B-088

Solithromycin for the Treatment of Anthrax

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2015 ICAAC, San Diego, CA
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ABSTRACT

Objective: Solithromycin, a fourth generation macrolide, is in Phase 3 clinical trials for community-acquired bacterial pneumonia (CABP) and is being developed for both oral and intravenous (IV) use. Through an ongoing partnership with BARDA, Cempra is developing SOLI for use in pediatrics, and has evaluated SOLI for use against bioterror threat pathogens. This study evaluated the therapeutic efficacy of solithromycin against a lethal inhalational challenge with *Bacillus anthracis* spores in cynomolgus macaques (CM).

Methods: CMs were challenged with a target dose of 200 *B. anthracis* (Ames strain) LD50 equivalents via aerosol exposure. Solithromycin or vehicle treatment (oral gavage) was initiated on an individual basis following detection of circulating protective antigen and administered once daily for 21 consecutive days. Solithromycin-treated animals were administered a humanized dosing regimen designed to mimic human exposures achieved with the oral CABP regimen (800 mg loading dose, 400 mg maintenance dose). Animals were observed for 36-37 days post-challenge (14 days post-last treatment) and evaluated by blood culture, PA-ECL, body temperature, toxin neutralizing antibody (TNA) and anti-PA IgG ELISA, assessment of bacterial load in the tissues, and histopathology.

Results: All CMs were confirmed bacteremic prior to treatment. All vehicle control-treated animals (7/7) succumbed following aerosol exposure to *B. anthracis*, while survival in the solithromycin group was 83% (10/12). Elevated body temperatures of solithromycin group survivors resolved to baseline levels by Days 4-5 post-challenge. Samples of the liver, brain, spleen, and mesenteric lymph node from all surviving solithromycin-treated animals were negative for *B. anthracis*. Bacteria consistent with *B. anthracis* was observed for 60% (6/10) or 20% (2/10) of the surviving solithromycin-treated animals in the samples of the lung and tracheobronchial lymph node, respectively, but the levels were below the limit of quantitation for all of these animals. By the end of the study (14 days post-last treatment), all survivors had developed positive anti-PA IgG and TNA titers, indicative of an immunologic response in the surviving animals.

Conclusion: Solithromycin is efficacious in the CM pulmonary anthrax therapeutic model.

BACKGROUND

- Solithromycin (SOLI), a 4th generation macrolide and the 1st fluoroketolide, is in Phase 3 clinical development for community-acquired bacterial pneumonia (CABP), and is being developed for both oral (capsule and suspension) and intravenous (IV) use. In addition, Cempra is developing SOLI for the treatment (Tx) of pneumonic disease in individuals exposed to aerosolized biowarfare/bioterrorism (BW/BT) agents.
- *B. anthracis* (BA), the etiologic agent of anthrax, is a gram-positive, rod shaped, aerobic and/or facultative anaerobic, spore-forming bacterium.
- Polyglutamate capsule prevents phagocytosis/killing of the bacterium, allowing vegetative cells to produce 3 key virulence factors: protective antigen (PA), lethal factor (LF), and edema factor (EF).
  - PA and LF combine to produce anthrax lethal toxin (LT)
  - PA and EF combine to produce edema toxin (ET)
- Initial clinical signs and symptoms of anthrax are nonspecific and may include malaise, headache, fever, nausea, and vomiting. These are followed by a sudden onset of respiratory distress with dyspnea, stridor, cyanosis and chest pain. The onset of respiratory distress is followed by shock and often death, with close to 100% mortality (caused by intoxication).
- Delayed onset of disease can occur following removal of therapeutic intervention due to prolonged persistence of BA spores in the lung. Relapse following antibiotic treatment may be due to antibiotics inhibiting germination of spores resulting in an insufficient protective immune response.
- SOLI has been shown to be active against *B. anthracis in vitro* (N=30; MIC range ≤0.008-0.015; $MIC_{50/90}$ ≤0.008)[1], concentrates in macrophages and pulmonary tissues[2], and has anti-inflammatory properties, which enhances its therapeutic potential. The SOLI MIC for the Ames strain is 0.25 µg/mL.
- In this study, SOLI was evaluated in a Tx model of *B. anthracis*-infected CMs using a humanized dose regimen that mimics exposures in humans administered the oral CABP regimen: 800 mg PO on Day 1 followed by 400 mg PO once daily on Days 2-5. This regimen was found to be non-inferior to the standard-of-care comparator moxifloxacin in a Phase 3 CABP trial[3].

METHODS

Study Design							
Tx Group	Test Material	N (1M:1F)	Dose	Treatment Initiation	Dosing Regimen	Tx Duration (d)	End of Study (EOS)
1	solithromycin	12	800/400[a]	PA-ECL+	SID (q24h)	21	14 Days post-last Tx
2	vehicle	8[b]	N/A	PA-ECL+	SID (q24h)	21	14 Days post-last Tx

[a] NHP dose equivalent to human oral CABP regimen; 800 mg loading dose on Day 1 followed by 400 mg once daily
[b] One control animal received a single dose of solithromycin on Tx Day 2 and was therefore excluded from data analyses

Experimental Test System: Naïve Chinese origin CMs (*Macaca fascicularis*). CMs were randomly assigned to one of two aerosol challenge days, then randomized by sex and weight into vehicle or solithromycin Tx groups.

