# Maternal Administration of Solithromycin, a New, Potent, Broad-Spectrum Fluoroketolide Antibiotic, Achieves Fetal and Intra-Amniotic Antimicrobial Protection in a Pregnant Sheep Model

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Solithromycin (CEM-101) is a new antibiotic that is highly potent against *Ureaplasma* and *Mycoplasma* spp. and active against many other antibiotic-resistant organisms. We have explored the maternal-amniotic-fetal pharmacokinetics of CEM-101 in a pregnant sheep model to assess its potential for treating intrauterine and antenatal infection. Chronically catheterized pregnant ewes ($n = 6$ or 7) received either a single maternal intravenous (i.v.) infusion of CEM-101 (10 mg/kg of body weight), a single intra-amniotic (i.a.) injection (1.4 mg/kg of estimated fetal weight), or a combined i.v. and i.a. dose. Maternal plasma (MP), fetal plasma (FP), and amniotic fluid (AF) samples were taken via catheter at intervals of 0 to 72 h postadministration, and concentrations of solithromycin and its bioactive polar metabolites (*N*-acetyl [NAc]–CEM-101 and CEM-214) were determined. Following maternal i.v. infusion, peak CEM-101 concentrations in MP, FP, and AF were 1,073, 353, and 214 ng/ml, respectively, representing a maternal-to-fetal plasma transfer efficiency of 34%. A single maternal dose resulted in effective concentrations ($>30$ ng/ml) in MP, FP, and AF sustained for $>12$ h. NAc–CEM-101 and CEM-214 exhibited delayed accumulation and clearance in FP and AF, resulting in an additive antimicrobial effect ($>48$ h). Intra-amniotic solithromycin injection resulted in elevated ($\sim50$ μg/ml) and sustained CEM-101 concentrations in AF and significant levels in FP, although the efficiency of amniotic-to-fetal transfer was low ($\sim1.5\%$). Combined i.v. and i.a. administration resulted in primarily additive concentrations of CEM-101 in all three compartments. Our findings suggest that CEM-101 may provide, for the first time, an effective antimicrobial approach for the prevention and treatment of intrauterine infection and early prevention of preterm birth.

Intrauterine infection and inflammation play a well-recognized role in the etiology of spontaneous preterm labor and birth, particularly in deliveries less than 32 weeks of gestation or those complicated by preterm prelabor rupture of membranes (PPROM) (1, 2). The origin of the infection is typically the vaginal flora: microorganisms are hypothesized to breach the cervical barrier, infect the fetal membranes, and eventually colonize the amniotic cavity (2–4). The vigorous inflammatory response that ensues is responsible for activation of myometrial contractions, membrane degradation, and rupture and cervical ripening, leading to labor and delivery (2, 5, 6). Intracellular organisms of the *Mollicutes* class, namely, *Ureaplasma* and *Mycoplasma* species, are the microorganisms most commonly isolated from the amniotic fluid (AF) of preterm deliveries (7, 8) and have been shown to be capable of eliciting preterm labor via a robust intrauterine inflammatory response in a dose-dependent fashion (9–11). Numerous other bacterial classes have also been identified in infected amniotic fluid samples, including streptococci, staphylococci, enterococci, *Fusobacterium* spp., *Bacteroides* spp., and *Haemophilus* spp. (8, 12).

A variety of clinical trials of maternal antibiotic administration have been performed to attempt to prevent or treat intrauterine infection with the aim of reducing the rates of preterm birth and associated neonatal morbidities; however, the benefits of these interventions have been unconvincing (13). The conclusions reached by the authors of several recent meta-analyses are that there is no evidence that treatment of women at risk of infection-driven preterm birth with antibiotics prophylactically or upon presentation with preterm labor reduces the rates of preterm delivery or improves neonatal outcomes (14–16). Not all researchers are in agreement, however. In some studies in which antibiotics (primarily clindamycin) have been administered to high-risk women prior to 22 weeks of gestation, significant reductions in rates of delivery before 37 and 33 weeks and in late miscarriage have been documented (17).

The reasons for the generally disappointing results of antibiotic interventions are likely severalfold, but the choice of antibiotic is a key factor. Macrolide antibiotics such as erythromycin and azithromycin are widely prescribed during pregnancy for the treatment of a variety of microbial infections, as they are perceived to be well tolerated, effective in treating important microorganisms such as *Ureaplasma* and *Mycoplasma* spp., and free of serious maternal and fetal side effects (18–21). Erythromycin is the most frequently administered antibiotic for treatment of PPROM based on the findings of the ORACLE I trial (22). However, there is strong evidence that systemic maternal erythromycin administra-

tion is largely ineffective in eradicating intrauterine *Ureaplasma* species infection (23). In an experimental sheep model of intrauterine *Ureaplasma* species colonization during pregnancy, we have shown that maternal intramuscular (i.m.) erythromycin administration does not eradicate *Ureaplasma* species infection from the amniotic fluid, chorioamnion, umbilical cord, or fetal lung (24). This is likely attributable to poor transplacental/transamniotic passage of macrolides. In the *ex vivo* perfused human placenta model, the transfer rate of macrolides is only 2 to 4% (25).

