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Eravacycline is Efficacious in a *Francisella tularensis*-Infected Cynomolgus Monkey Model

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ABSTRACT

Background: Eravacycline (ERV) is a novel broad-spectrum tetracycline being developed for the treatment of serious Gram-negative and Gram-positive aerobic and anaerobic bacterial infections including biothreat pathogens.

Methods: Cynomolgus monkeys were challenged with a target dose of 1000 F. tularensis (SCHU S4 NR-10492) CFU/animal using a head-only inhalation exposure chamber. Trigger-to-treat was defined as fever persisting for 9 consecutive 15 minute intervals and animals were dosed within 6 hours of the final elevated temperature reading. Humanized doses of 8 and 12 mg/kg/day ERV or 0.9% saline (control) were administered by intravenous infusion over 90 minutes for 21 consecutive days. Blood was collected for hematology, C-reactive protein, bacteremia and pharmacokinetics. Monkeys were observed for clinical signs post-challenge (PC). Necropsy was performed on all animals, including survivors 14 days after the final treatment administration. MIC analyses were done on bacteria from blood or tissue samples.

Results: The average aerosol challenge doses met or exceeded the target. The majority of animals developed a fever 2-3 days PC. As has been reported in human tularemia cases, none of the animals were bacteremic prior to dosing initiation. Control animals died (n=6/8) 9-14 days PC. A fever persisted for the two surviving control animals through ~14 days PC followed by resolution. Five of the 6 control animals that succumbed had a positive terminal blood culture and all 6 had positive tissue cultures. All animals treated with ERV resolved their fever within ~2 days of treatment initiation and remained non-bacteremic throughout the study. All animals receiving 8 mg/kg/day ERV and 7/8 animals that received 12 mg/kg/day ERV survived for the entire dosing/relapse period.

Conclusion: Further studies with ERV are warranted to confirm the efficacy seen in this study and to determine if ERV can be an important empiric therapy for the treatment of respiratory infections by biothreat pathogens.

BACKGROUND

Francisella tularensis, the causative agent of tularemia, is a small aerobic non-motile gram-negative coccobacillus. *F tularensis* subsp. *tularensis,* which includes the SCHU S4 strain, is one of the most infectious pathogenic bacteria known, since inhalation of 10 organisms can cause disease. Inhalation of *F. tularensis* bacteria causes pneumonia, respiratory failure, shock, and ultimately death. *F tularensis* has many characteristics that are suitable for its use as a biological weapon that include high infectivity, ease of growth in large quantities, stability in the environment and in weaponized preparations. *F. tularensis* isolates are resistant to penicillins, cephalosporins, carbapenems, and macrolides.

Eravacycline (ERV) is being developed as a broad-spectrum intravenous and oral antibiotic for use as a first-line empiric monotherapy for the treatment of multidrug-resistant (MDR) Gram-negative and Gram-positive bacteria. ERV is not subject to known tetracycline-specific resistance mechanisms for efflux, ribosomal protection, and inactivation. A successful Phase 2 clinical trial of eravacycline has been completed with IV administration for the treatment of patients with complicated intra-abdominal infections (cIAI) and a Phase 3 trial for the treatment of cIAI is ongoing. A second Phase 3 trial for the evaluation of IV to oral step-down ERV therapy for the treatment of complicated urinary tract infections has also initiated.

The *in vitro* potency of ERV against bacterial biothreats has been determined (Table 1). The degree to which ERV is impacted by well characterized resistance-nodulation-cell-division (RND) nonspecific efflux pumps (i.e., BpeEF-OprC in *Burkholderia pseudomallei*) was significantly lower than that observed with doxycycline and the observed MIC (2 µg/mL) may well be within the therapeutic range.

Table 1. Susceptibility ($MIC_{50/90}$ in µg/mL) of Biothreat Pathogens to Eravacycline and Comparators

Species (Number of Isolates)	MIC_{50} and MIC_{90} Values (µg/ml)						
	Eravacycline	Tetracycline	Penicillin	Ciprofloxacin	Gentamicin	Ceftazidime	Imipenem
***Francisella tularensis* (33)**	**0.12** 0.5	**0.25** 0.5	ND	**0.03** 0.12	**0.25** 0.5	ND	ND
***Yersinia pestis* (34)**	**0.06** 0.12	**1** 2	ND	**0.016** 0.03	**0.5** 1	ND	ND
***Bacilllus anthracis* (35)**	**≤0.016** 0.016	**0.03** 0.03	**0.03** 0.12	**0.03** 0.06	ND	ND	ND
***Burkholderia mallei* (30)**	**0.06** 0.25	**0** 0.12	ND[b]	ND	ND	**2** 4	ND
***Burkholderia pseudomallei* (41)**	**1** 2	**4**[a] 8	ND	ND	ND	**2** 4	**0.5** 2

[a]Doxycycline; [b]ND = not done

ERV was evaluated in a treatment model in *F. tularensis*-infected cynomolgus monkeys using IV dosages equivalent to either 1.5 mg/kg q24h or 1.0 mg/kg q12h that were used and found to be equivalent to the standard-of-care comparator ertapenem in a Phase 2 cIAI trial.

