Efficacy of Solithromycin (CEM-101) for Experimental Otitis Media Caused by Nontypeable *Haemophilus influenzae* and *Streptococcus pneumoniae*

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Solithromycin (CEM-101) is a “fourth-generation” macrolide, as it has three binding site and is acid stable. The three binding sites confer activity against bacteria resistant to the older macrolides and ketolides, including multidrug-resistant *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae* (NTHi). The objective of this study was to evaluate solithromycin pharmacokinetics (PK), middle ear fluid (MEF) concentrations, and microbiologic efficacy in a chinchilla model of experimental otitis media (EOM) due to strains of *S. pneumoniae* or NTHi. Plasma PK (maximum concentration of drug in serum [C_{max}] and area under the concentration-time curve from 0 to 24 h [AUC_{0-24}]) and middle ear fluid (MEF) concentrations were determined. Isolates with specified antimicrobial susceptibility patterns were inoculated directly into the middle ear (ME). Plasma and MEF were collected for PK and MEF cultures performed to determine efficacy. Solithromycin administered at 150 mg/kg of body weight/day resulted in C_{max} and AUC_{0-24} values of 2.2 $\mu\text{g/ml}$ and 27.4 $\mu\text{g} \cdot \text{h/ml}$ in plasma and 1.7 $\mu\text{g/ml}$ and 28.2 $\mu\text{g} \cdot \text{h/ml}$ in extracellular MEF on day 1. By day 3, C_{max} and AUC_{0-24} values had increased to 4.5 $\mu\text{g/ml}$ and 54 $\mu\text{g} \cdot \text{h/ml}$ in plasma and 4.8 $\mu\text{g/ml}$ and 98.6 $\mu\text{g} \cdot \text{h/ml}$ in extracellular MEF. For NTHi EOM, three isolates with MIC/minimal bactericidal concentration (MBC) ratios of 0.5/1 $\mu\text{g/ml}$ (isolate BCH1), 2/2 $\mu\text{g/ml}$ (isolate BMC1247C), and 4/4 $\mu\text{g/ml}$ (isolate BMC1213C) were selected. The MEF of >85% of animals infected with BCH1 and BMC1247C was sterilized. For NTHi BMC1213, >85% of MEF cultures remained positive. For *S. pneumoniae* EOM, 3 isolates with MIC/MBC ratios of 0.06/0.125 $\mu\text{g/ml}$ (*S. pneumoniae* 331), 0.125/1 $\mu\text{g/ml}$ (*S. pneumoniae* CP-645 [MLS_B phenotype]), and 0.5/2 $\mu\text{g/ml}$ (CP-712 [*mefA* subclass *mefA* resistance]) were selected. Solithromycin sterilized MEF in 100% of animals infected with *S. pneumoniae* 331 and *S. pneumoniae* CP-645. ME infection persisted in 60% of animals infected with CP-712. In a model of EOM, solithromycin sterilized MEF in >85% of animals challenged with NTHi with an MIC of $\leq 2 \mu\text{g/ml}$ and 100% of ME infected with *S. pneumoniae* with an MIC of $\leq 0.125 \mu\text{g/ml}$.

Randomized clinical trials continue to demonstrate that antimicrobial therapy reduces the time to resolution of symptoms (i.e., persistent otoscopic signs) of acute otitis media (AOM) in children 6 to 23 months of age (1–3). However, selection of effective antimicrobial therapy is complicated by emergence of resistance to β -lactam and macrolides among isolates of *Streptococcus pneumoniae* and intrinsic resistance to macrolides among isolates of nontypeable *Haemophilus influenzae* (NTHi). Thus, limited choices of antimicrobials that achieve effective sterilization of the middle ear contents are available, especially for children unable to tolerate β -lactams.

