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Solithromycin Pharmacokinetics in Plasma and Dried Blood Spots and Safety in Adolescents

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We assessed the pharmacokinetics and safety of solithromycin, a fluoroketolide antibiotic, in a phase 1, open-label, multicenter study of 13 adolescents with suspected or confirmed bacterial infections. On days 3 to 5, the mean (standard deviation) maximum plasma concentration and area under the concentration versus time curve from 0 to 24 h were 0.74 μg/ml (0.61 μg/ml) and 9.28 μg · h/ml (6.30 μg · h/ml), respectively. The exposure and safety in this small cohort of adolescents were comparable to those for adults. (This study has been registered at ClinicalTrials.gov under registration no. NCT01966055.)

Invasive infections due to drug-resistant bacteria are increasingly common and often fatal. In the United States, approximately 2 million people have drug-resistant infections, resulting in 23,000 deaths annually (1). Solithromycin is a new fluoroketolide antibiotic with activity against a wide array of bacteria causing respiratory tract infections and other pathogens. Solithromycin is under investigation for oral and intravenous use in children. We performed a phase 1, open-label, multicenter pharmacokinetics (PK) and safety study of oral solithromycin in adolescents.

We enrolled male and female adolescents, aged 12 to 17 years (inclusive), with suspected or confirmed bacterial infections (ClinicalTrials.gov registration number NCT01966055). Adolescents were enrolled and administered solithromycin (capsules) as an add-on therapy (12 mg/kg of body weight on day 1 [800-mg adult maximum] and 6 mg/kg daily on days 2 to 5 [400-mg adult maximum]) for up to 5 days. Solithromycin was taken without regard to food. Written informed consent was obtained from the parent or other legally authorized representative and informed assent from the patient (if age appropriate according to local requirements). All study sites had the protocol reviewed and approved by their institutional review boards. The first adolescent was enrolled on 17 February 2014, and the last adolescent completed the study on 5 September 2014. An independent data monitoring committee (DMC) assessed the overall study status and safety of patients. The DMC met prior to the first patient enrollment, after the first four subjects had completed enrollment, and after the study completion to review the trial data.

Paired plasma and dried blood spot (DBS) PK samples were collected at 0.5 to 1.5, 2 to 4, 8 to 10, and 23 to 24 h after the first and multidose administrations of solithromycin. Samples for both matrices were analyzed for solithromycin by a central laboratory (MicroConstants, San Diego, CA, USA) using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods for both matrices. The accuracy and precision were within the Food and Drug Administration bioanalytical assay validation criteria for both methods (e.g., ±15 to 20%). The solithromycin lower limit of quantitation was 0.01 μg/ml, and the calibration range was 0.01 to 20 μg/ml for both matrices.

A noncompartmental PK analysis was performed with Phoenix WinNonlin (version 6.3; Certara, St. Louis, MO, USA) using solithromycin plasma concentration versus time data. Following the first dose and on days 3 to 5, the maximum concentration (C_{max}) and the area under the concentration versus time curve from 0 to 24 h (AUC_{0-24}) were determined. The AUC_{0-24} was calculated using the trapezoidal method. The solithromycin concentrations in traditional plasma and DBS samples were compared using weighted linear regression, and the overall presence of bias and imprecision was assessed through the calculation of the median percentage prediction error (MPPE) and the median absolute percentage prediction error (MAPE) (2). MPPE and MAPE values of <15% were considered acceptable (3, 4). Also, we repeated the analyses after correcting the DBS concentrations for hematocrit (3).

Thirteen adolescents were enrolled, and all completed the clinical trial. The demographic and clinical laboratory variables are summarized in Table 1. The most frequently reported primary medical conditions were cystic fibrosis (3 [23%]), skin infection (3

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TABLE 1 Adolescent characteristics and study dosing

Variable	Value[a]
Dose (mg)	
Day 1	800 (400–800)
Days 2–5	400 (200–400)
Dose (mg/kg)	
Day 1	12.3 (9.5–13.3)
Days 2–5	6.3 (4.8–6.8)
Age (yr)	16 (12–17)
Weight (kg)	64 (30–84)
Hematocrit (%)	38 (22–45)
Male gender	10 (77)
Race/ethnicity	
White	11 (85)
Non-Hispanic or Latino	10 (77)

[a] Values are median (range) or no. (%).

