Bioorganic & Medicinal Chemistry 24 (2016) 6429–6439

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Plazomicin is effective in a non-human primate pneumonic plague model

William M. Mega [a], Melanie Doyle-Eisele [a], Robert T. Cass [b,†], Corwin F. Kostrub [b,†], Robert L. Sherwood [a], Matthew A. Metz [b,‡], Ryan T. Cirz [b,*,‡]

[a] Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr. SE, Albuquerque, NM 87108, United States
[b] Former or Current Employees of Achaogen Inc., 7000 Shoreline Ct., South San Francisco, CA 94080, United States

ARTICLE INFO

Article history:
Received 15 April 2016
Revised 17 August 2016
Accepted 26 August 2016
Available online 27 August 2016

Keywords:
Plazomicin
Aminoglycoside
Yersinia pestis
Plague
Pneumonia
Animal rule

ABSTRACT

The efficacy of plazomicin for pneumonic plague was evaluated in a non-human primate model. African Green monkeys challenged with a lethal aerosol of *Yersinia pestis* [median (range) of 98 (15–331) LD_{50s}] received placebo ($n = 12$) or 'humanized' dose regimens (6.25, 12.5 or 25 mg/kg every 24 h) of plazomicin ($n = 52$) after the onset of fever for a duration of 5 or 10 days. All animals treated with placebo died, while 36 plazomicin-treated animals survived through study end. The majority (33/36) were either in the 10-day (high-/mid-/low-dose) or 5-day high-dose groups. The findings suggest an exposure range of plazomicin for treatment of pneumonic/bacteremic *Y. pestis* infection in humans.

1. Introduction

Plazomicin (plazomicin sulfate) is a novel compound in the aminoglycoside class of antibiotics that is being developed for the treatment of serious Gram-negative infections. Plazomicin contains structural modifications that allow it to maintain activity in the presence of the common aminoglycoside-modifying enzymes (AMEs) that inactivate currently marketed aminoglycosides[1] (Fig. 1). Furthermore, plazomicin has demonstrated in vitro activity against clinical isolates that possess a broad range of resistance mechanisms, including beta-lactamases and fluoroquinolone target site mutations that limit the utility of other classes of antibiotics. At the time of this publication, plazomicin is being studied in two phase 3 clinical trials (NCT01970371 and NCT02486627), one of which is specifically focused on serious infections due to carbapenem-resistant Enterobacteriaceae (CRE), a family of multidrug resistant (MDR) pathogens that has been identified as an urgent health need.[2]

In partnership with the Biomedical Advanced Research and Development Authority (BARDA), plazomicin is also being investigated for the treatment of two diseases caused by potential bioterrorism agents, pneumonic Tularemia, caused by *Franciscella tularensis*, and pneumonic plague, caused by *Yersinia pestis*. In this article, we describe the evaluation of plazomicin in a primate model of pneumonic plague.

Y. pestis is considered to be one of the most threatening bioweapons due to the virulence of the bacterium, the availability of virulent strains in natural environmental reservoirs, the ease of preparation of aerosols and the rapid onset of symptoms and death associated with primary pneumonic plague.[3] Also, unlike diseases such as anthrax, pneumonic plague has a greater potential to spread from person-to-person.[3,4] While a handful of plague cases are reported annually in the United States, they are predominantly the bubonic form, which is generally caused by contact with an infected animal.[5] The severity of disease and high associated mortality of primary pneumonic plague is not adequately captured in these bubonic plague patients. The sparse case reports of primary pneumonic plague that are available in the literature indicate that it is a rapidly progressing and most often lethal disease, even with antibiotic treatment.[3] While it is fortunate that the incidence of pneumonic plague is low, there is a critical need for new therapies in the event of an attack with weaponized, aerosolized plague.

Levofloxacin and moxifloxacin are the only two FDA-approved antibiotics for the treatment of primary pneumonic plague. Both were recently approved (2012 and 2015 respectively) using an animal model similar to the one described in this study.[6,7] While

* Corresponding author. Tel.: +1 650 800 3636; fax: +1 650 434 3765.
E-mail address: rcirz@achaogen.com (R.T. Cirz).
† Former employee.
‡ Current employee.

http://dx.doi.org/10.1016/j.bmc.2016.08.049
0968-0896/

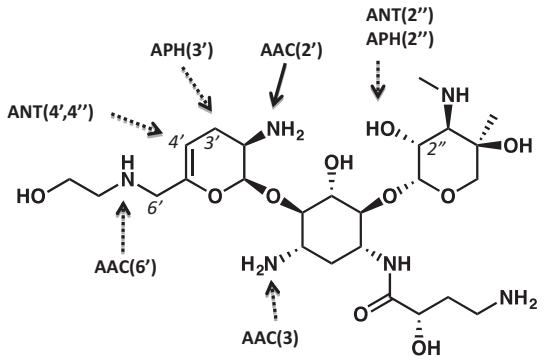

Figure 1. Plazomicin structure and AME families from Gram-negative and Gram-positive organisms. AME families shown with dotted arrows cannot modify plazomicin. AAC, aminoglycoside acetyltransferase; ANT, aminoglycoside nucleotidyltransferase; APH, aminoglycoside phosphotransferase.

it is encouraging that two agents are now approved for treatment, both are members of the fluoroquinolone class of antibiotics and therefore share common resistance mechanisms. In the event that a fluoroquinolone-resistant strain was disseminated, either naturally or intentionally, the efficacy of both approved drugs would be compromised. The ideal armamentarium for any infectious agent would consist of several drugs possessing different modes of action. Plazomicin is an aminoglycoside antibiotic that targets the bacterial ribosome and therefore has a different cellular target and mechanism of bacterial killing than the fluoroquinolones. Plazomicin has also been chemically modified to maintain activity in the presence of many of the AMEs that inactivate older aminoglycosides (Fig. 1).[1] Based on its differential mode of action compared with the fluoroquinolones and its ability to kill strains resistant to other members of the aminoglycoside class, plazomicin would significantly add to our armamentarium against *Y. pestis*.