We recently showed that in sheep, maternal macrolide administration fails to deliver effective chemotherapeutic levels to either the fetal circulation or the amniotic cavity (26). *In vivo* studies confirm that the degree of erythromycin passage to the human fetus is low and variable (27, 28), while the extent of maternal-to-amniotic fluid transfer is more uncertain. A recent study in pregnant women at term reported that the transfer of azithromycin from maternal circulation to AF was more efficient than the maternal-to-fetal plasma transfer, although resulting AF concentrations (~150 ng/ml) still failed to reach the $MIC_{90}$ for *Ureaplasma* spp. (~500 ng/ml) (29). Interestingly, a recent study in pregnant primates with intra-amniotic (i.a.) *Ureaplasma* infection showed that a sustained 10-day maternal course of azithromycin (12.5 mg/kg of body weight) achieved effective antimicrobial levels in the amniotic fluid and low levels in the fetal circulation (30); however, although the infection was cleared in 90% of animals, intra-amniotic and fetal inflammation remained evident following azithromycin treatment, and pregnancy length was extended by only 7 to 10 days, with all animals delivering preterm (30). From these studies, it is clear that intrauterine infections are difficult to eradicate and a more potent antibiotic with better maternal-amniotic-fetal transfer properties is required to eliminate both fetal and amniotic infection, in combination with an effective anti-inflammatory therapeutic to prevent the adverse consequences of inflammation within the amniotic cavity.

Solithromycin (CEM-101) is a novel macrolide/fluoroketolide antibiotic which exhibits broad-spectrum activity against Gram-positive and some Gram-negative organisms, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Haemophilus* spp., streptococci, and enterococci (31–37). It is exceptionally potent against *Ureaplasma parvum*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* (125 to 250 times more potent than azithromycin) (31). The fluoro group incorporated into its structure imparts activity against even highly resistant strains of multiple classes of microorganisms (38). It is acid stable with excellent oral bioavailability and demonstrates excellent tissue uptake and accumulation (39); it is also bactericidal at concentrations 2 to 8 times its $MIC_{90}$ for some pathogens (40). Solithromycin has a plasma half-life ($t_{1/2}$) in humans of approximately 7 h and has two polar phase I metabolites, both of which are bioactive, although there are significant species differences in the extent of metabolism (41, 42). Currently in clinical trials (43), solithromycin appears to be well tolerated and free of the adverse effects associated with its ketolide predecessor telithromycin (43, 44).

Due to its excellent spectrum of activity against a wide range of susceptible and resistant microorganisms, we believe solithromycin may represent a significant therapeutic advance for the treatment and prevention of intrauterine infections, depending on its ability to cross the placenta and fetal membranes and reach the fetus and amniotic cavity. The aim of the present study, therefore, was to determine the pharmacokinetics and maternal-to-fetal plasma transfer of solithromycin in a pregnant ovine model to assess its potential for treating intrauterine and antenatal infection. An intravenous (i.v.) route of administration was selected to avoid species differences in gastrointestinal uptake and metabolism associated with oral administration. We also assessed amniotic-to-fetal transfer following i.a. administration for comparison with our previous studies of azithromycin and erythromycin biodistribution using the same model (26).

## MATERIALS AND METHODS

**Surgical procedures and antibiotic administration.** All experimental procedures described in this study were approved by the Animal Ethics Committee of The University of Western Australia. Details of animal management, anesthesia, surgical catheterization, and recovery have been described previously (26). Five days after surgery, at 116 ± 1 days gestation, chronically catheterized pregnant ewes (~65 kg) were randomly selected to receive either (i) a single maternal i.v. infusion of 650 mg solithromycin (10 mg/kg maternal weight; $n = 6$ ewes) over 60 min in 325 ml infusion buffer (5.77 g/liter tartaric acid, 50 g/liter mannitol, 5 g/liter thioglycerol, buffered to pH 4.2 with NaOH), (ii) a single i.a. injection of 3.5 mg solithromycin (1.4 mg/kg fetal weight) in 3 ml perfusion buffer ($n = 6$ ewes), or (iii) a combination of maternal i.v. infusion and i.a. injection ($n = 7$ ewes). At this gestational age, fetal weight was estimated to be 2.5 kg, and amniotic fluid volume was approximately 250 ml. Maternal and fetal arterial blood and amniotic fluid (two sets of 1 ml) samples were collected into heparinized tubes 30 min before and immediately prior to the administration of the macrolide antibiotics as described above; after the completion of antibiotic administration, samples were taken at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h. Fetal arterial $PO_2$ ($PaO_2$), $PCO_2$ ($PaCO_2$), $O_2$ saturation ($SaO_2$), pH (pHa), and electrolytes were measured with a Rapid Lab 1265 blood gas analyzer (Siemens, Germany). Fetal and maternal plasma and amniotic fluid samples were stored at −80°C until they were shipped frozen to MicroConstants, Inc. (San Diego, CA), for analysis of solithromycin and its metabolites.