TABLE 2 Solithromycin exposure in adolescents and historically healthy adult subjects[a]

Day(s)	Parameter	Mean (SD) results for: Adolescents ($n = 13$)[b]	Healthy adults ($n = 5/10$)[c]
1	C_{max} (μg/ml)	0.97 (0.73)	1.32 (0.92)
	$AUC_{0–24}$ (μg · h/ml)	11.62 (8.55)	13.67 (9.56)
3–5	C_{max} (μg/ml)	0.74 (0.61)	1.09 (0.52)
	$AUC_{0–24}$ (μg · h/ml)	9.28 (6.30)	13.27 (7.36)

[a] Data are means (SD).
[b] For the maximum concentration (C_{max}), all subjects contributed data. For the area under the concentration versus time curve from 0 to 24 h ($AUC_{0–24}$), 12 and 10 adolescents contributed data on day 1 and days 3 to 5, respectively.
[c] Day 1 adult estimates were obtained from healthy subjects that received an 800-mg single dose ($n = 5$) (5). The area under the concentration versus time curve (AUC) estimate reported represents AUC from time zero to the last sample time point. The day 3 to 5 adult estimate used for comparison represents observed exposure on day 7 in healthy adults receiving 400 mg/day ($n = 10$) (5).

[23%]), and systemic infection (2 [15%]). One adolescent (8%) received oxcarbazepine, and another adolescent received nafcillin throughout solithromycin treatment.

On day 1, 8 of the 13 adolescents (62%) received an 800-mg loading dose (adult maximum). The median (range) loading dose was 800 mg (400 to 800 mg) or 12.3 mg/kg (9.5 to 13.3 mg/kg). Thereafter, all adolescents received a 400-mg daily maintenance dose except for two patients, who received 200-mg or 300-mg daily doses. The median (range) maintenance dose was 400 mg (200 to 400 mg) or 6.3 mg/kg (4.8 to 6.8 mg/kg). Treatment duration was 3, 4, and 5 days for 46% (6/13), 23% (3/13), and 31% (4/13) of the adolescents, respectively. A total of 118 plasma and 117 DBS samples were collected, of which 96 and 95 samples (both 81%), respectively, had quantifiable solithromycin concentrations; 16 (73%) of the 22 samples with concentrations below the quantification limit were collected from three adolescents. Solithromycin concentration versus time curves are shown in Fig. 1.

Overall, the C_{max}, and $AUC_{0–24}$ values for solithromycin were within the range of the observed values (mean [standard deviation]) in healthy adult subjects (Table 2). Four adolescents in this study had lower than expected day 3 to 5 solithromycin plasma exposures. Two of these adolescents had cystic fibrosis, and one adolescent (without cystic fibrosis) received blood transfusions on the day of the PK sampling. One adolescent had therapeutic exposures following a loading dose, but low exposures after multiple dosing (for both the parent drug and metabolites); a review of this adolescent's medical history and concomitant medications did not provide insight into the cause of this observation.

A total of 92 matched pairs of plasma and DBS sample solithromycin concentrations from 12 adolescents were included in the comparability analysis. The median (range) hematocrit was 38% (22 to 45%). Weighted linear regression showed a linear relationship between the DBS and plasma sample solithromycin concentrations (slope 0.91 [95% confidence interval, 0.82 to 0.99]) (Fig. 2). Similar results were observed using nonparametric regression. The MPPE for the comparison of