Solithromycin (CEM-101) is a “fourth-generation” macrolide and the first fluoroketolide with *in vitro* antibacterial activity against multidrug-resistant *Streptococcus pneumoniae*, including erythromycin-resistant (ER) isolates, and both β -lactamase-positive and -negative nontypeable *Haemophilus influenzae* (NTHi) (4, 5). It has also demonstrated *in vitro* activity against *Moraxella catarrhalis*, a Gram-negative respiratory tract pathogen (6). A recent study of 272 pneumococcal isolates demonstrated high potency (MIC_{50/90}, 0.06/0.25 $\mu\text{g/ml}$) and inhibition of all strains at MICs of $< 0.5 \mu\text{g/ml}$, including the two most prevalent macrolide-resistant serotypes (19A and 35B). This spectrum of activity and the lack of resistance among pneumococcal isolates support the idea of a potential role in the treatment of AOM (4). We evaluated the pharmacokinetics, middle ear penetration, and microbiologic efficacy of solithromycin in an experimental chinchilla model of otitis media due to *S. pneumoniae* and NTHi.

MATERIALS AND METHODS

Isolates. Respiratory isolates of NTHi and *S. pneumoniae* with specified antimicrobial susceptibility patterns were selected for evaluation of solithromycin. MIC values were determined using tube-dilution methods (7, 8), and the minimal bactericidal concentration (MBC) determinations were performed as recommended in CLSI M26-A (9). The MBC is defined as a 3 log reduction in CFU per milliliter compared to the inoculum. The Etest method was used for evaluation of ampicillin, azithromycin, clarithromycin, and erythromycin susceptibility. Our designated names and the antibiotic resistance patterns of the NTHi and *S. pneumoniae* strains used in this study are reported in Tables 1 and 2. Four isolates of NTHi previously recovered from nasopharyngeal cultures in children were selected. β -Lactamase-negative NTHi isolates 1086 and BMC1247C had an amoxicillin MIC of 0.75 $\mu\text{g/ml}$ and solithromycin MICs/MBCs of 8/16 and 2/2 $\mu\text{g/ml}$, respectively. β -Lactamase-positive isolates BCHi and BMC1213C had an amoxicillin MIC of $> 256 \mu\text{g/ml}$ and solithromycin MICs/MBCs of 0.5/1 and 4/4 $\mu\text{g/ml}$, respectively.

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TABLE 1 NTHi strains used^a

NTHi strain ID	β -Lactamase test result	MIC (μ g/ml)				Solithromycin MIC/MBC
		Azithromycin	Erythromycin	Amoxicillin	Amox/Clav	
1086	Neg	4	12	0.75	1	8/16
BMC1247C	Neg	1.5	3	0.75	0.75	2/2
BCH1	Pos	2	6	>256	24	0.5/1
BMC1213C	Pos	1.5	4	>256	1.5	4/4

^a NTHi, nontypeable *Haemophilus influenzae*; ID, identifier; Amox/Clav, amoxicillin-clavulanic acid; Neg, negative; Pos, positive.

Three pneumococcal isolates were selected. *S. pneumoniae* 331 (type 14) was obtained from nasopharyngeal cultures from a child at Boston City Hospital with an erythromycin MIC of 0.25 μ g/ml and a solithromycin MIC of 0.06 μ g/ml. Two erythromycin-resistant isolates of *S. pneumoniae* were provided by Michael Jacobs' laboratory in Cleveland, Ohio: CP-645 (type 14), with the MLS_B phenotype, was shown to be PCR positive for *ermB* by gene probe analysis; and CP-712 (type 19F), with the M phenotype (*mefA* subclass *mefA*), was sequenced by David J. Farrell at JMI Laboratories ST6793 and was shown to be PCR positive for *mefA*, *msrD*, and *tetM* (10). Both isolates had erythromycin MICs of >256 μ g/ml. The solithromycin MICs were 0.125 μ g/ml for *S. pneumoniae* CP-645 and 0.5 μ g/ml for *S. pneumoniae* CP-712, and the MBCs were 1 μ g/ml and 2 μ g/ml, respectively.

