Efficacy of Solithromycin against *Enterococcus faecalis* in a Neutropenic Mouse Thigh Abscess Model

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ABSTRACT

Objectives: Solithromycin (SOLI) is a fluoroketolide that is in Phase 3 clinical development for community acquired bacterial pneumonia. It has demonstrated a wider spectrum than the older macrolides with activity against most macrolide-resistant strains. SOLI is unlike older macrolides in that it has in vitro activity against *Enterococcus faecalis* and *Enterococcus faecium*. In this study we determined its in vivo activity in a neutropenic mouse thigh abscess model.

Methods: Female CD-1 mice (18 to 20 gms) were made neutropenic with two doses of cyclophosphamide (150 and 100 mg/kg) on days -4 and -1 prior to infection. On day 0 mice were infected with *E. faecalis* A2302971 via intramuscular administration of 0.1 mL under light anesthesia. Treatment was administered 1.5 hours post infection for 3 days. SOLI was administered either orally or intravenously (IV), BID or once a day. Linezolid (LZ) administered orally at 10 mg/kg BID was the comparator. At 1.5 and 74 hours post infection mice were euthanized and the infected thighs were removed, weighed, homogenized, serially diluted, and plated on bacterial growth media. The plates were incubated overnight at 37°C in 5% CO_2. CFU per gram of thigh was calculated by enumerating the plated colonies then adjusting for serial dilutions and the weight of the thigh. The MICs of SOLI and LZ for this strain were 1 mcg/mL and 2 mcg/mL, respectively.

Results: SOLI when administered IV demonstrated an efficacious response with a 2.07 log_{10} CFU change at the 12.5 mg/kg BID dose and 3.85 log_{10} CFU reduction at the 25 mg/kg BID dose compared to the vehicle control (T=74 hours). Linezolid demonstrated a 3.50 log_{10} CFU reduction. When administered orally, SOLI demonstrated an efficacious response compared to the vehicle control (T=74 hours). SOLI treated twice daily at 50 mg/kg BID demonstrated a 2.99 log_{10} CFU reduction, whereas SOLI treated once daily at 100 mg/kg produced a 2.77 log_{10} CFU reduction. Linezolid demonstrated a 3.37 log_{10} CFU reduction when treated twice daily at 10 mg/kg.

Conclusions: SOLI demonstrated activity against *E. faecalis* in the abscess model in neutropenic mice. SOLI, especially when administered by the IV route, demonstrated comparable activity to linezolid, an antibiotic that is approved for use against this pathogen. The side chain of SOLI undergoes greater metabolism in mice than in humans when administered orally, but not IV. Therefore, this study was conducted using both oral and IV routes of administration in mice. Unlike older macrolides, SOLI has activity against *E. faecalis,* a difficult-to-treat pathogen.

INTRODUCTION

Solithromycin (CEM-101) is a fourth generation macrolide and the first fluoroketolide that is in development for treatment of moderate to moderately severe community acquired bacterial pneumonia (CABP) in monotherapy. The first Phase 3 trial (Solitaire-Oral) was completed successfully where it was non-inferior to the potent fluoroquinolone, Moxifloxacin. The second Phase 3 trial in CABP (Solitaire-IV) is evaluating intravenous solithromycin with the ability to switch to oral treatment and is currently enrolling.

Unlike older macrolides, solithromycin has three sites of interaction with the bacterial ribosome, which confers activity against most macrolide-resistant respiratory pathogens and also includes pathogens such as enterococci that are not in the spectrum of activity of older macrolides [**1, 2, 3, 4, 5**]. The MIC_{90} for solithromycin against enterococci is 2 mg/L, including against vancomycin-resistant strains. The MIC_{90} for linezolid is also 2 mg/L for enterococci. There is an increasing need for new options for treating enterococci. Therefore, in this study we have determined the efficacy of solithromycin against *E. faecalis* in a thigh abscess model in neutropenic mice.

MATERIALS AND METHODS

Solithromycin was supplied by Cempra Inc. The experiments were conducted at Avastus, Waltham, MA, USA (formerly Vivisource).

Animals: CD-1 female mice weighing 18-20 grams were purchased from Harlan Laboratories, Indianapolis, In. USA. There were four mice in each group.

Immunosuppression: Mice were made neutropenic by injecting cyclophosphamide IP at a 150 mg/Kg four days prior to infection. A second treatment was performed 24 hours prior to infection at 100 mg/Kg injected IP. The reduction in the leukocyte count was in the order of 85% compared with the normal animals.

Bacterial strain and in vitro susceptibility: In vitro susceptibility of *E. faecalis* A2302971 to solithromycin (MIC 1 µg/mL) and linezolid (MIC 2 µg/mL) were determined by standard CLSI methods using microtiter broth dilution.

Bacterial Inoculum into the thigh muscle: *E. faecalis* A2302971 was grown overnight and a single colony was re-suspended in 0.9% Saline. The bacterial suspension was diluted in saline to achieve a final inoculum concentration of 3.6 x10^5 CFU/ mouse. Mice were infected via intramuscular injection of 0.1 mL per mouse.

Treatment- Intravenous: Solithromycin for Injection was delivered at 6.3, 12.5 or 25mg/kg twice daily for three days, starting at 1.5 hours post infection. Infection control mice were dosed with test article vehicle.

Treatment- Oral: Solithromycin was delivered orally as a suspension at 12.5, 25 and 50 mg/kg twice daily for three days or 100 mg/kg once daily for three days, starting at 1.5 hours post infection. Infection control mice were dosed with test article vehicle. Linezolid was used as the positive control at a dose of 10 mg/kg BID, orally.

