P0109

24th ECCMID
10-13 May, 2014
Barcelona, Spain

Eravacycline Protects in a *Bacillus anthracis*-Infected New Zealand White Rabbit Treatment Model

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Abstract

Objectives: 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 those by biothreat pathogens. ERV was previously shown to be efficacious in a phase 2 complicated intra-abdominal infection trial and is currently in phase 3 clinical trials. This study in New Zealand white (NZW) rabbits challenged with lethal doses of *Bacillus anthracis* was performed to determine the efficacy of ERV in a biothreat treatment model.
Methods: NZW rabbits were challenged with a target dose of 200 LD_{50} (2 x 10^7 cfu *B. anthracis* Ames spores/animal) in a plethysmography exposure chamber. Trigger-to-treat was defined as fever persisting for 3 consecutive hourly intervals or serum-positive for protective antigen (PA). Animals were dosed within 6 hours of the final elevated temperature reading or positive PA-ECL response (an electrochemiluminescence assay to detect PA). Humanized dose regimens of 0.8 and 1.6 mg/kg/day ERV or 0.9% saline (control) were administered intravenously over 1-2 minutes daily for 28 consecutive days. Blood was collected for hematology, C-reactive protein, toxin neutralization assay, bacteremia and pharmacokinetics. Rabbits were observed for clinical signs post-challenge (PC). Necropsy was performed on all animals, including survivors 28 days after the final treatment administration, and included histopathological evaluations of select organs and bacterial burdens in the lung, brain, spleen and cerebrospinal fluid.
Results: The average aerosol challenge doses exceeded the target, delivering an average of 270 LD_{50} per animal. All but one animal (23/24) developed a fever by 42 hours PC, and all but one animal was bacteremic prior to dosing initiation. Control animals (n= 2F/2M) died 4-5 days PC. All animals treated with ERV (n = 5F/5M per dose group) resolved their fever within ~2 days of treatment initiation and all survived, remaining non-bacteremic throughout the 28-day treatment and 28 day post-treatment periods.
Conclusions: ERV administered in doses that produced equivalent exposures to those used in clinical trials was 100% effective at protecting NZW rabbits from the lethal effects of pneumonic anthrax, preventing any relapse. 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 caused by biothreat pathogens.

Background

Bacillus anthracis, the etiologic agent of anthrax, is a Gram-positive, rod-shaped, aerobic and/or facultative anaerobic, spore-forming bacterium that can cause human disease via the gastrointestinal, cutaneous, or inhalation (pulmonary) routes with different clinical manifestations of disease, pulmonary being the most lethal. The incubation period usually varies from 12 hr to 5 or more days depending upon the dose received and the route of infection. Following an anthrax inhalation exposure, the initial human clinical signs and symptoms 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, leading to shock and death with close to 100% mortality. The ability to generate high titer anthrax spores using basic microbiological techniques combined with the ability of these spores to be disseminated by aerosolization combine to produce an effective biological weapon. Following the 2001 civilian attacks, efforts to develop and license the use of medical countermeasures for therapeutic and post-exposure prophylaxis were increased. This study was designed to assess the therapeutic efficacy of the novel antibiotic eravacycline for the treatment of inhalational anthrax in the NZW rabbit anthrax model. Eravacycline was administered intravenously once a day for 28 consecutive days.

Materials and Methods

Experimental test system. New Zealand white rabbits (*Oryctolagus cuniculus*) surgically implanted with two vascular access ports for dosing (femoral vein) and collecting blood samples (jugular vein) were randomized and challenged by head-only aerosol with a targeted dose of 200 LD_{50} *B. anthracis* Ames spores in a plethysmography chamber. There were 3 dose groups (DG): DG1 (n = 5M/5F), 1.6 mg/kg/day of eravacycline; DG2 (n = 5M/5F), 0.8 mg/kg/day eravacycline; DG3 (n = 2M/2F), vehicle (0.09% saline). All doses were administered IV using cGMP-reconstituted eravacycline within 6 hours following the first of either a significant increase in body temperature (SIBT) or confirmation of protective antigen (PA) in circulation by electrochemiluminescence (ECL). All animals were treated for 28 consecutive days or until death. All survivors were monitored for 28 days following the last dose. All study animals received a complete necropsy with tissue collection. Target tissues including meninges, sternal bone marrow, thymus, GI tract (stomach, duodenum, jejunum, ileum, cecum, colon, rectum), lymph nodes (mesenteric, mediastinal, and submandibular), brain, lungs, liver and spleen along with gross lesions were evaluated. All experiments with *Bacillus anthracis* were performed under BSL-3 laboratory conditions.

Dosage of eravacycline. Eravacycline displays atypical nonlinear plasma protein binding in all species[1]. *f*AUC/MIC is calculated from the fraction of free drug and the total AUC and is the key PK/PD parameter for eravacycline[2]. Target *f*AUC/MIC values were converted into respective daily doses by the equation:

Daily Dose (mg/kg) = Target (fAUC/MIC) x MIC x CLu, where CLu is the unbound clearance that can be calculated as dose/fAUC.

The MIC of eravacycline against *B. anthracis* Ames is 0.008 µg/ml. From phase 2 trials in the treatment of complicated intra-abdominal infections[3], the clinically effective doses of eravacycline are 1.5 mg/kg/day and 2 mg/kg/day. The equivalent dosages of eravacycline in rabbit that were predicted to give the same *f*AUC/MIC ratios achieved in humans were 1.3 and 1.8 mg/kg/day respectively. Dosages of 0.8 and 1.6 mg/kg/day were chosen for the efficacy experiment in NZW rabbits.