In vitro activity of solithromycin against NTHi respiratory isolates. The solithromycin MIC₅₀ and MIC₉₀ for a collection of 165 nontypeable *Haemophilus influenzae* (NTHi) strains were obtained from a study of nasopharyngeal carriage of *H. influenzae* conducted in children (aged 3 months to 5 years) by S. I. Pelton, Boston University Medical Center, Boston, MA, between 1 July 2010 and 2014. The determinations of the MICs of solithromycin and comparator drugs were performed by Dwight Hardy, University of Rochester, NY, using broth microdilution in *Haemophilus* Test Medium as recommended in CLSI M7-A8 (11). MBCs were determined as recommended in CLSI M26-A.

The MIC₅₀ and MIC₉₀ for solithromycin were 1 and 2 μ g/ml, respectively (Table 3). The MBCs of solithromycin were either equal to the MIC or only 1 2-fold dilution greater than the MIC (data not shown).

Animals. An experimental chinchilla model of experimental otitis media (EOM) was used (12). Adult chinchillas (*Chinchilla lanigera*) were obtained from Ryerson's Chinchilla Ranch (Plymouth, OH).

Experimental otitis media model. The tympanic membranes of adult chinchillas were first examined by tympanometry and otomicroscopy to confirm a normal middle ear. Overnight cultures of NTHi or *S. pneumoniae* were diluted to 30 to 50 CFU/100 μ l and inoculated directly into the left superior bullae of the chinchillas. At 24 to 72 h after inoculation, tympanometry, otomicroscopy, and direct and quantitative middle ear fluid (MEF) cultures were performed to document culture-positive infection as previously described (13).

Following the middle ear culture, animals were randomly assigned to either the solithromycin group or the control group and antimicrobial therapy was initiated. A previous experiment (data not shown) performed to evaluate plasma, middle ear pellet, and extracellular fluid samples for three escalating oral doses (25, 50, and 150 mg/kg of body weight) of solithromycin in the chinchilla showed that a dose of 150 mg/kg correlated best with pharmacokinetics (PK) parameters in adults administered

a therapeutic dose of solithromycin (400 mg/day [5 to 6 mg/kg/day]). Solithromycin was administered at 150 mg/kg/day once daily through an orogastric feeding tube. The untreated control group for studies of EOM due to NTHi did not receive antimicrobial agents. The control group in studies of EOM due to *S. pneumoniae* 331, an amoxicillin-sensitive isolate, received amoxicillin at 40 mg/kg/dose, intramuscularly, once a day; control groups in studies of EOM due to amoxicillin- and erythromycin-resistant *S. pneumoniae* were treated differently. Animals challenged with *S. pneumoniae* CP-645 received ceftriaxone at 50 mg/kg/dose, intramuscularly, once a day. Animals challenged with CP-712 received solithromycin at 90 mg/kg/day once daily by orogastric feeding tube; no antibiotic controls were included in this study group.

Therapy with each antibiotic was continued for a 3-day course. Middle ear examinations and cultures were repeated on day 3 of therapy and 2 to 5 days after completion of therapy.

In order to measure solithromycin concentrations, plasma and MEF were collected after dosing on day 1 and day 3 of therapy by cephalic sinus puncture and aspiration of middle ear contents through a 20-gauge angiocatheter. MEF was centrifuged after collection to separate extracellular fluid from the cellular content. Plasma and middle ear samples were frozen at -80°C .

Antibiotic concentration determinations. Plasma, middle ear pellet, and extracellular fluid concentrations of solithromycin were measured at MicroConstants, Inc. (San Diego, CA), with a previously developed method using liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS) (analytical method MN08060) for the analysis of solithromycin in animal and human plasma (14). The method was applied directly to the matrices generated from this study without modification.

The middle ear pellet and extracellular fluid samples were analyzed after doses 1 and 3 of the 3-day dosing regimen.

