Maternal Administration of Solithromycin, a New, Potent, Broad-Spectrum Fluoroketolide Antibiotic, Achieves Fetal and Intraamniotic Antimicrobial Protection In a Pregnant Sheep Model.

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Running title: Solithromycin pharmacokinetics in ovine pregnancy
ABSTRACT

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=6-7) received either a single maternal IV infusion of CEM-101 (10 mg/kg), a single intra-amniotic (IA) injection (1.4 mg/kg estimated fetal weight), or a combined IV and IA dose. Maternal plasma (MP), fetal plasma (FP) and amniotic fluid (AF) samples were taken via catheter at intervals 0-72 h post administration and concentrations of solithromycin and its bioactive polar metabolites (NAc-CEM-101 and CEM-214) determined. Following maternal IV infusion peak CEM-101 concentrations in MP, FP and AF were 1073, 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). IA solithromycin injection resulted in elevated (~50 µg/mL) and sustained CEM-101 concentrations in AF and significant levels in FP, although the efficiency of amniotic-to-fetal transfer was low (~1.5%). Combined IV and IA administration resulted in primarily additive concentrations of CEM-101 in all three compartments. Our findings suggest that solithromycin/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.

**KEY WORDS:** Antibiotics; intrauterine infection; pharmacokinetics; pregnancy; Ureaplasma; sheep
INTRODUCTION

Intrauterine infection and inflammation play a well-recognised role in the etiology of spontaneous preterm labor and birth, particularly in deliveries less than 32 weeks’ gestation or those complicated by preterm pre-labor 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 colonise 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 Mollicute class, namely *Ureaplasma* and *Mycoplasma* species, are the microorganisms most commonly isolated from the amniotic fluid 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 metaanalyses 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 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 many-fold, 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 administration is largely ineffective in eradicating intrauterine *Ureaplasma* spp. infection (23). In an experimental sheep model of intrauterine *Ureaplasma* spp. colonisation during pregnancy, we have shown that maternal intramuscular (IM) erythromycin administration does not eradicate *Ureaplasma* spp. infection from the amniotic fluid, chorioamnion, umbilical cord or the 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 2-4% (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 transfer is more uncertain. A recent study in pregnant women at term reported that the transfer of azithromycin from maternal circulation to amniotic fluid (AF) was more efficient than the maternal-to-fetal transfer, although resulting AF
concentrations (~150 ng/mL) still failed to reach the MIC\textsubscript{90} for \textit{Ureaplasma} spp. (~500 ng/mL) (29). Interestingly, a recent study in pregnant primates with intraamniotic \textit{Ureaplasma} infection showed that a sustained 10-day maternal course of azithromycin (5 mg/kg) 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 intraamniotic and fetal inflammation remained evident following azithromycin treatment and pregnancy length was only extended by 7-10 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 \textit{Neisseria gonorrhoeae}, \textit{Chlamydia trachomatis}, \textit{Haemophilus} spp., \textit{Streptococci} and \textit{Enterococci} (31-37). It is exceptionally potent against \textit{Ureaplasma parvum}, \textit{Ureaplasma urealyticum} and \textit{Mycoplasma hominis} (125-250 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 2-8 times its MIC\textsubscript{90} for some pathogens (40). Solithromycin has a plasma half-life 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 transfer of solithromycin in a pregnant ovine model to assess its potential for treating intrauterine and antenatal infection. An intravenous (IV) 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 intraamniotic (IA) 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, anaesthesia, surgical catheterization and recovery have been described previously (26). Five days after surgery, at 116 ± 1 days gestation, chronically catheterised pregnant ewes (~65 kg) were randomly selected to receive either: (i) a single maternal IV infusion of 650 mg solithromycin
(10 mg/kg maternal weight; n=6) over 60 minutes in 325 mL infusion buffer (5.77 g/L tartaric acid, 50 g/L mannitol, 5 g/L thioglycerol, buffered to pH 4.2 with NaOH); (ii) a single IA injection of 3.5 mg solithromycin (1.4 mg/kg fetal weight) in 3 mL perfusion buffer (n=6) or (iii) a combination of maternal IV infusion and IA injection (n=7). At this gestational age fetal weight was estimated to be 2.5 kg and amniotic fluid volume approximately 250 mL. Maternal and fetal arterial blood and amniotic fluid (2 x 1 mL) samples were collected into heparinised 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 analyser (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-tandem tandem quadrupole mass spectrometry (MicroConstants’ analytical method MN12084). K$_3$EDTA (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 reversed-phase HPLC using a Phenomenex Luna CN 100Å 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.
B (0.1% formic acid in methanol:acetonitrile [50:50, v/v]) 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 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 v. 4.1 (Waters, Milford, MA). All samples were analysed in a total of nine assay runs. The coefficient of variation (CV) of the mean plasma QC values (low, medium and high) for solithromycin, CEM-214 and NAc-CEM-101 ranged from 3.86 - 4.93%, 9.92 - 23.4% and 3.59 - 10.4%, respectively; in AF the CV of the QC values ranged from 4.54 - 6.91%, 5.43 - 10.1% and 3.34 - 7.96%, respectively. Methodological accuracy for all three analytes was 6.7-10.0%.

