
<td>0.25</td>
<td>8.0</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

Concentration of linezolid in serum and middle ear fluid. Linezolid concentrations in serum were determined by Dennis Stalker at Pharmacia & Upjohn using a sensitive and selective high-performance liquid chromatographic (HPLC) system (12). The method was validated and run for Pharmacia & Upjohn at AVTech Laboratories, Inc., Kalamaoo, Mich. Briefly, serum specimens (0.1 ml) were spiked with an internal standard (IS) and extracted using a solid phase cartridge. Samples were eluted with methanol and injected onto the reversed-phase analytical column (C18, 4.6 by 150 mm, 5 µm). The mobile phase was acetonitrile and water (20:80, vol/vol), and a Beckman Gold HPLC system was used for monitoring the samples. Peak height ratios, based on UV detection at 251 nm, were used for quantitative analysis. A nine-point calibration standard curve was linear over the range of 0.02 to 100 µg/ml, using a weighted (1/concentration) linear least-squares regression. Coefficients of variation for quality control samples were <8.3%, and the mean accuracy was from 99 to 105%.

Linezolid in middle ear fluid was quantitated using a selective HPLC system coupled with a triple-quadrupole mass spectrometer (LC/MS/MS) (PE Sciex API 300) developed and validated for Pharmacia & Upjohn for quantitation of linezolid in human plasma (proprietary method). Because of the low calibration range of this method, the chinchilla middle ear fluid (0.1 ml) was diluted 1:200 with blank human plasma before it was spiked with a deuterated IS and extracted using liquid-liquid techniques. Mean recoveries for linezolid and the IS were 106 and 101% respectively. Calibration standard curves were created from eight calibration points and were linear over the range of 1.0 to 250 ng/ml, using a weighted (1/concentration) linear least-squares regression. Coefficients of variation for quality control samples were <11.5%, with a mean accuracy of 98.7 to 101%.

**RESULTS**

Concentration of linezolid in serum and middle ear during experimental otitis media. Serum specimens collected 2 1/2 to 6 h after the third dose had a mean linezolid concentration of 17.5 µg/ml (range, 13.0 to 23.6 µg/ml). Middle ear specimens obtained simultaneously had a mean concentration of 12.8 µg/ml (range, 7.3 to 19.9 µg/ml) (Fig. 1). Middle ear specimens obtained 16 to 17 h after the third dose had a mean concentration of 4.1 µg/ml (range, 0.96 to 13.6 µg/ml). The mean ratio of the linezolid concentration in middle ear fluid to that in serum was 81% (range, 48 to 120%).

**Treatment of experimental otitis media due to Streptococcus pneumoniae**: linezolid (25 mg/kg b.i.d., p.o.) compared with amoxicillin (40 mg/kg b.i.d., i.m.). Amoxicillin failed to eradicate middle ear infection or nasopharyngeal carriage due to *S. pneumoniae* 645, while linezolid sterilized 100% of middle ear cultures by day 2 and 100% of nasopharyngeal cultures by day 4 (Table 2). Four of the linezolid-treated animals had 1 to 5 CFU reappear in the middle ear culture performed 72 h after completion of therapy. The proportions of animals with middle ear fluid on the final day of therapy (10 of 15 treated with linezolid versus 9 of 9 treated with amoxicillin) and 3 days after completing therapy (6 of 12 versus 5 of 6) suggested a more rapid resolution in the linezolid-treated group, but the difference was not statistically significant. Nasopharyngeal carriage of *S. pneumoniae* was rapidly cleared in the linezolid-treated cohort but persisted in the cohort treated with amoxicillin.

**Treatment of experimental otitis media due to NTHI isolate BCH3** (linezolid MIC = 16 µg/ml): linezolid (25 mg/kg/dose b.i.d.) versus amoxicillin (40 mg/kg/dose b.i.d.). Linezolid failed to eradicate middle ear infection due to BCH3. Infection persisted through the entire course of therapy. Amoxicillin rapidly eradicated infection, and by day 5 middle ear cultures had been sterilized in all amoxicillin-treated animals (Table 3).