**Solithromycin concentration determination.** Concentrations of solithromycin and its two side-chain metabolites (CEM-214 and *N*-acetyl [NAc]–CEM-101) in ovine plasma (maternal and fetal) and amniotic fluid were assayed using high-performance liquid chromatography(HPLC)-tandem quadrupole mass spectrometry (MicroConstants' analytical method MN12084). $K_3EDTA$ (anticoagulant) and internal standards were added to the plasma and amniotic fluid samples, which were then diluted with water, extracted using solid-phase extraction well plates, and analyzed by reverse-phase HPLC using a Phenomenex Luna CN 100-Å column maintained at 25°C. The mobile phase was 35% solvent A (20.0 mM ammonium formate, 0.2% formic acid, 0.0002% citric acid in water) and 65% solvent B (0.1% formic acid in methanol-acetonitrile [50:50, vol/vol]) at a flow rate of 0.3 ml/min. The retention times of CEM-101, NAc–CEM-101, and CEM-214 were 2.1, 2.0, and 1.95 min, respectively. The mobile phase was nebulized using heated nitrogen in a Z spray source/interface set to electrospray positive ionization mode; the ionized compounds were detected using tandem MS (MS/MS). The calibration range of the assay was from 10 to 20,000 ng/ml for solithromycin and from 1 to 2,000 ng/ml for both CEM-214 and NAc–CEM-101. CEM-101–d3 (BioLink Life Sciences, Inc.) and NAc–CEM-101–d6 (Microconstants Inc.) were used as the internal standards. The peak heights of solithromycin, CEM-214, NAc–CEM-101, and the internal standards and subsequent calibration curves were acquired using MassLynx version 4.1 (Waters, Milford, MA). All samples were analyzed in a total of nine assay runs. The coefficient of variation (CV) of the mean plasma quality control (QC) values (low, medium, and high) for solithromycin, CEM-214, and NAc–CEM-101 ranged from 3.86 to 4.93%, 9.92 to 23.4%, and 3.59 to 10.4%, respectively; in AF, the CV of the QC values ranged from 4.54 to 6.91%, 5.43 to 10.1%, and 3.34 to 7.96%, respectively. Methodological accuracy for all three analytes was 6.7 to 10.0%.
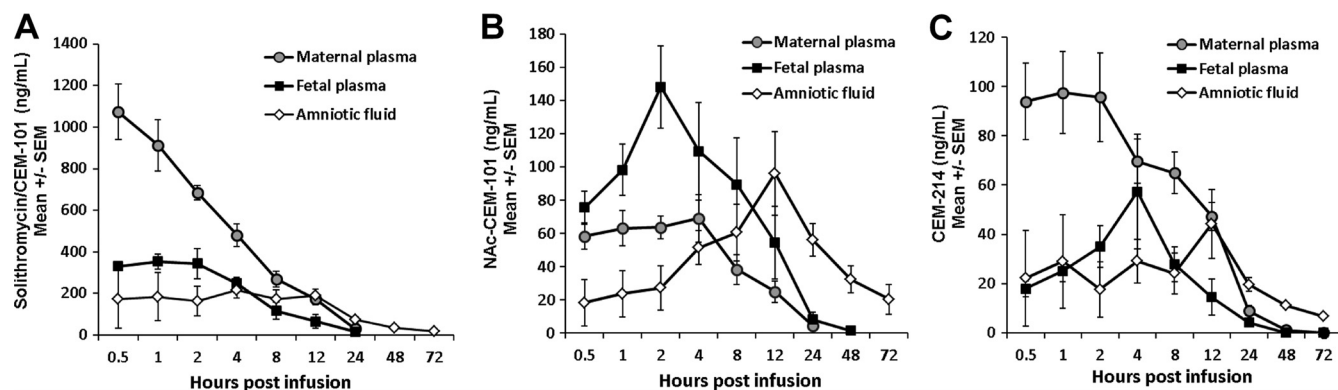
**FIG 1** Concentrations of solithromycin (CEM-101) (A), *N*-acetyl solithromycin (NAc–CEM-101) (B), and CEM-214 (C) in maternal plasma, fetal plasma, and amniotic fluid after a single maternal dose of solithromycin (650 mg, 10 mg/kg maternal weight) by i.v. infusion. Data are shown as means ± standard errors of the means (SEM) (*n* = 6 or 7 animals).

**Statistical and pharmacokinetic analysis.** Antibiotic concentration data from *n* = 6 or 7 animals per time point were grouped, and the mean, standard deviation, and standard error of the mean were calculated. Pharmacokinetic analysis was performed using PKSolver software (45). Maternal and fetal solithromycin pharmacokinetic data were fitted using a two-compartment model, whereas the i.a. solithromycin administration data and all metabolite data were best described by a noncompartmental model.