Aerosol Challenge: On Study Day 0, CMs were aerosol-challenged with a targeted 200 LD_{50} dose [1.24 x 107 spores] of *B. anthracis* spores (Ames strain).

Treatment Trigger: Treatments were initiated within 6h of detection of PA in blood (PA-ECL+), a surrogate marker for viable *B. anthracis*.

Treatment Administration: Macaques were administered a humanized solithromycin dose regimen or vehicle (0.5% methylcellulose) by oral gavage once daily for 21 consecutive days

Clinical Monitoring: Cage-side clinical observations were performed every 6 hours from Day 1-9 post-challenge (PC), and twice daily thereafter. CMs were monitored for 14 days following the last Tx for signs of relapse.

Assessments: Blood culture, clinical chemistry and hematology, CRP analysis, bacterial tissue burden, formulation analysis, bioanalysis, population PK analysis, gross necropsy and histopathology on select tissues, IgG ELISA and Toxin Neutralization Assay (TNA).

The TNA assay measures and qualifies the functional ability of serum to neutralize BA lethal toxin activity using an in vitro cytotoxicity assay (J774A.1 cell line).

- Effective Dilution-50 (ED50) is the reciprocal of the serum dilution that results in 50% neutralization of anthrax lethal toxin.
- Neutralization Factor-50 (NF50) is the quotient of the ED50 of the test sample and the ED50 of the reference standard.

[1]Heine HS, et al. Antimicrobial Activity of CEM-101 a New Macrolide, Tested Against Diverse Collections of Bacterial Biowarfare/Bioterrorism Agents. Abstr F1-3980, 48th Annual ICAAC and 46th IDSA, 25-28 October 2008.
[2]Rodvold KA, et al. Comparison of plasma, epithelial lining fluid, and alveolar macrophage concentrations of solithromycin (CEM-101) in healthy adult subjects. Antimicrob. Agents Chemother. 2012;56:5076-5081.
[3]Oldach DW, et al. Oral solithromycin vs oral moxifloxacin for treatment of adult community-acquired bacterial pneumonia (CABP): results of the global phase 3 trial, Solitaire-Oral. Abstr A6424 Int. Conf. Am. Thorac. Soc., 15-20 May 2015.

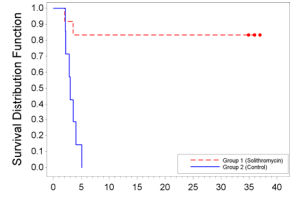
RESULTS

Challenge Doses and Treatment Initiation

Challenge Day	Average Dose (Standard Deviation, SD)
A	167 (34)
B	197 (48)
Treatment Group	**Average Dose (SD)**
1	187 (44)
2	175 (43)

- Average time from challenge until treatment was 45.8h (solithromycin) and 47.8h (vehicle control).
- All animals were confirmed bacteremic by culture and toxemic by PA-ECL prior to treatment.

Time to Death & Survival of *B. anthracis*-infected CMs



- All vehicle control-treated animals (7/7) succumbed within 5 days following aerosol exposure to *B. anthracis*, while survival in the solithromycin group was 83% (10/12).
- Not depicted in Kaplan-Meier curve: there was one CM in the vehicle control group that received solithromycin for the 2nd treatment and was therefore excluded from statistical analysis. This animal survived to EOS.

Tx Group	Number Survived/N	Median Time to Death in Hours Post-Challenge (95% Confidence Interval)	Log-rank test P-value
1	10/12	-- (85.5, --)	0.0004*
2	0/7	72.5 (51.3, 96.7)	

-- Not enough animals died to calculate the estimate.
* Significant at the 0.05 level. [The Log-Rank Test compares time to death and overall survival.]

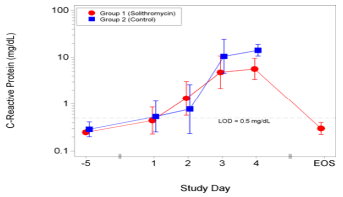
- One solithromycin-treated CM was euthanized 1h 46min after the first dose (48.5h PC) and prior to the anticipated Cmax (~4h). A second solithromycin-treated CM was euthanized after the second dose (85.5h PC).
- There was significant protection in the solithromycin-treated group compared to the control group.

Time from Challenge to Bacteremia

Tx Group	Number Abnormal/N	Kaplan-Meier Median Time from Challenge to Abnormal in Hours (95% Confidence Interval)	Log-Rank test P-value
1	12/12	35.1 (23.3, 40.8)	0.2615
2	7/7	36.3 (28.6, 42.1)	

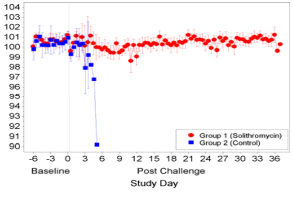
- **Non-survivors**: Eighty-nine percent (8/9) of the terminal samples were positive for *B. anthracis*. The only terminal sample that was negative for *B. anthracis* was from the solithromycin-treated animal that was euthanized ~14h after the second dose.
- **Survivors**: Complete resolution of bacteremia occurred by 7 days post-last treatment (next time point assessed after prior to treatment). All survivors remained negative for bacteremia at the end of study time point.