Pharmacokinetic studies. Blood and MEF samples were collected concurrently when possible at 0 h (prior to administration of antibiotic) and 1, 2, 4, 6, 16, and 24 h after dosing on days 1 and 3 of therapy. MEF was centrifuged immediately after collection at 10,000 rpm at 4°C to separate extracellular fluid from cellular content. Plasma and middle ear fluid supernatant samples were frozen at -80°C . Pharmacokinetic parameters included the area under the concentration-time curve from time 0 to time *t* (last measurable concentration) for plasma ($\text{AUC}_{\text{plasma } 0-t}$) and MEF ($\text{AUC}_{\text{MEF } 0-t}$), using the trapezoidal method. The maximum concentration of drug in serum (C_{max}) for solithromycin in plasma and MEF corresponded to the highest measured values.

Time-kill studies. A time-kill experiment was performed for the two erythromycin-resistant isolates of *S. pneumoniae*, CP-645 and CP-712. Tubes containing 2.5 ml of brain heart infusion broth (BHIB) with soli-

TABLE 2 *Streptococcus pneumoniae* strains used

<i>S. pneumoniae</i> strain ID	Serotype/resistance gene	MIC (μ g/ml)				Solithromycin MIC/MBC
		Azithromycin	Erythromycin	Amoxicillin	Ceftriaxone	
331	14 (none)	0.2	0.1	0.064	0.047	0.064/0.125
CP-645	14 (<i>ermB</i>)	>256	>256	12	2	0.125/1
CP-712	19F (<i>mefA</i>)	>256	>256	12	3	0.5/2

TABLE 3 *In vitro* solithromycin activity against 165 nontypeable *Haemophilus influenzae* respiratory isolates^a

MIC	Solithromycin	Telithromycin	Azithromycin	Erythromycin	Ampicillin	Amox/Clav	Cefdinir	Trim/Sulfa
Range	0.12 to 8	0.12 to 16	0.06 to 4	0.12 to 16	0.06 to >64	0.12/0.06 to 8/4	≤0.03 to 2	≤0.015/0.3 to 32/608
MIC ₅₀	1	1	0.5	2	0.25	0.5/0.25	0.25	0.03/0.6
MIC ₉₀	2	2	1	8	64	2/1	1	8/152

^a Amox/Clav, amoxicillin-clavulanic acid; Trim/Sulfa, trimethoprim-sulfamethoxazole.

thromycin at a specified concentration were inoculated with an equal volume of approximately 5×10^5 CFU/ml and incubated at 37°C in a shaking water bath. Final antibiotic concentrations were calculated to include the MIC, 2 and 4 times the MIC, and half the MIC. Viable bacterial counts of antibiotic-containing suspensions were performed at 0, 2, 4, 6, 12, and 24 h by plating 0.1-ml aliquots from a 10-fold dilution onto blood agar. Time-kill assays were analyzed by determining the change in the viable CFU count at each time point compared to the CFU count at time 0 h. The concentration was considered bactericidal if there was a ≥ 3 log₁₀ decrease in the CFU count per milliliter compared to that measured at time 0 h (15).

Ethics statement. The Institutional Animal Care and Use Committee at Boston University Medical Center approved our animal care protocols as being consistent with humane treatment of laboratory animals and with standards set forth in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act (Animal Welfare Assurance approval number A-3316-01). Thus, our study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Statistical methods. The levels of mean bacterial density were compared across two groups, treated and untreated animals. The geometric mean bacterial density was calculated for each organism from MEF samples on day 6 and days 8 to 10 and 13 after inoculation. Differences in the means were compared using *t* tests. In all analyses, *P* values of <0.05 were considered to be statistically significant.

RESULTS

Pharmacokinetics of solithromycin in the chinchilla model. Pilot studies in animals infected with NTHi 1086 (MIC/MBC, 8/16 µg/ml [believed to be above the susceptible breakpoint]) administered at 25, 50, or 150 mg/kg per orogastric tube were performed to obtain plasma and middle ear fluid samples for determination of the appropriate dose for future studies. Solithromycin administered at 150 mg/kg/dose daily for 3 days was used in all subsequent studies except where a different dose is specified.