**Statistical and pharmacokinetic analysis**

Antibiotic concentration data from n=6-7 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 IA solithromycin administration data and all metabolite data were best described by a non-compartmental model.
Maternal IV administration

The pharmacokinetics of maternal IV 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 1073 ng/mL were obtained 30 min after maternal IV infusion (t=1.5 h), which declined steadily with a T_{1/2} of 6 hours, resulting in an AUC_{0-∞} of greater than 6500 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 post infusion 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 solithromycin, although the mean residence time (MRT) was slightly longer (8.5 h vs. 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 NAc-CEM-101 (97.6 ng/mL at 1 h post infusion, 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 IV administration) (41).

Solithromycin concentrations in fetal plasma peaked 1-2 h post infusion, 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-∞} in the fetal compartment was...
In the fetal compartment, NAc-CEM-101 levels reached almost half those of CEM-101 at 2 h post-infusion (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). Levels of CEM-214 were lower than NAc-CEM-101 (35 ng/mL at 2 h post infusion) but exhibited a similar pharmacokinetic profile (Table 3).

Amniotic fluid concentrations of solithromycin had the lowest \( C_{\text{max}} \) of the three compartments, 214 ng/mL at 4-8 h post 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: 6458 ng/mL.h (Table 1). Concentrations of NAc-CEM-101 in AF rose steadily during the first 12 h post infusion, reaching a \( C_{\text{max}} \) 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 (4226 ng/mL.h) (Table 2). CEM-214 also slowly accumulated in AF during the first 12 h post infusion (Fig 1C), exhibiting a \( T_{1/2} \) of 31.6 h and a \( C_{\text{max}} \) at 13 h of 44.2 ng/mL (Table 3). Both metabolites were still detectable in AF at the 72 h time point.

**Intraamniotic administration**

Administration of solithromycin (3.5 mg) into the amniotic cavity resulted in peak AF concentrations ranging from 18.9 – 92.8 \( \mu \)g/mL (mean, 52.7 \( \mu \)g/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 post infusion). Nevertheless, fetal plasma solithromycin levels were sustained above 30 ng/mL for 24 h (T₁/₂ 7 h; MRT >10 h) (Fig 2A). Maternal concentrations barely exceeded the limit of quantitation (10 ng/mL) and only at the 4-12 h time-points, indicating a very low efficiency of AF-to-maternal transfer (<0.05%).

NAc-CEM-101 was readily detectable after IA solithromycin administration in all three compartments, peaking at 8 h in amniotic fluid at levels nearly 5% 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 the parent drug (11 h). Fetal concentrations were lower (Cₘₐₓ: 33 ng/mL at 4 h post infusion), but showed a similar profile, falling after t=9 h post infusion 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 IA solithromycin administration were on the borderline of detectability and data could not be analysed.

Combined maternal and intra-amniotic administration

The combination of maternal IV and IA 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 post injection/infusion, fetal plasma levels reached 511 ng/mL and then declined with a T₁/₂ of 5.2 h; the AUC₀-∞ was 4,880 ng/mL.h (Table 1). In the amniotic cavity, the combination of IV plus IA administration did not initially alter CEM-101 concentrations above expected levels, but between 12-28 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 IV and IA doses (Fig 3B). However, CEM-214 concentrations in AF showed an interesting and unexpected response to the combination dose (Fig 3C; Table 3). With combined IV plus IA CEM-101 administration, AF levels of CEM-214 showed a marked late-onset rise, reaching ~200 ng/mL at 8-24 h post-infusion. This is four-fold higher than observed in the IV only group, despite the fact that in the IA group CEM-214 concentrations in AF were undetectable. The AF AUC₀-∞ in the combined IV plus IA group was much higher than in the maternal IV group (7,782 vs. 1,582 ng/mL.h, respectively). 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 to fetal or amniotic compartments (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 post maternal IV 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 and maternal-to-AF 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 to fetal compartments (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 ($C_{\text{max}}$, $T_{1/2}$, and $\text{AUC}_{0-\infty}$) following a 600 mg oral dose are 862 ng/mL, 5.5 h and 9049 ng/mL.h, respectively (42). These data are reasonably similar to the present figures in maternal sheep plasma at a similar dose (1073 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 ($T_{1/2} \sim 1.3$ h), although the $\text{AUC}_{0-\infty}$ was similar ($6,227 \text{ ng/mL.h}$)(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 appears 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 (~350-400 ng/mL) 1 h post-maternal infusion, suggesting a relatively rapid rate of transplacental passage. The efficiency of transfer (30-50%) 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 trans-placental and trans-chorioamnion diffusion, in conjunction with a slow rate of clearance ($T_{1/2} \sim 20$ h, similar to the $T_{1/2}$ of azithromycin in AF of 17.5 h (26)). Hence, despite the $C_{\text{max}}$ values 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\textsubscript{90} for solithromycin against most macrolide susceptible strains of \textit{Ureaplasma} spp, \textit{Mycoplasma} spp, streptococci and staphylococci is <30 ng/mL (31, 34, 38). Farrell and colleagues tested solithromycin \textit{in-vitro} against a large number (>10,000) of clinical bacterial pathogens and reported that the large majority of isolates of \textit{Streptococcus pneumoniae}, \textit{Staphylococcus aureus}, coagulase-negative Staphylococci, beta-haemolytic Staphylococci, viridans group Streptococci and \textit{Moraxella catarrhalis} were inhibited by 60 ng/mL solithromycin (34). Based on this cut-off, 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 \textit{Haemophilus influenzae} and Enterococci, required higher concentrations (500-2000 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 intraamniotic dose would be sufficient in theory to eliminate even highly resistant microorganisms from the amniotic cavity. A combined IV/IA regimen might, therefore, have significant therapeutic advantages in terms of eradication of resistant or persistent intraamniotic 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, 5-10% 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 IV 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 currently underway to assess the transfer and metabolism of solithromycin by the human placenta.