Group geometric mean CRP with 95% CI



- The elevated CRP levels through Day 4 post-challenge is consistent with inflammation due to infection, and the return to baseline for Group 1 is indicative of recovery.

Group Mean Body Temperature with 95% CI



- Elevated body temperatures of solithromycin group survivors resolved to baseline levels by Days 4-5 post-challenge.

Tissue Bacterial Load

Animal ID	Tx Group	Time to Death (h)	Tissue (cfu/g)					
			Lung	Liver	Brain	Spleen	TLN	MLN
C55796	1: Solithromycin	EOS	0	0	0	0	0	0
C59632		85:30	3.03E+07	2.47E+07	6.52E+05	5.62E+05	4.57E+06	2.82E+06
C64148		EOS	0	0	0	0	0	0
C64150		EOS	+	0	0	0	0	0
C64159		EOS	0	0	0	0	0	0
C64783		48:31	2.65E+08	1.49E+08	2.48E+06	4.48E+08	NR	3.14E+06
C59676		EOS	+	0	0	0	0	0
C60879		EOS	+	0	0	0	0	0
C60880		EOS	+	0	0	0	0	0
C64047		EOS	+	0	0	0	0	0
C64091		EOS	0	0	0	0	+	0
C64136		EOS	+	0	0	0	+	0
C61520	2: Vehicle Control	85:36	1.07E+08	6.38E+07	3.05E+06	2.20E+08	2.61E+07	7.42E+06
C61534		52:45	4.15E+04	1.81E+07	5.63E+05	1.31E+09	4.89E+06	3.18E+06
C64106		96:40	2.76E+05	1.02E+06	3.10E+05	1.12E+06	1.14E+06	8.33E+06
C64163		EOS	+	0	0	0	0	0
C58926		72:30	1.63E+04	1.80E+04	+	8.46E+05	5.00E+04	2.90E+06
C61517		121:59	3.59E+08	1.28E+08	1.72E+06	7.86E+08	1.87E+08	3.99E+07
C61519		51:16	3.29E+08	1.51E+08	3.56E+08	7.04E+08	3.75E+07	1.81E+07
C64140		68:36	6.36E+04	1.62E+04	2.64E+07	2.88E+05	3.06E+04	1.80E+07

+ = Mean *B. anthracis* colony count, or majority of CFU for dilution set (2 of 3 plates) was less than 10.
NR = no result; TLN = Tracheobronchial Lymph Node; MLN = Mesenteric Lymph Node

- **Non-survivors**
  - All animals that died during the study had quantifiable levels in all tissues assessed.
- **Survivors**
  - Samples of the liver, brain, spleen, and mesenteric lymph node from all surviving solithromycin-treated animals were negative for *B. anthracis*. 60% (6/10) and 20% (2/10) of the solithromycin-treated animals were positive for *B. anthracis* in the lung and tracheobronchial lymph node samples, respectively.
  - The one control survivor that received one dose of SOLI was also positive for *B. anthracis* in the lung sample assessed (below LOQ).

Pathology/Histology

- **Non-survivors**: Among animals that died or were euthanized due to moribund condition, all lesions were consistent with acute *B. anthracis* infection.
- **Survivors**: Occasionally, minimal chronic inflammation was present in various organs among animals surviving to study termination.

Toxin Neutralization Assay (TNA)

- None of the animals had a TNA titer prior to aerosol challenge.
- By the end of the study (14 days post-last treatment), all survivors had developed a TNA ED50 and NF50 titer, indicative of an adaptive immune response.

CONCLUSIONS

- Solithromycin corresponding to clinical doses of 800 mg on Day 1 followed by 400 mg once daily and administered by oral gavage (SID) for 21 days protected CMs from death due to *B. anthracis* infection.
- No solithromycin-treated animals relapsed after completion of treatment and all animals developed TNA titers suggesting that solithromycin therapy does not interfere with development of an adaptive immune response.
- Future studies with solithromycin are warranted to determine the minimal dose and duration of treatment necessary to treat anthrax.

We would like to acknowledge the Study Microbiologist (Addie Payne) and Pathologist (Kristin Patton, DVM, PhD), as well as The Institute for Clinical Pharmacodynamics (ICPD) and MicroConstants, Inc. for their contributions to this project. ICPD developed the humanized dose regimen and conducted population PK analysis. MicroConstants conducted formulation analysis and bioanalysis.

The project described herein has been funded by the Biomedical Advanced Research and Development Authority (BARDA), Office of the Assistant Secretary for Preparedness Response, Office of the Secretary, Department of Health and Human Services, under Contract HHSO100201300009C.