The United States Food and Drug Administration's (FDA) 'animal rule' enables a path to regulatory approval for new therapies directed at rare infectious agents by establishing efficacy in animals when the study drug cannot be feasibly/ethically tested in humans.[8] Therefore this study aimed to test the hypothesis that plazomicin could improve the survival of non-human primates after a lethal aerosol dose of *Y. pestis* strain Colorado 92.[9] The African Green monkey (AGM) was chosen as the test system predominately due to historical precedent. AGMs are highly susceptible to *Y. pestis* aerosol infection and the timing and evolution of disseminated disease following aerosol exposure is similar to humans.[10] AGM was the primate species utilized in the levofloxacin and moxifloxacin studies to support approval,[6,7] and the FDA advisory committee from the levofloxacin approval unanimously agreed the model is able to 'provide substantial evidence of efficacy. . .for the treatment of humans with pneumonic plague'.[11,12]

In addition, an important feature of this study is that treatment with plazomicin was delayed until the disease advanced from a primary pneumonia to a systemic disease hallmarked by spread of *Y. pestis* from the lungs into the bloodstream and surrounding organs, a process that takes between 2 and 3 days. In the event of an outbreak or intentional release of aerosolized plague, it is likely that many exposed individuals will not present to a medical facility for treatment until they exhibit clear signs and symptoms of infection. We therefore sought to evaluate the efficacy of plazomicin under these more challenging conditions in which the disease has already progressed.

The current phase 3 plazomicin studies are using a once-daily 15 mg/kg dose, administered as a 30-min intravenous (IV) infusion, with dose adjustments required for patients with compromised renal function.[13] Based on a population PK model generated from healthy volunteer data and data collected in patients with complicated urinary tract infections or acute pyelonephritis, the mean daily steady state area under the curve (AUC_{24}) associated with this dose is 262 mg/L × h.[14] We sought to determine if this AUC_{24} was sufficient to treat pneumonic plague in AGMs.

We also investigated the impact of duration of plazomicin therapy on outcome. There are very few antibiotic studies that examine the optimal duration of therapy and, as a result, one of the biggest knowledge gaps in the antibacterial field is a clear understanding of the importance of treatment duration on outcome.[15–17] The AGM study provides an opportunity to empirically examine the impact of treatment duration on outcomes in otherwise healthy animals with a life-threatening, multi-organ infection with a large bacterial burden when therapy is initiated, akin to the disease state in critically ill patients with pneumonia and associated bacteremia/sepsis.

2. Material and methods

2.1. Overview of plague study design

Plazomicin was evaluated in three separate studies referred to below as FY-105A, FY-105B and FY-036A. These studies were similar in design but each explored different aspects of plazomicin dosing and duration of therapy. Due to animal handling limitations, each study was separated into two exposure cohorts. In total, 64 animals were evaluated across the six cohorts. A placebo group was included in each cohort and all attempts were made to ensure that each cohort was handled identically. Briefly, AGMs were anesthetized and exposed, head only, via aerosol to lethal doses of *Y. pestis*. Treatment was delayed until the disease had progressed from an isolated pneumonia to a multi-organ, systemic disease marked by spreading of the bacteria through the bloodstream to other body sites and the occurrence of sustained fever. Since our intent was to treat animals when they were bacteremic, and knowing that the presence of bacteremia takes >24 h to confirm, we required a surrogate marker. It had previously been established that the systemic phase of disease is associated with fever.[10] Therefore, animals were continuously monitored for development of fever via implanted telemetry. Treatment with placebo or 'humanized' doses of plazomicin (6.25, 12.5 or 25 mg/kg 30-min infusion every 24 h) was initiated within 6 h of onset of sustained fever and was continued for 5 or 10 days, depending on the study cohort. The total observation period post-aerosol challenge was between 28 and 30 days (Fig. 2). Blood samples were taken to evaluate plazomicin PK on the first, third and last day of treatment and compared with results from healthy animals. In addition, blood was sampled pre-challenge and at time of death or study end, to evaluate serum chemistry, hematology and quantitative bacteriology. Upon death, euthanasia due to meeting moribund criteria, or euthanasia due to study end, histopathological examination of multiple organs and determination of bacterial burden were performed.

2.2. Animal assurance and procedures

All studies complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR Parts 1, 2 and 3) and the Guide for the Care and Use of Laboratory Animals (8th Ed., 2010, National Academies Press, Washington, DC). All in-life portions of the studies were performed in Lovelace Respiratory Research Institutes (LRRI) facilities, which are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).