## RESULTS

**Maternal i.v. administration.** The pharmacokinetics of maternal i.v. solithromycin displayed characteristics in the pregnant sheep that are broadly similar to those reported in adult humans and other animal models (Fig. 1A). Peak concentrations of 1,073 ng/ml were obtained 30 min after maternal i.v. infusion (*t* = 1.5 h), which declined steadily with a half-life ($t_{1/2}$) of 6 h, resulting in an area under the concentration-time curve from 0 to infinity ($AUC_{0-\infty}$) of greater than 6,500 ng/ml · h (Table 1). The acetylated metabolite NAc–CEM-101 was present in low but readily detectable levels in maternal plasma, reaching peak concentrations (69.2 ng/ml) at 4 h postinfusion that were approximately 7% of the maximal parent drug concentrations (Fig. 1B). The maternal plasma half-life of NAc–CEM-101 was similar to that of solithro-

mycin, although the mean residence time (MRT) was slightly longer (8.5 h compared to 7.4 h) (Table 2). The bioactive metabolite CEM-214, which is formed by loss of the aminophenyl-1,2,3-triazole chain of solithromycin, was detected at levels higher than those of NAc–CEM-101 (97.6 ng/ml at 1 h postinfusion, 10.7% of the solithromycin concentration) and remained detectable for at least 24 h (Fig. 1C) with a $t_{1/2}$ of 7.9 h (Table 3). At *t* = 2 h, the combined level of side-chain metabolites was 18% of the parent drug, somewhat higher than that reported for humans (approximately 8% after i.v. administration) (41).

Solithromycin concentrations in fetal plasma peaked 1 to 2 h postinfusion, reaching concentrations of ~350 ng/ml (Fig. 1A), representing a placental transfer efficiency of ~34%. Clearance from the fetal compartment was similar to that in the maternal circulation, with a $t_{1/2}$ of 6.2 h; fetal circulating concentrations were stable for the first 2 h after infusion and remained at therapeutic levels for over 12 h following a single maternal dose. The $AUC_{0-\infty}$ in the fetal compartment was 2,971 ng/ml · h (Table 1). In the fetal compartment, NAc–CEM-101 levels reached almost half those of CEM-101 at 2 h postinfusion (148 ng/ml); however, it was cleared more slowly, with a half-life of 7.6 h and an MRT of 9.7 h (Table 2) (Fig. 1B).

**TABLE 1** Pharmacokinetic parameters of solithromycin in maternal, fetal, and amniotic compartments after administration of solithromycin via maternal i.v. infusion or i.a. injection[a]

| Parameter | Value | | | | | |
|---|---|---|---|---|---|---|
| | Maternal i.v. | | | i.a. injection | | |
| | MP | FP | AF | MP | FP | AF |
| $C_{max}$ (ng/ml) | 1,073.1 | 353.4 | 214.4 | 11.3 | 81.0 | 52,700 |
| $T_{max}$ (h) | 1.5 | 2 | 5 | 5 | 4 | 1.5 |
| $t_{1/2}$ (h) | 6.0 | 6.2 | 21.5 | NC | 7.0 | 16.8 |
| $AUC_{0-\infty}$ (ng/ml · h) | 6,545 | 2,971 | 6,458 | NC | 981.5 | 317,808 |
| $MRT_{0-\infty}$ (h) | 7.4 | 8.5 | 30.6 | NC | 10.1 | 11.2 |
| CL (mg/ng/ml/h) | 0.099 | 0.22 | 0.101 | NC | 0.004 | <0.001 |
| $V_{ss}$ (mg/ng/ml) | 0.633 | 1.64 | 2.981 | NC | 0.033 | <0.001 |

[a] MP, maternal plasma; FP, fetal plasma; AF, amniotic fluid. The start of infusion was designated as *t* = 0. $AUC_{0-\infty}$, area under the curve from 0 to infinity; MRT, mean residence time; CL, clearance; $V_{ss}$, volume of distribution at steady state; $T_{max}$, time to maximum concentration of drug in serum; NC, not calculated due to insufficient data; $C_{max}$, maximum concentration of drug in serum.

**TABLE 2** Pharmacokinetic parameters of *N*-acetyl–CEM-101 in maternal, fetal, and amniotic compartments after administration of solithromycin via maternal i.v. infusion or i.a. injection[a]

| Parameter | Value | | | | | |
|---|---|---|---|---|---|---|
| | Maternal i.v. | | | i.a. injection | | |
| | MP | FP | AF | MP | FP | AF |
| $C_{max}$ (ng/ml) | 69.12 | 148.3 | 96.2 | 2.7 | 33.2 | 512.2 |
| $T_{max}$ (h) | 5 | 3 | 13 | 9 | 5 | 9 |
| $t_{1/2}$ (h) | 6.0 | 7.6 | 33.8 | 12.2 | 6.7 | 21.8 |
| $AUC_{0-\infty}$ (ng/ml · h) | 789.5 | 1,635.7 | 4,226.5 | 64.2 | 490.8 | 18,414 |
| $MRT_{0-\infty}$ (h) | 8.5 | 9.7 | 49.2 | 19.1 | 11.5 | 33.0 |
| CL (mg/ng/ml/h) | 0.823 | 0.397 | 0.154 | 0.055 | 0.007 | 0.0002 |
| $V_{ss}$ (mg/ng/ml) | 6.16 | 3.47 | 7.41 | 0.99 | 0.075 | 0.0061 |

[a] The start of infusion was designated as *t* = 0. MP, maternal plasma; FP, fetal plasma; AF, amniotic fluid; $AUC_{0-\infty}$, area under the curve from 0 to infinity; MRT, mean residence time; CL, clearance; $V_{ss}$, volume of distribution at steady state; $T_{max}$, time to maximum concentration of drug in serum; $C_{max}$, maximum concentration of drug in serum.