The concentration-time curves of solithromycin in plasma and MEF are illustrated in Fig. 1. Solithromycin (at 150 mg/kg/day for 3 days) demonstrated peak plasma and extracellular MEF concentrations of 2.2 and 1.7 µg/ml at 2 and 8 h, respectively, after administration of the first dose. By day 3, the *C*_{max} values increased to 4.5 µg/ml in plasma and 4.28 µg/ml in extracellular MEF at 4 and 8 h, respectively. The 24-h plasma AUC_{0–24} values were 27.4 and 54 µg · h/ml after dose 1 and dose 3, respectively. The 24-h extracellular MEF AUC_{0–24} values were 28.2 and 98.6 µg · h/ml after dose 1 and dose 3, respectively.

Treatment of EOM due to NTHi. For nontypeable *Haemophilus influenzae* (NTHi) EOM, three isolates whose MIC/MBC values had been determined (BCH1, 0.5/1 µg/ml; BMC1247C, 2/2 µg/ml; BMC1213C, 4/4 µg/ml) were selected for study. On day 3 of therapy, 1 of 7 animals challenged with BCH1 and 1 of 8 challenged with NTHi BMC1247C remained MEF positive. At 48 h after completing 3 days of solithromycin treatment, all BCH1-challenged middle ears were sterile (Fig. 2A); 1/8 animals challenged with NTHi BMC1247C remained MEF positive (Fig. 2B).

For NTHi BMC1213C, 6 (86%) animals remained MEF culture positive at both day 3 of therapy and 48 h after completion (Fig. 2C).

Treatment of experimental otitis media due to *S. pneumoniae*. For *S. pneumoniae* EOM, three isolates were studied. For *S. pneumoniae* 331 (erythromycin [E] susceptible, with an erythromycin MIC of 0.06 µg/ml), 7 of 7 solithromycin-treated animals had sterilized MEF by day 3. Animals in the untreated control group developed bacteremia on day 1 of therapy and were euthanized (Fig. 3A). For two erythromycin-resistant isolates of *S. pneumoniae*, CP-645 (type 14; MIC, 0.125 µg/ml; MLS_B phenotype and PCR positive for *ermB*) and CP-712 (type 19F; MIC, 0.5 µg/ml; M phenotype [*mefA* subclass *mefA*] and PCR positive for *mefA*, *msrD*, and *tetM*), we observed discordant results. For CP-645, 6 of 6 solithromycin-treated animals had sterilized MEF by day 3 (Fig. 3B). For CP-712, only 40% (3 of 5) of solithromycin-treated animals had sterile MEF on day 3 (Fig. 3C).

Time-kill studies. Time-kill analysis demonstrated bactericidal activity at 8 h for solithromycin at one-half the MIC for *S. pneumoniae* CP-645 with *ermB* resistance (Fig. 4A) and survival of *S. pneumoniae* CP-712 with *mefA* subclass *mefA* resistance at both the MIC and twice the MIC (Fig. 4B).

DISCUSSION

Solithromycin is a new, fourth-generation macrolide with improved *in vitro* antimicrobial activity against respiratory pathogens. It binds to the large 50S subunit of the ribosome and inhibits protein biosynthesis, presumably preventing ribosomal subunit formation (16). Rodgers and colleagues reported that solithromy-

FIG 1 Plasma PK parameters (*C*_{max} and AUC_{0–24}) and MEF concentrations were determined on day 1 and day 3 after administration of 150 mg of solithromycin/kg of body weight once per day. Open circles and open triangles represent plasma and MEF concentrations, respectively, after the first dose of solithromycin, and filled circles and filled triangles represent plasma and MEF concentrations, respectively, after the third dose of solithromycin.

FIG 2 Comparison of levels of middle ear fluid bacterial density in chinchillas treated with solithromycin challenged with NTHi BCH1 (A), BMC1247C (B), and BMC1213C (C). Triangles represent animals treated with 150 mg/kg/day of solithromycin for 3 days and circles represent untreated animals.

cin inhibits ribosomal biosynthesis of *S. pneumoniae* and NTHi at nanogram concentrations, albeit *S. pneumoniae* was nearly 20-fold more sensitive to its action than to that of NTHi. *In vitro*, the solithromycin MIC₉₀ for *S. pneumoniae* was 0.5 µg/ml and the MIC₉₀ for NTHi was 2 µg/ml, consistent with the observed concentrations of solithromycin required for ribosomal inhibition.