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REFERENCES


Figure legends

**FIGURE 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 IV infusion. Data are shown as mean ± SEM (n=6-7 animals).

**FIGURE 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 intraamniotic (IA) injection. Data are shown as mean ± SEM (n=6-7 animals). Note log concentration axes. CEM-214 concentrations were at or below the limit of detection (data not shown).

**FIGURE 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 intraamniotic (1.4 mg/kg) administration of solithromycin. Data are shown as mean ± SEM (n=6-7 animals). Note the log concentration axis in (A).

**FIGURE 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 IV infusion (Note: 10 mg/kg solithromycin, 5 mg/kg azithromycin; single dose).
TABLE 1. Pharmacokinetic parameters of solithromycin in maternal, fetal and amniotic compartments after administration of solithromycin via maternal intravenous (IV) infusion or intraamniotic (IA) injection. MP, maternal plasma; FP, fetal plasma; AF, amniotic fluid. The start of infusion was designated as t=0. AUC, area-under-the-curve; MRT, mean residence time; CI, clearance; V_{ss}, volume of distribution at steady state. NC, not calculated due to insufficient data.

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### TABLE 2. Pharmacokinetic parameters of N-Acetyl-CEM-101 in maternal, fetal and amniotic compartments after administration of solithromycin via maternal intravenous (IV) infusion or intraamniotic (IA) injection. The start of infusion was designated as t=0. MP, maternal plasma; FP, fetal plasma; AF, amniotic fluid. AUC, area-under-the-curve; MRT, mean residence time; Cl, clearance; \( V_{ss} \), volume of distribution at steady state.

<table>
<thead>
<tr>
<th></th>
<th>Maternal IV</th>
<th>IA injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MP</td>
<td>FP</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>69.12</td>
<td>148.3</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>( T_{\frac{1}{2}} ) (h)</td>
<td>6.0</td>
<td>7.6</td>
</tr>
<tr>
<td>( \text{AUC } o-\infty ) (ng/mL.h)</td>
<td>789.5</td>
<td>1,635.7</td>
</tr>
<tr>
<td>( \text{MRT } o-\infty ) (h)</td>
<td>8.5</td>
<td>9.7</td>
</tr>
<tr>
<td>( \text{Cl } ) (mg/ng/mL/h)</td>
<td>0.823</td>
<td>0.397</td>
</tr>
<tr>
<td>( V_{ss} ) (mg/ng/mL)</td>
<td>6.16</td>
<td>3.47</td>
</tr>
</tbody>
</table>
TABLE 3. Pharmacokinetic parameters of CEM-214 in maternal, fetal and amniotic compartments after administration of solithromycin via maternal intravenous (IV) infusion or a combination of IV infusion plus intraamniotic (IA) injection. Concentrations of CEM-214 with IA injection alone were low or non-detectable 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, area-under-the-curve; MRT, mean residence time; Cl, clearance; Vss, volume of distribution at steady state.

<table>
<thead>
<tr>
<th></th>
<th>Maternal IV</th>
<th>Combined IV + IA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MP</td>
<td>FP</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>9.6</td>
<td>57.3</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>7.9</td>
<td>6.9</td>
</tr>
<tr>
<td>AUC o→∞ (ng/mL.h)</td>
<td>1,292</td>
<td>538</td>
</tr>
<tr>
<td>MRT o→∞ (h)</td>
<td>10.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Cl (mg/ng/mL/h)</td>
<td>0.503</td>
<td>1.21</td>
</tr>
<tr>
<td>Vss (mg/ng/mL)</td>
<td>4.74</td>
<td>10.2</td>
</tr>
</tbody>
</table>