**TABLE 3** Pharmacokinetic parameters of CEM-214 in maternal, fetal, and amniotic compartments after administration of solithromycin via maternal i.v. infusion or a combination of i.v. infusion and i.a. injection[a]

| Parameter | Value | | | | | |
| | Maternal i.v. | | | Combined i.v. and i.a. | | |
| | MP | FP | AF | MP | FP | AF |
|---|---|---|---|---|---|---|
| $C_{max}$ (ng/ml) | 9.6 | 57.3 | 44.2 | 125.8 | 48.3 | 198.2 |
| $T_{max}$ (h) | 2 | 5 | 13 | 2 | 9 | 25 |
| $t_{1/2}$ (h) | 7.9 | 6.9 | 31.6 | 12.4 | 8.1 | 14.9 |
| $AUC_{0-\infty}$ (ng/ml · h) | 1,292 | 538 | 1,582 | 2,800 | 868 | 7,782 |
| $MRT_{0-\infty}$ (h) | 10.4 | 9.4 | 42.3 | 15.9 | 12.9 | 29.9 |
| CL (mg/ng/ml/h) | 0.503 | 1.21 | 0.411 | 0.232 | 0.748 | 0.084 |
| $V_{ss}$ (mg/ng/ml) | 4.74 | 10.2 | 16.95 | 3.46 | 8.93 | 2.41 |

[a] Concentrations of CEM-214 with i.a. injection alone were low or nondetectable in all compartments and insufficient for pharmacokinetic calculations. The start of infusion was designated as $t = 0$. MP, maternal plasma; FP, fetal plasma; AF, amniotic fluid; $AUC_{0-\infty}$, area under the curve from 0 to infinity; MRT, mean residence time; CL, clearance; $V_{ss}$, volume of distribution at steady state; $T_{max}$, time to maximum concentration of drug in serum; $C_{max}$, maximum concentration of drug in serum.

Levels of CEM-214 were lower than NAc–CEM-101 (35 ng/ml at 2 h postinfusion) but exhibited a similar pharmacokinetic profile (Table 3).

Amniotic fluid concentrations of solithromycin had the lowest maximum concentration of drug in serum ($C_{max}$) of the three compartments, 214 ng/ml at 4 to 8 h after maternal infusion; however, due to its very slow clearance and long half-life (21.5 h), therapeutic levels were sustained at >30 ng/ml for over 48 h (Fig. 1A). Accordingly, the $AUC_{0-\infty}$ in amniotic fluid was high: 6,458 ng/ml · h (Table 1). Concentrations of NAc–CEM-101 in AF rose steadily during the first 12 h postinfusion, reaching a $C_{max}$ of 96 ng/ml at 12 h, before declining slowly to 20 ng/ml at 72 h (Fig. 1B). This resulted in a long MRT (49 h) and a high AUC (4,226 ng/ml · h) (Table 2). CEM-214 also slowly accumulated in AF during the first 12 h postinfusion (Fig. 1C), exhibiting a $t_{1/2}$ of 31.6 h and a $C_{max}$ at 13 h of 44.2 ng/ml (Table 3). Both metabolites were still detectable in AF at the 72-h time point.

**Intra-amniotic administration.** Administration of solithromycin (3.5 mg) into the amniotic cavity resulted in peak AF concentrations ranging from 18.9 to 92.8 µg/ml (mean, 52.7 µg/ml) (Fig. 2A). Concentrations declined slowly but steadily, with a $t_{1/2}$ of >16 h; the $AUC_{0-\infty}$ was extremely high at 317,000 ng/ml · h, and concentrations remained well above therapeutic levels (>100 ng/ml) throughout the 72-h experimental period (Fig. 2A). Transfer from the amniotic to fetal compartment was poor, however, with fetal plasma levels peaking at ~1.5% of maximal amniotic fluid levels (81 ng/ml at 2 h postinfusion). Nevertheless, fetal plasma solithromycin levels were sustained above 30 ng/ml for 24 h ($t_{1/2}$ of 7 h; MRT of >10 h) (Fig. 2A). Maternal concentrations barely exceeded the limit of quantitation (10 ng/ml) and only at the 4- to 12-h time points, indicating a very low efficiency of AF-to-maternal plasma transfer (<0.05%).

NAc–CEM-101 was readily detectable after i.a. solithromycin administration in all three compartments, peaking at 8 h in amniotic fluid at levels nearly 5% of those of the parent drug at the same time point (512 ng/ml) (Fig. 2B). The MRT in amniotic fluid (33 h) was considerably longer than that for the parent drug (11 h). Fetal concentrations were lower ($C_{max}$ of 33 ng/ml at 4 h postinfusion) but showed a similar profile, falling after $t = 9$ h postinfusion and remaining detectable until after 24 h. Maternal levels also peaked at 9 h but never exceeded 3 ng/ml. The levels of CEM-214 in all three compartments following i.a. solithromycin administration were on the borderline of detectability and data could not be analyzed.