Clinical studies have already demonstrated that solithromycin achieves high exposure in serum and epithelial lining fluid and within alveolar macrophages (17). Our report provides evidence of excellent middle ear penetration with middle ear fluid AUC concentrations (ignoring intracellular diffusion) that exceeded plasma values and provided high cure rates with strains of NTHi and *S. pneumoniae* with solithromycin MICs of ≤2 µg/ml and 0.125 µg/ml, respectively.

In a pivotal phase 3 trial, oral solithromycin demonstrated statistical noninferiority to oral moxifloxacin in adults with community-acquired bacterial pneumonia (18). Consistent with *in vitro* susceptibility and time-kill studies, pneumococci in the chinchilla middle ear infection model that had a solithromycin MIC of ≤0.5 µg/ml were rapidly sterilized whereas those with an MIC of 0.5 µg/ml persisted in the middle ear. Similarly, also consistent with time-kill studies, middle ear infections caused by *Haemophilus* strains with a solithromycin MIC of <0.5 µg/ml were rapidly sterilized, while those with an MIC of 0.5 µg/ml had delayed killing and strains with an MIC between 0.5 and 2 µg/ml persisted. Sequencing the strain of *S. pneumoniae* with a solithromycin MIC of 0.5 µg/ml identified *mefA*, *msrD*, and *tetM* as the mechanism(s) of resistance (10).

Solithromycin treatment, at the PK parameters reported in our model, would be expected to eradicate middle ear infection due to the majority of community strains, as the MIC₉₀ has been demonstrated to be 2 µg/ml (19). In addition, the potent anti-inflammatory activity described by Kobayashi et al. may be beneficial in the resolution of signs and symptoms and potentially in the treatment of persistent middle ear effusion (20). The PK profile that was observed, in combination with high rates of sterilization of middle ear infection, supports further evaluation of solithromycin for treatment of respiratory tract infection, including acute otitis media.

A limitation in our studies was the differences in PK between chinchillas and humans, specifically, the accumulation of solithromycin over time in our chinchilla model. Initial studies in our chinchilla model were designed to select a treatment dose that would achieve C_{max} values that were comparable to those reported in adolescents (1.8 µg/ml) and in adults (2.2 µg/ml) (21, 22). In retrospect, we relied on available single-dose studies in humans. However, it is now clear that accumulation is more substantial in the chinchilla than in humans, resulting in greater drug exposure. Although accumulation at day 3 resulted in higher C_{max} and greater AUC levels, in fact, the middle ear fluid was sterilized prior to administration of the third dose with both pathogens (NTHi, MIC = ≤2 µg/ml; *S. pneumoniae*, MIC = <0.125 µg/ml) and before significant accumulation was observed after administration of the third dose on day 3. Our study demonstrated how middle ear fluid concentrations relate to serum concentrations for solithromycin and the potential of solithromycin against strains of

FIG 3 Comparison of levels of middle ear fluid bacterial density in chinchillas treated with solithromycin and challenged with *S. pneumoniae* 331 (A), CP-645 (B), and CP-712 (C). In panels A and B, triangles represent animals treated with 150 mg of solithromycin/kg/day for 3 days and circles represent animals treated with either amoxicillin or ceftriaxone. In panel C, triangles represent animals treated with 90 mg of solithromycin/kg/day and circles represent animals treated with 150 mg of solithromycin/kg/day.

FIG 4 Time-kill assessment of solithromycin versus *S. pneumoniae* CP-645 *ermB* resistance was performed at 0.5× MIC, 1× MIC, and 2× MIC (A) and versus *S. pneumoniae* CP-712 *mefA* subclass *mefA* resistance was performed at 0.5× MIC, 1× MIC, 2× MIC, and 4× MIC (B).

NTHi and *S. pneumoniae* with drug MICs at or below those used in our model.

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