MATERIALS & METHODS

Strain source. *Francisella tularensis* SCHU S4 (NR10492, BEI Resources). All experiments with *F. tularensis* were performed under BSL-3 laboratory conditions.

Experimental test system. A total of 28 (50% male, 50% female) Cambodian origin cynomolgus macaques (*Macaca fascicularis*) were procured from SNBL, a USDA approved facility. Prior to shipment, macaque serum were screened for anti-*F. tularensis* antibodies by tube agglutination assay. Non-human primates (NHPs) were verified as negative for tuberculosis, Simian Immunodeficiency Virus, Simian T-Lymphotrophic Virus-1, *Macacine herpesvirus* 1, and Simian Retroviruses 1 and 2. Animals were also tested for antibodies to *Trypanosoma cruzi* using serum obtained during the quarantine period.

NHPs were quarantined for approximately five weeks after which one TA-D70 telemetry transmitter [Data Sciences International (DSI)] and two VAPs (CATH in CATH 2 ALAT-6; AVA Biomedical) per animal were surgically implanted at JM-10 (one VAP in right jugular vein and one VAP in left jugular vein).

Monkeys were randomized initially by weight (prior to challenge) into one of 3 groups of 8 animals (with each group containing 4 males and 4 females) according to Table 1. Since aerosol challenge could not be accomplished in a single day, animals were randomized to one of two aerosol challenge days and the final randomization was to determine a challenge order per day.

Aerosol Challenge. On Study Day 0, animals were challenged with an average of 1.3x 10^3 cfu *F. tularensis* SCHU S4.

Treatment. Cynos were treated with eravacycline or saline by infusion after aerosol challenge. Treatment was initiated within six hours of a confirmed fever. Animals received a 90 minute IV infusion of eravacycline (either 8 or 12 mg/kg/day) or saline daily for 21 consecutive days. Animals were monitored for 14 days following the last infusion.

Assays. Blood culture, bacterial tissue assessment, hematology, C-reactive protein, MICs

Fever criteria and treatment initiation. Fever was defined as 9, consecutive 15 minute intervals of elevated temperature reading. Animals were treated within 6 hours of the final elevated temperature reading

Clinical Monitoring. Cage-side observations were performed every 8 hours from Day 2-10 post-challenge, twice daily on days 11-end of study. Body weights were measured weekly.

Pathology. Gross necropsy (all animals) and histopathology (animals that died during study)

MIC analysis. All testing of *F. tularensis* and comparators strains was performed in Cation-adjusted Mueller Hinton broth (CAMHB) + 2% IsovitaleX, with pH adjusted to 7.1 following CLSI guidance (see footnote (6) to Table 16 in the Clinical and Laboratory Standards Institute (CLSI) M45-A2 Vol. 30 No. 18, 2010). Antibiotic controls included in each run were tetracycline HCl (Sigma T3383-25G) (8-0.016 µg/ml), ciprofloxacin HCl (Sigma (RTC) PHR1044-1G) (8-0.016 µg/ml), tigecycline hydrate (Sigma PZ0021-5MG) (8-0.016 µg/ml), gentamicin (Sigma G1397-10mL) (8-0.016 µg/ml), and doxycycline hyclate (Sigma, D9891-5G) (8-0.0168 µg/ml). QC strains included in each run were *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853. MICs from each bacterial strain were evaluated in duplicate using two different independently prepared inocula and weighing of each compound. Inocula were verified by serial dilution of inoculum suspensions and plating. Microtiter test plates were incubated at 35 ± 2°C, ambient air for 48 hours prior to reading endpoints.

RESULTS

Table 2. Study Design

Group	Treatment	No. of Animals	Eravacycline Dose (mg/kg/day)	Dosing Concentration (mg/mL)	Dosing Volume (mL/kg)	Treatment Initation[a]	Therapy Duration (IV)	End of study
1	Eravacycline as a 90 min infusion	8 (4M/4F)	12	0.6	20	Temperature Increase	SID for 21 consecutive days (21 total doses)	14 Days after last treatment
2	Eravacycline as a 90 min infusion	8 (4M/4F)	8	0.6	13	Temperature Increase	SID for 21 consecutive days (21 total doses)	14 Days after last treatment
3	Vehicle Control as a 90 min infusion	8 (4M/4F)	N/A	N/A	13	Temperature Increase	SID for 21 consecutive days (21 total doses)	14 Days after last treatment

N/A – Not Applicable
[a] Within 6 hrs of confirmed increase in temperature for each animal

Figure 1. Kaplan-Meier curve illustrates that cynomolgus macaques treated with eravacycline survive *F. tularensis* challenge.