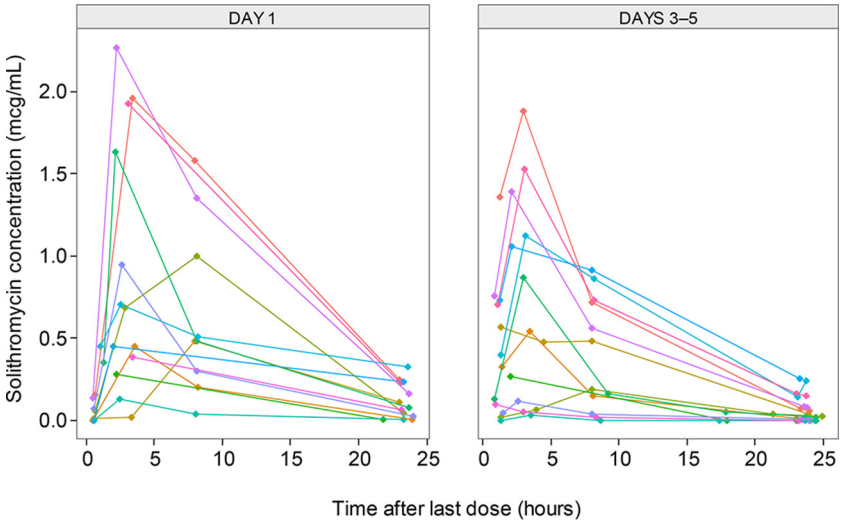

FIG 1 Solithromycin plasma concentration versus time after dose in adolescents. Each line denotes an individual subject concentration versus time curve.

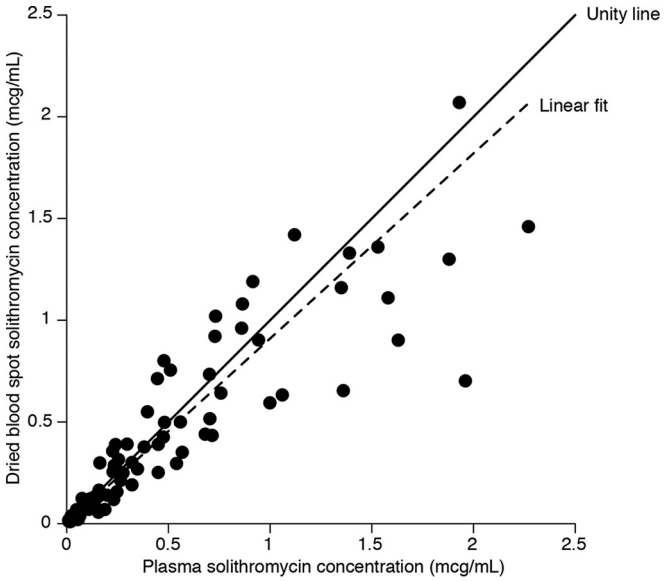

FIG 2 Dried blood spot versus plasma solithromycin concentrations are displayed. The solid and dashed black lines denote the line of unity and linear fit, respectively.

the DBS to plasma sample solithromycin concentrations was −6.9%, and the MAPE was 29.0%; the latter is outside our predefined acceptable cutoff. Correcting for hematocrit did not provide any additional improvement in the agreement between plasma and DBS sample concentrations.

Twelve adverse events were reported in eight adolescents; nine (75%) of these events were unrelated to solithromycin (Table 3). Two separate episodes of mild headache and one episode of increased hepatic transaminases (<3× upper limit of normal) seemed to be related to the study drug in three subjects. All three drug-related adverse events subsided upon discontinuation of solithromycin. The adolescent with increased hepatic transaminases had a medical history of cystic fibrosis and pancreatic insufficiency and received concomitant medications that might potentially alter hepatic transaminases (i.e., azithromycin and cefepime).

In this study, due to impending hospital discharge, ~50% of the adolescents in our study had multiple-dose PK assessments on day 3, which limited the ability to compare these data to adult solithromycin exposures collected in healthy volunteers after at least 5 days of dosing. Despite the early PK sampling (day 3 of therapy), on average, the solithromycin exposures in the adolescents with quantifiable PK data after multiple doses were within the range of exposures observed in these healthy adult volunteers. In adults, exposures in the epithelial lining fluid were approximately 10-fold higher (6), and similar penetration may be seen in adolescents although this was not directly measured. Therefore, these data support the use of a 12-mg/kg loading dose (up to 800 mg) and 6-mg/kg maintenance doses (up to 400 mg) in future safety studies of solithromycin in adolescents.