Tissue Processing: Thigh tissues were collected at 0 and 72 hours post start of treatment. Mice were euthanized by CO_2 inhalation and thighs of the mice were aseptically removed, weighed, homogenized, serially diluted, and plated on TSA media. The plates were incubated overnight at 37°C with 5% CO_2.

Data Analysis: CFU per gram of thigh was calculated by enumerating the plated colonies then adjusting for serial dilutions and the weight of the thigh.

RESULTS

Efficacy of Oral Treatment: Solithromycin demonstrated an efficacious response against the 74 hour infection control at both the 50 mg/kg BID and 100 mg/kg QD dosing levels. The 12.5 and 25 mg/kg doses produced similar efficacious responses with 1.35 and 1.01 log_{10} CFU reduction respectively, compared to the vehicle control (T=74). Solithromycin treatment twice daily at 50 mg/kg demonstrated a 2.99 log change whereas solithromycin treated once daily at 100 mg/kg produced a 2.77 log change compared to the vehicle control (T=74). Linezolid was used as the antibiotic control and demonstrated a 3.37 log_{10} CFU reduction when treated twice daily at 10 mg/kg for three days.

Table 2a. In Vivo Efficacy after Oral Treatment of Solithromycin In the Neutropenic Mouse Thigh Abscess Model

Treatment	Concentration (mg/kg/dose)	Route (Dosing Regimen)	Log CFU/ g of Thigh	St. Dev.	Log change from T=74	Log change from T=1.5
Control T=Rx	-	-	6.08	0.18	-2.32	-
Control T=74	-	-	8.40	0.51	-	2.32
Solithromycin	12.5	PO (BID for 3 days)	7.05	0.30	-1.35	0.97
	25		7.40	0.07	-1.01	1.31
	50		5.42	0.27	-2.99	-0.67
	100	PO (QD for 3 days)	5.63	0.69	-2.77	-0.45
Linezolid	10	PO (BID for 3 days)	5.04	0.27	-3.37	-1.05

PO: oral dosing; BID: twice daily; QD: once daily

Efficacy of Intravenous Treatment: Solithromycin demonstrated an efficacious response at both the 12.5 and 25 mg/kg BID intravenous doses, with 2.07 and 3.85 log changes respectively, compared to the vehicle control (T=74). Linezolid was administered as the positive control and demonstrated a 3.50 log_{10} CFU reduction compared to the vehicle control (T=74.)

Table 2. In Vivo Efficacy after Intravenous Treatment of Solithromycin In the Neutropenic Mouse Thigh Abscess Model

Treatment (BID for 3 days)	Concentration (mg/kg/dose)	Route	Log CFU/ g of Thigh	St. Dev.	Log change from T=74	Log change from T=1.5
Control T=Rx	-	-	5.95	0.04	-1.99	-
Control T=74	-	-	7.94	0.22	-	1.99
Solithromycin	6.3	IV	7.36	0.66	-0.58	1.41
	12.5		5.87	0.72	-2.07	-0.08
	25		4.10	0.08	-3.85	-1.85
Linezolid	10	PO	4.44	0.08	-3.50	-1.51

DISCUSSION AND CONCLUSIONS

Solithromycin demonstrated an efficacious response in the neutropenic mouse thigh infection model against the *E. faecalis* A2302971 strain at doses of 12.5 mg/kg BID and 25 mg/kg BID dosing (intravenous). The 25 mg/kg BID intravenous dosing of CEM-101 was equally efficacious to the 10 mg/kg BID oral dosing of linezolid. Solithromycin also showed an efficacious response against *E. faecalis* A2302971 at the 50 mg/kg BID and the 100 mg/kg QD oral dosing regimens. In mice, bioavailability of solithromycin by the intravenous route could be better predictive of bioavailability by the intravenous and oral routes in human because of greater side chain metabolism in mice when solithromycin is administered orally, unlike in humans.

Unlike older macrolides, solithromycin has activity in vitro and in vivo against enterococci. Solithromycin could be considered as a treatment option for enterococcal infections.

REFERENCES

1). Farrell DJ, Castanheira M, Sader HS, Jones RN. 2010. The in vitro evaluation of solithromycin (CEM-101) against pathogens isolated in the United States and Europe (2009). J. Infect. 61: 476-483. **2**). Hardy D, Vicino D, Fernandes P. 2013. Potent in vitro activity of Solithromycin (CEM-101) against vancomycin-resistant and -susceptible enterococci. Abstr. 53rd Intersc. Conf. Antimicrob. Agents Chemother., E-144a, Denver, CO. **3**). Morrissey I, Fernandes P, Lemos B, Hawser SP. 2014. Activity of solithromycin against azithromycin-resistant *Streptococcus pneumoniae* with characterized resistance mechanisms. Abstr. 24th Eur. Congr. Clin. Microb. Infect. Dis., P1585, Barcelona, Spain. **4**). Putnam SD, Sader HS, Farrell DJ, Biedenbach DJ, Castanheira M. 2011. Antimicrobial characterisation of solithromycin (CEM-101), a novel fluoroketolide: activity against staphylococci and enterococci. Int. J. Antimicrob. Agents 37: 39-45. **5**). Woosley LN, Castanheira M, Jones RN. 2010. CEM-101 activity against Gram-positive organisms. Antimicrob. Agents Chemother. 54: 2182-2187.