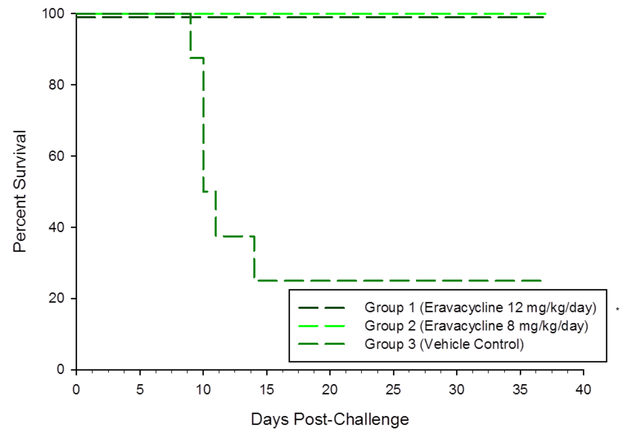


Table 3. Survival of Cynomolgus Monkeys in *F. tularensis* Treatment Model

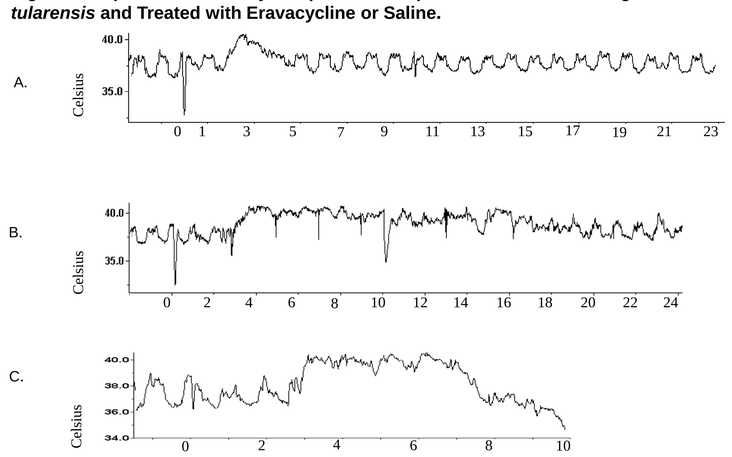
Group	Dose Regimen	Number Assigned to Group	Number of Survivors	Survival Percent
1	Eravacycline 12 mg/kg/day	8	7/7*	100%
2	Eravacycline 8 mg/kg/day	8	8/8	100%
3	Vehicle Control 0.9% saline, pH 6.5	8	2/8	25%

* One animal was moribund secondary to a prolapsed rectum and euthanized. Since this euthanization was not attributed to *F. tularensis* infection or eravacycline treatment failure, the animal was excluded from the survival analysis.

Bacteremic Results

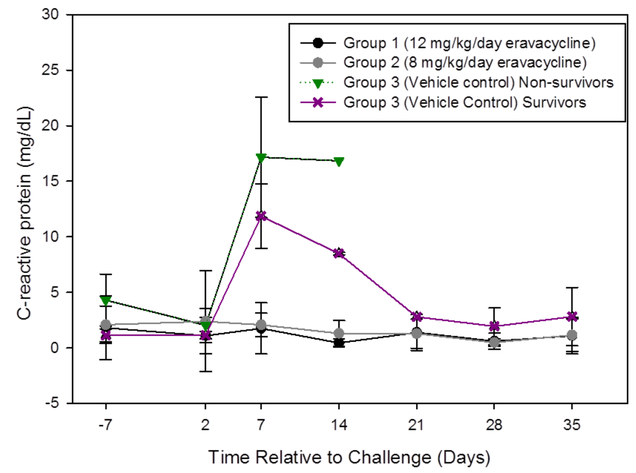
- Eravacycline-treated animals:
  - Blood cultures were negative at all time points during the study
  - Samples of lung, liver, mediastinal lymph node, and spleen collected at the end of the study from all survivors were negative for *F. tularensis*
- Vehicle control-treated animals:
  - Five out of six terminal blood cultures were positive for *F. tularensis*
  - Blood cultures were negative at all time points for the two survivors
  - Samples of lung, liver, mediastinal lymph node, and spleen collected at the time of death were positive for *F. tularensis* (except mediastinal lymph node was negative for one terminal animal)
  - Samples of lung, liver, mediastinal lymph node, and spleen collected at the end of the study from the two control survivors were negative for *F. tularensis*

Figure 2. Representative Body Temperature Graphs for Animals Challenged with *F. tularensis* and Treated with Eravacycline or Saline.