**Combined maternal and intra-amniotic administration.** The combination of maternal i.v. and i.a. administration resulted in peak fetal concentrations about 25% higher than levels that would be predicted on a simple additive basis (Fig. 3A). At 1 h postinjection/postinfusion, fetal plasma levels reached 511 ng/ml and then declined with a $t_{1/2}$ of 5.2 h; the $AUC_{0-\infty}$ was 4,880 ng/ml · h (Table 1). In the amniotic cavity, the combination of i.v. plus i.a. administration did not initially alter CEM-101 concentrations above the expected levels, but between 12 to 28 h the levels were sustained at >2-fold-higher concentrations than the simple combination would have predicted (Fig. 3A). Maternal concentrations were similar to those expected.

Levels of NAc–CEM-101 in all three compartments were within the range expected based on a simple combination of the i.v. and i.a. doses (Fig. 3B). However, CEM-214 concentrations in
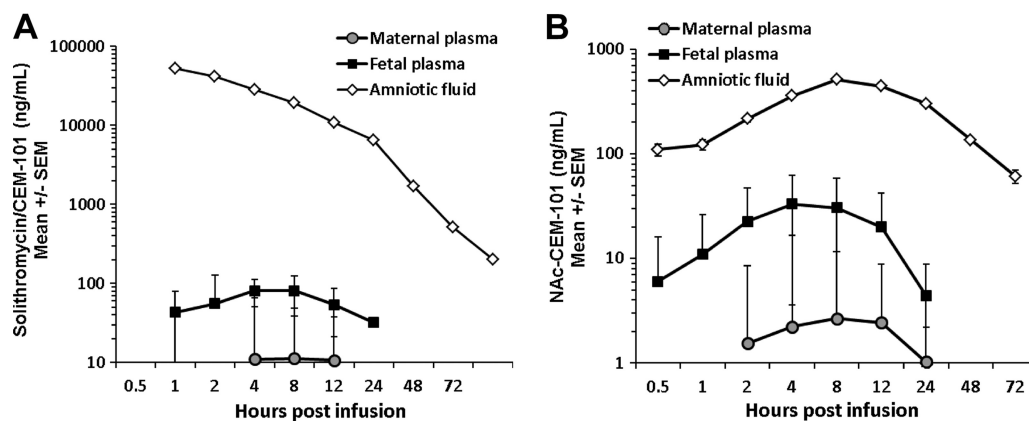


**FIG 2** Concentrations of solithromycin (A) and its metabolite NAc–CEM-101 (B) in maternal plasma, fetal plasma, and amniotic fluid after a single amniotic dose (3.5 mg, 1.4 mg/kg fetal weight) by intra-amniotic (i.a.) injection. Data are shown as means ± SEM ($n = 6$ or 7 animals). Note log concentration axes. CEM-214 concentrations were at or below the limit of detection (data not shown).
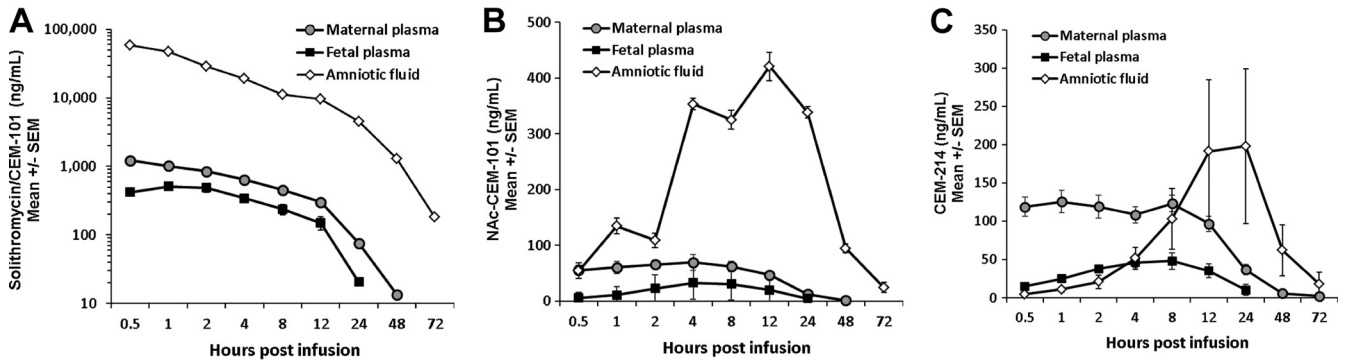
**FIG 3** Concentrations of solithromycin (CEM-101) (A), NAc–CEM-101 (B), and CEM-214 (C) in maternal plasma, fetal plasma, and amniotic fluid after combined maternal (10 mg/kg) and intra-amniotic (1.4 mg/kg) administration of solithromycin. Data are shown as means ± SEM (*n* = 6 or 7 animals). Note the log concentration axis in panel A.

AF showed an interesting and unexpected response to the combination dose (Fig. 3C and Table 3). With combined i.v. and i.a. CEM-101 administration, AF levels of CEM-214 showed a marked late-onset rise, reaching ~200 ng/ml at 8 to 24 h postinfusion. This is 4-fold higher than that observed in the i.v.-only group, despite the fact that in the i.a. group, CEM-214 concentrations in AF were undetectable. The AF $AUC_{0-\infty}$ in the combined i.v. and i.a. group was much higher than in the maternal i.v. group (7,782 compared to 1,582 ng/ml · h). Plasma CEM-214 levels were predictable and did not show this counterintuitive response to the combined dose.