**Treatment of experimental otitis media due to NTHI isolate no. 2** (linezolid MIC = 8.0 µg/ml): high-dose (37.5 mg/kg) versus low-dose (25 mg/kg) linezolid. We selected a second isolate of NTHI and evaluated dosing at both 25 mg/kg b.i.d. and 37.5 mg/kg b.i.d. in a small pilot experiment to determine if an isolate for which the MIC is lower or treatment with a

**TABLE 2. Treatment of experimental otitis media due to *S. pneumoniae*: linezolid (25 mg/kg b.i.d., p.o.) compared with amoxicillin (40 mg/kg b.i.d., i.m.)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Middle ear culture</th>
<th>Nasopharyngeal culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linezolid</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>0</td>
<td>18/18</td>
<td>9/9</td>
</tr>
<tr>
<td>2</td>
<td>0/18</td>
<td>9/9</td>
</tr>
<tr>
<td>4</td>
<td>0/17</td>
<td>9/9</td>
</tr>
<tr>
<td>8</td>
<td>4/14</td>
<td>Not evaluated</td>
</tr>
</tbody>
</table>

aP < 0.001 for comparisons of middle ear cultures on day 2 or 4 and nasopharyngeal cultures on day 4.

bAll four culture-positive animals has less than 5 CFU; P < 0.02 for comparisons of middle ear culture on day 8.
higher dose would successfully eradicate in middle ear infection or nasopharyngeal carriage with NTHI. Neither dose was successful, as middle ear infection and nasopharyngeal carriage persisted throughout the course of therapy (Table 4).

**DISCUSSION**

The chinchilla model of experimental otitis media has proven valuable for assessing the potential efficacy of antimicrobial agents. Studies demonstrating the efficacy of amoxicillin, broad-spectrum cephalosporins, and macrolides (1, 10, 15) for isolates of *S. pneumoniae* susceptible to penicillin and of cefotibuten and ampicillin-sulbactam (13, 14) for isolates of NTHI all predated human studies of acute otitis media by using microbiologic endpoints to affirm the efficacy of these agents. Studies of macrolides for acute otitis media due to NTHI in chinchillas failed to demonstrate rapid sterilization of middle ear infection (2) and correlate with observations of persistent infection at day 3 to 5 in children treated with macrolides, as reported by Howie (8) and more recently by Dagan et al. (R. Dagan, E. Leibovitz, M. Jacobs, D. Fliss, A. Leiberman, and P. Yagupsky, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. K-102, 1997).

The present study suggests that linezolid will be a valuable antibiotic in the treatment of respiratory infection due to *S. pneumoniae*, including those isolates resistant to high levels of amoxicillin. The dose utilized in these studies, 25 mg/kg b.i.d., gives peak levels in serum similar to those reported for adults administered 625 mg p.o. b.i.d. Concentrations in the middle ear were 80% of those in serum and exceeded the MIC for amoxicillin. The dose utilized in these studies, 25 mg/kg b.i.d., including those isolates resistant to high levels of *S. pneumoniae* antibiotic in the treatment of respiratory infection due to *S. pneumoniae*, including highly resistant isolates. This is especially relevant given the recent report of increased numbers of isolates of *S. pneumoniae* for which the MIC was 8.0 µg/ml was not possible. Craig has suggested that the middle ear antimicrobial levels need to exceed the MIC for greater than 40 to 50% of the dosing interval (3). Although the limited pharmacokinetic studies performed suggest that we achieved that goal for at least one isolate of NTHI, infection persisted. Potential explanations include the high MBC for NTHI with linezolid. Craig’s model may fit better for β-lactamase antibiotics which are bactericidal. Antimicrobial agents that are bacteriostatic, such as linezolid against NTHI, may need to achieve different parameters for successful bacterial eradication. Second, failure to eradicate nasopharyngeal colonization may result in continuous reinfection within the middle ear. Finally, the biologic activity of linezolid may be less than that determined by chemical detection methods. Further studies will be necessary to precisely define the contributions these factors make to antibiotic failure in this model.

Our results support the need for clinical studies of linezolid for the treatment of acute otitis media due to *S. pneumoniae*, including highly resistant isolates. This is especially relevant given the recent report of increased numbers of isolates of *S. pneumoniae* for which the MICs are ≥4.0 µg/ml (7a). If studies with children confirm our observations, linezolid may provide an effective method for treating infection and reducing the risk of spread of resistant *S. pneumoniae* within the community. However, since linezolid is unlikely to be effective in disease due to NTHI, its use should be limited to patients with documented or high suspicion of infection with *S. pneumoniae*, especially resistant isolates.

**ACKNOWLEDGMENT**

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**REFERENCES**