(A) Eravacycline-treated animals developed a fever 1-3 days following aerosol challenge. Fever resolved within 1-2 days of treatment initiation and returned to diurnal rhythm. All animals survived to the end of the study, except for one animal that was euthanized; death not attributed to F. tularensis infection or eravacycline treatment. (B) The two saline-control surviving animals developed a fever 3 days following aerosol challenge. These animals continued to exhibit body temperature readings above the temperature threshold for fever (and no diurnal rhythm) while receiving daily saline infusions. Approximately 3 weeks post-challenge, the fevers resolved and diurnal rhythm returned. These control animals survived to the end of the study. (C) The six saline-control non-surviving animals developed a fever 2-3 days following aerosol challenge. These animals continued to exhibit fevers through for 6-8 days following challenge and then the body temperatures declined until the animals succumbed to infection on Days 9-14 post-challenge.

Figure 3. Group average kinetics of C-reactive protein (CRP) for animals challenged with *F. tularensis* and then treated with eravacycline or saline.



While CRP levels remain relatively constant for the eravacycline-treated animals, the CRP levels greatly increase by Day 7 post-challenge for the vehicle control group (survivors and non-survivors). The CRP levels for the two vehicle control survivors decreases after Day 7 and returns close to baseline values by Day 21 post-challenge. The limit of detection is 0.5 mg/dL. Group average with standard deviation is shown.

Table 4. MICs[a] of *F. tularensis* Isolates from Blood and Tissue Samples[b]

Animal Number	Description	MICs (µg/ml)					
		Eravacycline	Tetracycline	Ciprofloxacin	Tigecycline	Gentamicin	Doxycycline
NR-10492	Challenge Strain	0.125	2	0.016	2	0.06	1
101814-01LU	Female Control	0.06	0.5	0.016	0.25	0.016	0.125
101814-01L	Female Control	0.125	1	0.016	0.25	0.06	0.25
101814-01N	Female Control	0.031	0.25	0.016	0.06	0.031	0.25
101814-01S	Female Control	0.06	1	0.031	0.5	0.06	0.5
101814-02LU	Female Control	0.06	0.5	0.016	0.125	0.016	0.25
101814-02L	Female Control	0.125	1	0.031	0.25	0.125	1
101814-02N	Female Control	0.125	1	0.016	0.25	0.06	0.5
101814-02S	Female Control	0.125	1	0.031	0.25	0.125	1
101814-03LU	Female Control	0.125	1	0.016	0.25	0.06	0.5
101814-03L	Female Control	0.06	1	0.016	0.5	0.06	0.5
101814-03N	Female Control	0.125	1	0.016	0.25	0.125	0.5
101814-03S	Female Control	0.25	0.5	0.016	0.25	0.06	0.5
110797-01	Female Control	0.031	0.25	0.016	0.06	0.016	0.25
110797-02	Female Control	0.031	0.25	0.031	0.06	0.031	0.25
110804-01	Female Control	0.031	0.5	0.016	0.125	0.06	0.25
110804-02	Female Control	0.031	0.5	0.016	0.125	0.031	0.5
110804-03	Female Control	0.125	0.5	0.016	0.25	0.016	0.125
130051-01	Male Control	0.06	0.5	0.016	0.25	0.06	0.5
130051-02	Male Control	0.06	0.5	0.016	0.25	0.06	0.5
130063-01	Male Control	0.06	0.25	0.016	0.125	0.016	0.125
130063-02	Male Control	0.06	0.25	0.016	0.125	0.016	0.125
130063-03	Male Control	0.06	0.5	0.016	0.25	0.031	0.5
130068-01	Male Control	0.25	1	0.016	0.5	0.06	0.5
130068-02	Male Control	0.125	0.5	0.016	0.25	0.031	1
130068-03	Male Control	0.06	0.5	0.016	0.25	0.06	0.5

[a] MICs were determined from 4 evaluations (duplicates of two different compound weighings), with the reported MIC determined according to the following rules:
- when there was more than two replicate values, the most frequent value (the mode) was selected
- when there was more than two replicate values, and all values are different, the value closest to the middle (the median) was selected

[b] Isolate numbers containing LU, L, N or S were obtained from lung, liver, mediastinal lymph node or spleen, respectively

CONCLUSIONS

- Eravacycline administered at dosages corresponding to clinical doses of 1.5 mg/kg q24h or 2 mg/kg q12h protected all cynomolgus monkeys from death due to *F. tularensis* infection.
- *F. tularensis* was isolated from six of the eight control animals post-mortem and no isolate had increased MICs to any antibiotic tested.
- Significant increase in fever and loss of diurnal pattern did not always foreshadow death in *F. tularensis*-infected cynomolgus monkey.
- CRP levels were not always predictive of mortality in *F. tularensis*-infected cynomolgus monkeys.
- Further studies with eravacycline are warranted to confirm the efficacy seen in this study and to determine if eravacycline can be an important empiric therapy for the treatment of respiratory infections by biothreat pathogens.

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