**Comparison with azithromycin.** Our previous studies of macrolide biodistribution in pregnancy using the same model at the same gestational age observed very low rates of transfer of macrolides from the maternal compartment to the fetal or amniotic compartment (26). To highlight the differences between solithromycin and azithromycin, maternal plasma, fetal plasma, and AF concentrations of both antibiotics were plotted on the same graph for 48 h after maternal i.v. infusion. As shown in Fig. 4, the concentrations of solithromycin in the fetal and amniotic compartments were dramatically higher than azithromycin, with solithromycin achieving and sustaining therapeutic concentrations for at least 12 h (48 h in the amniotic cavity). At the 4-h time point, the maternal-to-fetal plasma and maternal-to-AF plasma transfer rates of azithromycin were 1.4% and 13.3%, respectively,

whereas the equivalent figures for solithromycin were 51.6% and 47.9%.

## DISCUSSION

The central finding of this study is that a single dose of solithromycin given maternally can be an effective mode of delivery of antibiotic to both the fetal and amniotic compartments. Assuming this finding is replicated in human pregnancy, this is an extremely significant observation clinically, as currently available macrolides have limited oral bioavailability and do not transfer efficiently from the maternal compartment to the fetal compartment (unless given over extended periods of time). Consequently, maternal therapy provides poor antimicrobial protection for the amniotic cavity (including the fetus). This is likely to be a major factor contributing to the poor success rates of macrolides in the prevention of preterm birth and reduction of associated neonatal morbidity and mortality. The fact that solithromycin is considerably more potent than existing macrolides, has a high level of oral bioavailability, exhibits significant cellular accumulation, and, most importantly, is very effective against most macrolide-resistant strains of target organisms (31) makes maternal solithromycin administration an extremely attractive therapeutic prospect for treating and preventing intrauterine infections in pregnancy.

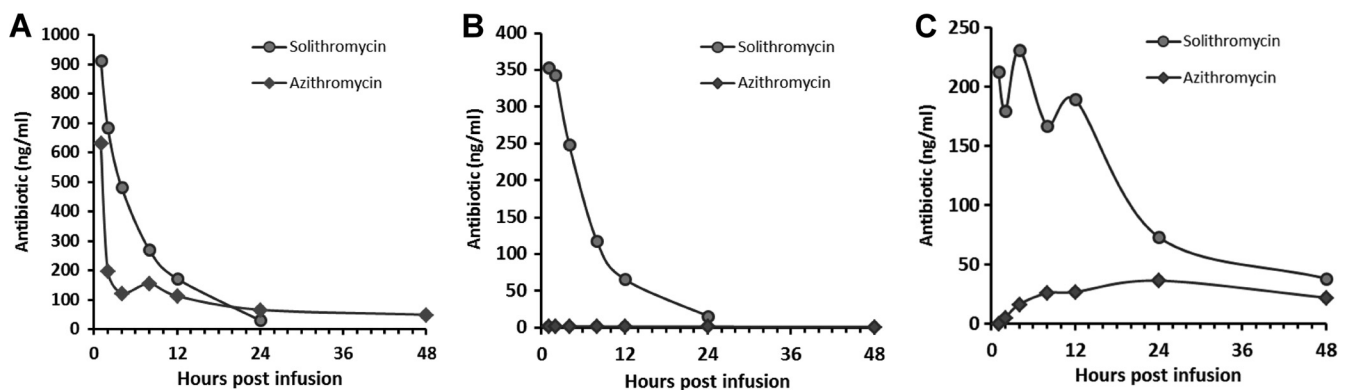In adult humans, solithromycin pharmacokinetic parameters



**FIG 4** Comparison of solithromycin and azithromycin pharmacokinetic profiles in maternal plasma (A), fetal plasma (B), and amniotic fluid (C) after a single maternal dose by i.v. infusion (10 mg/kg solithromycin, 5 mg/kg azithromycin; single dose).

($C_{max}$, $t_{1/2}$, and $AUC_{0-\infty}$) following a 600-mg oral dose are 862 ng/ml, 5.5 h, and 9,049 ng/ml · h, respectively (42). These data are reasonably similar to the present figures in maternal sheep plasma at a similar dose (1,073 ng/ml, 6.0 h, 6,545 ng/ml · h, respectively), suggesting that the pharmacokinetics are broadly similar in both species. While the ovine placenta differs structurally and anatomically from the human placenta in a number of important ways (46), the pregnant ewe remains a useful model to study placental transport and fetal growth and development (47). In our earlier studies in the same model, azithromycin (at half the dose) was cleared from maternal plasma considerably more rapidly than solithromycin ($t_{1/2}$ of ~1.3 h), although the $AUC_{0-\infty}$ was similar (6,227 ng/ml · h) (26). Surprisingly, in light of the immaturity of the fetus in terms of drug metabolic and secretory capacity, the $t_{1/2}$ and MRT of solithromycin in fetal plasma appear to be only slightly longer than in the maternal circulation. This may relate to the mode of excretion of solithromycin. In adults, there is some evidence to suggest that solithromycin and an *N*-demethylated metabolite are eliminated in the bile (Cempra Inc., unpublished findings). Both human and ovine fetuses are known to have an immature but partially functional biliary secretion system (48, 49), presumably capable of eliminating small amounts of solithromycin. Repeated doses, therefore, may be expected to result in delayed elimination and associated changes in pharmacokinetic parameters. Future studies will need to explore this to ensure that fetal concentrations do not increase to toxic levels following multiple doses.