Notably, the range of solithromycin exposures on day 1 and days 3 to 5 varied substantially between adolescents. This effect is likely multifactorial and might be related to the inherent intersubject variability in drug concentrations characteristic of macrolides (7–9), underlying disease (e.g., cystic fibrosis) (10), concomitant medications (e.g., CYP3A4 inducers and pH modifiers), limitations of our sparse sampling approach, and/or timing of PK sampling (e.g., sampling on day 3 versus day 5). In the three patients with cystic fibrosis, there was a trend toward lower solithromycin exposure with multiple dosing compared with that in patients without cystic fibrosis and with adult values. This finding may be due to the drug absorption limitations of cystic fibrosis (10, 11). Nonetheless, the current sample size limits our ability to make robust conclusions with regard to the comparison between cystic fibrosis and non-cystic fibrosis patients. Another potential confounding variable may have been the concomitant exposure to oxcarbazepine and nafcillin, which are CYP3A4-inducing drugs, in two adolescents. Although clinical data available to evaluate the effect of nafcillin on the PK of CYP3A4 substrates are limited, *in vitro* data suggest that nafcillin may induce the protein expression of CYP3A4 (12).

The solithromycin concentrations in DBS and plasma samples were comparable, albeit with substantial variability, particularly at the low end of the concentration range (see Fig. S1 in the supplemental material). This variability may have resulted from variability in red blood cell partitioning, nonhomogeneous distribution across the blood spot sample, inherent physicochemical properties of the molecule, or sample hematocrit (13). However, accounting for sample hematocrit in our study did not improve agreement between the two matrices. A slope near unity of the DBS to plasma concentration ratio indicates that significant red blood cell partitioning occurs, which is in agreement with previously observed data (~75% whole blood/plasma partitioning based on total radioactivity; sponsor data [Cempra, Inc., Chapel Hill, NC] on file) (14).

We found solithromycin to be well tolerated in a small sample of adolescents. Although we concluded that these three adverse events were related to the study drug, these adolescents were receiving a variety of concomitant medications, which might also account for the adverse events. The favorable safety profile of solithromycin is consistent with that in phase 1 and 2 adult studies, where reports of headache were mild, and mean changes in laboratory parameters were not deemed clinically significant. A hepatic impairment study found no difference in safety relative to healthy adults (15) and reported that no dosage adjustment is needed in patients with mild, moderate, or severe disease. A future phase 2/3 study will be performed to assess the safety of solithro-

TABLE 3 Reported adverse events[a]

Adverse event	No. (%) in all patients (n = 13)
Total no.	8 (61.5)
Serious (limb abscess not related to treatment)	1 (7.7)
Fatal outcome	0
Resulting in permanent treatment discontinuation	0
Related to study treatment	3 (23.1)
Headache (mild severity)	2 (15.4)
Increased transaminases (<3× ULN[a])	1 (7.7)
Severe	0

[a] ULN, upper limit of normal.

mycin in children with community-acquired bacterial pneumonia (CABP).