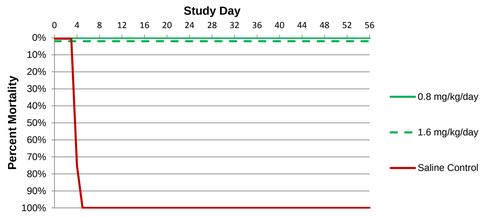
Fever criteria and treatment initiation. Each animal had two temperature transponder chips injected subcutaneously while under sedation. A threshold for elevated temperature (critical temperature threshold) was calculated for each animal (equaling the baseline average for each animal plus two times the standard deviation). An SIBT was defined as the third consecutive hourly reading, or the second occurrence of two consecutive hourly readings, above the critical temperature threshold. Alternatively, an increase in PA by ECL was used as a trigger.

Blood collection. Blood was collected to assess bacteremia, hematology, C-reactive protein (CRP), and levels of circulating PA 7 days before challenge, 24, 30, 36, 42, and 48 ± 1 hours post-challenge and within 30 minutes of the first treatment administration. Bacteremia was monitored for Days 1, 2, 3, 7, 14, and 21 post-treatment initiation and on Day 28 post-challenge; hematology and CRP collections were taken weekly. Samples for toxin neutralization assay (TNA) analysis by suppression of *in vitro* cytotoxicity[4] were collected on Day -7, Day 21 post-first treatment and Days 14, 28, 36, 42, and 56 post-challenge. TNA ED_{50} is the reciprocal of the dilution of a serum sample that results in 50% neutralization of anthrax lethal toxin and NF_{50} is the quotient of the ED_{50} of the test sample and the ED_{50} of the reference standard. Additional samples were taken post-last treatment (24 and 72 hrs) and post-challenge (35, 42 and 56 days) to determine if bacteria were present.

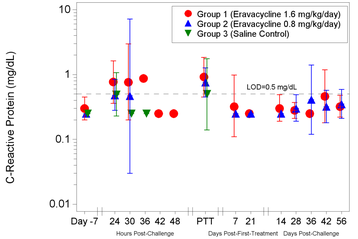
Bacteremia. Approximately 200 µL of each sample was spread plated onto blood agar and incubated for a minimum of 48 hours at 37±2°C and assessed for the presence of colonies consistent with *B. anthracis* morphology.

Results

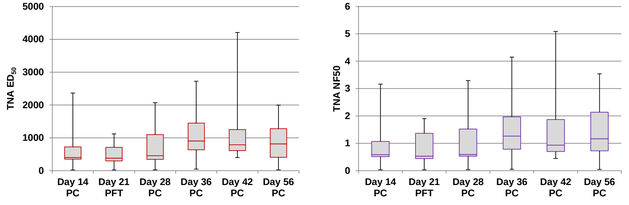
Eravacycline is 100% Efficacious in Treating *B. anthracis*-infected Rabbits



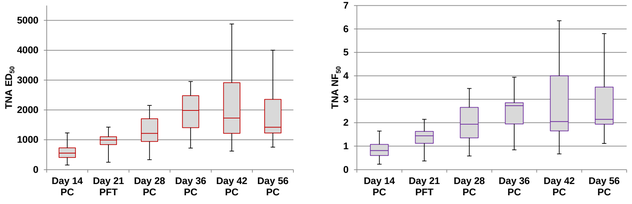
Plot of Mean CRP Levels Over Time (with 95%Confidence Intervals) by Group



TNA ED_{50} and NF50 Levels Over Time in Monkeys Administered 0.8 mg/kg/day Eravacycline



TNA ED_{50} and NF50 Levels Over Time in Monkeys Administered 1.6 mg/kg/day Eravacycline



The median is the middle line. The lower bar represents the difference between the median and the first quartile while the upper bar is the difference between the third quartile and the median. The whiskers represent the minimum and maximum values observed.

Other Results

- All except one rabbit was bacteremic prior to treatment and 23/24 rabbits started treatment by 42 hours post-challenge (71% triggered by ↑ temperature; 29% by ↑ PA)
- Rabbits treated with either dose of eravacycline had fever resolution within 48 hours of first dose and remained free of *B. anthracis* in blood or tissue throughout the 28-day treatment and 28 day post-treatment periods with no abnormal autopsy findings
- All rabbits in the eravacycline treatment groups developed neutralizing immune responses to anthrax lethal toxin demonstrating that all were infected at the time of treatment

Conclusions

- Eravacycline at dosages equivalent to free exposures seen in human subjects receiving either 1.5 mg/kg/day or 2.0 mg/kg/day successfully treated 100% of New Zealand white rabbits infected by *B. anthracis* spores via the aerosol route while all rabbits receiving vehicle control succumbed to anthrax 4-5 days after exposure.
- Eravacycline has been shown to be 100% effective as a treatment in two biothreat pathogen respiratory models: *Francisella tularensis*-infected non-human primates[5] and *B. anthracis*-infected New Zealand white rabbits (this poster).
- Eravacycline continues to show promise as a broad-spectrum countermeasure that could be used in the event of a bioterrorist attack, in addition to its use in treatment of serious hospital infections

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This project has been funded in whole or in part with Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, Department of Health and Human Services, under Contract No HHSO100201200002C.