Fetal plasma solithromycin concentrations reached peak levels (~350 to 400 ng/ml) 1 h after maternal infusion, suggesting a relatively high rate of transplacental passage. The efficiency of transfer (30 to 50%) was much greater than existing macrolides, which are <1% in the same ovine model (26) and <4% in humans (25). The relatively static levels of solithromycin found in the amniotic fluid probably reflects the initial accumulation of the antibiotic from fetal excretion and transplacental and transchorioamnion diffusion, in conjunction with a low rate of clearance ($t_{1/2}$ of ~20 h, similar to the $t_{1/2}$ of azithromycin in AF of 17.5 h [26]). Hence, despite the $C_{max}$ in AF barely exceeding 200 ng/ml, the AUC was equivalent to that in MP (>6,000 ng/ml · h) and more than twice that in FP (2,971 ng/ml · h). Collectively, these data suggest that a single maternal 650-mg solithromycin dose is sufficient to give adequate therapeutic antibiotic levels in the fetal and amniotic compartments.

The $MIC_{90}$ for solithromycin against most macrolide-susceptible strains of *Ureaplasma* spp., *Mycoplasma* spp., streptococci, and staphylococci is <30 ng/ml (31, 34, 38). Farrell and colleagues tested solithromycin *in vitro* against a large number (>10,000) of clinical bacterial pathogens and reported that the large majority of isolates of *Streptococcus pneumoniae*, *Staphylococcus aureus*, coagulase-negative staphylococci, beta-hemolytic streptococci, viridans group streptococci, and *Moraxella catarrhalis* were inhibited by 60 ng/ml solithromycin (34). Based on this cutoff, a single maternal dose would be expected to provide antimicrobial coverage in maternal plasma, fetal plasma, and AF for >12, >12, and >24 h, respectively. More resistant strains of these organisms and of other less susceptible species, such as *Haemophilus influenzae* and enterococci, required higher concentrations (500 to 2,000 ng/ml) to achieve effective inhibition (34). Repeated maternal administration is likely to be needed to achieve these concentrations in the amniotic cavity. Alternatively, as we have shown in this study, a single intra-amniotic dose would be sufficient in theory to eliminate even highly resistant microorganisms from the amniotic cavity. A combined i.v.-i.a. regimen might, therefore, have significant therapeutic advantages in terms of eradication of resistant or persistent intra-amniotic infections unresponsive to maternal administration alone.

Detailed studies of solithromycin metabolism and the bioactivity of its metabolites have not yet been carried out. In preliminary studies, the two known urinary excretion products of solithromycin metabolism, NAc–CEM-101 and CEM-214, have been found to possess approximately 50% and 25% of the activity of the parent compound against susceptible strains of Gram-positive organisms, although against resistant organisms they are markedly less effective (41). Their relative efficacy against *Ureaplasma* and *Mycoplasma* spp. is currently being evaluated, although these studies are not yet complete; therefore, definitive assessment of their bioactivity is currently lacking. These metabolites were both present in the maternal circulation at low but readily detectable levels, 5 to 10% of the concentrations of the parent drug. In this regard, the sheep and mouse are similar. Humans, on the other hand, produce less of both metabolites, while monkeys produce ~10-fold more and rats produce more NAc–CEM-101 but almost no CEM-214 after oral administration (41). The delayed clearance of the metabolites in AF results in extended duration of their effects (i.e., high AUC) in this compartment. Taking into account the preliminary data available on the relative potency of CEM-101 and its metabolites (approximately 1:0.5:0.25) and applying this to the individual AUC values for CEM-101 and its metabolites, the net combined AUCs in maternal plasma, fetal plasma, and AF following the maternal i.v. dose are 7,263, 3,923, and 8,967 ng/ml · h, respectively. Hence, the metabolites are likely to play a particularly significant contribution to solithromycin's antimicrobial effects in the amniotic cavity.

Solithromycin's spectrum of activity is particularly pertinent to the treatment of bacterial genital tract infections. Not only is it extremely effective against *Mollicutes* (*Ureaplasma* and *Mycoplasma* spp.), the organisms most commonly isolated from amniotic fluid following preterm labor or PPROM, but it is also highly effective against other important genital tract pathogens, such as group B streptococci, *N. gonorrhoeae*, and *C. trachomatis* (32, 34–40). Assuming that the present findings can be replicated in human pregnancy, then administration of solithromycin to women in preterm labor or PPROM would be expected to constitute a markedly more effective antenatal antimicrobial therapy than currently used macrolides, with commensurate benefits in the reduction of neonatal infections and associated morbidity and mortality. For asymptomatic women early in pregnancy at risk of infection-driven preterm birth, maternal solithromycin administration may offer an effective therapeutic approach for the prevention of preterm birth. Studies are under way to assess the transfer and metabolism of solithromycin by the human placenta.

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