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REFERENCES

1. **US Centers for Disease Control and Prevention.** 2013. Antibiotic resistance threats in the United States, 2013. http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf. Accessed 19 October, 2015.
2. **Gonzalez D, Melloni C, Poindexter BB, Yogev R, Atz AM, Sullivan JE, Mendley SR, Delmore P, Delinsky A, Zimmerman K, Lewandowski A, Harper B, Lewis KC, Benjamin DK, Jr, Cohen-Wokowiez M, Best Pharmaceuticals for Children Act—Pediatric Trials Network Administrative Core Committee.** 2015. Simultaneous determination of trimethoprim and sulfamethoxazole in dried plasma and urine spots. Bioanalysis **7:**1137–1149. http://dx.doi.org/10.4155/bio.15.38.
3. **Parsons TL, Marzinke MA, Hoang T, Bliven-Sizemore E, Weiner M, Mac Kenzie WR, Dorman SE, Dooley KE.** 2014. Quantification of rifapentine, a potent anti-tuberculosis drug, from dried blood spot samples using liquid chromatographic-tandem mass spectrometric analysis. Antimicrob Agents Chemother **58:**6747–6757. http://dx.doi.org/10.1128/AAC.03607-14.
4. **Ting LS, Villeneuve E, Ensom MH.** 2006. Beyond cyclosporine: a systematic review of limited sampling strategies for other immunosuppressants. Ther Drug Monit **28:**419–430. http://dx.doi.org/10.1097/01.ftd.0000211810.19935.44.
5. **Still JG, Schranz J, Degenhardt TP, Scott D, Fernandes P, Gutierrez MJ, Clark K.** 2011. Pharmacokinetics of solithromycin (CEM-101) after single or multiple oral doses and effects of food on single-dose bioavailability in healthy adult subjects. Antimicrob Agents Chemother **55:**1997–2003. http://dx.doi.org/10.1128/AAC.01429-10.
6. **Rodvold KA, Gotfried MH, Still JG, Clark K, Fernandes P.** 2012. Comparison of plasma, epithelial lining fluid, and alveolar macrophage concentrations of solithromycin (CEM-101) in healthy adult subjects. Antimicrob Agents Chemother **56:**5076–5081. http://dx.doi.org/10.1128/AAC.00766-12.
7. **Sampson MR, Dumitrescu TP, Brouwer KL, Schmith VD.** 2014. Population pharmacokinetics of azithromycin in whole blood, peripheral blood mononuclear cells, and polymorphonuclear cells in healthy adults. CPT Pharmacometrics Syst Pharmacol **3:**e103. http://dx.doi.org/10.1038/psp.2013.80.
8. **Stevens RC, Reed MD, Shenep JL, Baker DK, Foulds G, Luke DR, Blumer JL, Rodman JH.** 1997. Pharmacokinetics of azithromycin after single- and multiple-doses in children. Pharmacotherapy **17:**874–880.
9. **Shi J, Pfister M, Jenkins SG, Chapel S, Barrett JS, Port RE, Howard D.** 2005. Pharmacodynamic analysis of the microbiological efficacy of telithromycin in patients with community-acquired pneumonia. Clin Pharmacokinet **44:**317–329. http://dx.doi.org/10.2165/00003088-200544030-00007.
10. **Rey E, Tréluyer JM, Pons G.** 1998. Drug disposition in cystic fibrosis. Clin Pharmacokinet **35:**313–329. http://dx.doi.org/10.2165/00003088-199835040-00004.
11. **Han K, Capitano B, Bies R, Potoski BA, Husain S, Gilbert S, Paterson DL, McCurry K, Venkataramanan R.** 2010. Bioavailability and population pharmacokinetics of voriconazole in lung transplant recipients. Antimicrob Agents Chemother **54:**4424–4431. http://dx.doi.org/10.1128/AAC.00504-10.
12. **Yasuda K, Ranade A, Venkataramanan R, Strom S, Chupka J, Ekins S, Schuetz E, Bachmann K.** 2008. A comprehensive in vitro and in silico analysis of antibiotics that activate pregnane X receptor and induce CYP3A4 in liver and intestine. Drug Metab Dispos **36:**1689–1697. http://dx.doi.org/10.1124/dmd.108.020701.
13. **O'Mara M, Hudson-Curtis B, Olson K, Yuey Y, Dunn J, Spooner N.** 2011. The effect of hematocrit and punch location on assay bias during

quantitative bioanalysis of dried blood spot samples. Bioanalysis **3:**2335–2347. http://dx.doi.org/10.4155/bio.11.220.

14. **Cohen-Wolkowiez M, Sampson M, Bloom BT, Arrieta A, Wynn JL, Martz K, Harper B, Kearns GL, Capparelli EV, Siegel D, Benjamin DK, Jr, Smith PB, Best Pharmaceuticals for Children Act—Pediatric Trials Network.** 2013. Determining population and developmental pharmacokinetics of metronidazole using plasma and dried blood spot samples from premature infants. Pediatr Infect Dis J **32:**956–961. http://dx.doi.org/10.1097/INF.0b013e3182947cf8.
15. **Jamieson BD, Ciric S, Fernandes P.** 2015. Safety and pharmacokinetics of solithromycin in subjects with hepatic impairment. Antimicrob Agents Chemother **59:**4379–4386. http://dx.doi.org/10.1128/AAC.04652-14.