Journal of Pharmaceutical Sciences xxx (2016) 1-10

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Nanotechnology

Formulation and Characterization of Nanocluster Ceftazidime for the Treatment of Acute Pulmonary Melioidosis

Sara I. Ruiz [1, 2], Nashwa El-Gendy [3], Larry E. Bowen [1, 2, 4], Cory Berkland [3, 5], Mark M. Bailey [1, *]

[1] Aerobiology, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702
[2] Aerobiology, Ke'aki Technologies, Frederick, Maryland 21704
[3] Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, Kansas 66047
[4] Aerobiology, Clinical Research Management, Frederick, Maryland 21701
[5] Department of Chemical & Petroleum Engineering, The University of Kansas, Lawrence, Kansas 66045

ARTICLE INFO

Article history:
Received 28 March 2016
Revised 28 July 2016
Accepted 29 July 2016

Keywords:
NanoCluster
ceftazidime
pulmonary melioidosis
aerosols
dry powder

ABSTRACT

Melioidosis is an infectious disease caused by *Burkholderia pseudomallei*. The disease is responsible for a high proportion of human pneumonia and fatal bacteremia in the endemic areas of the world and is highly resistant to most commonly available antibiotics. Studies have shown that prophylactic antibiotic treatment, when administered 24 h following bacterial challenge, can prevent infection in a murine model. Prophylactic treatment against this disease using a pulmonary antibiotic formulation has not previously been examined, but may reduce the number of treatments required, allow for the delivery of higher doses, eliminate the need for intravenous administration, and help to minimize systemic side effects. Ceftazidime was formulated as a dry powder aerosol suitable for pulmonary delivery using previously developed NanoCluster dry powder technology. Pharmacokinetics of aerosolized ceftazidime was analyzed in a mouse model. This study demonstrates that ceftazidime can be formulated using NanoCluster technology as a dry powder aerosol suitable for pulmonary delivery to humans. We have also demonstrated the retention of nebulized ceftazidime in mouse lungs for up to 6 h after exposure. The results indicate that this treatment may be useful as a prophylactic treatment against melioidosis. Future work will examine the efficacy of this treatment against *B. pseudomallei* aerosol challenge.

Introduction

Melioidosis is an infectious disease caused by the Gram-negative, motile, non−spore-forming bacillus, *Burkholderia pseudomallei*. The bacterium is responsible for a high proportion of human pneumonia and fatal bacteremia in the endemic areas of Southeast Asia and Northern Australia. The disease has a variable and inconsistent clinical presentation, but typically manifests as a febrile illness with an acute pulmonary infection, acute fulminant septicemia, or chronic suppurative infection.[1] Pneumonia can occur after inhalation or after hematogenous dissemination of infection to the lungs.[2] Overall mortality is around 40%-50% in endemic regions.[1] *B. pseudomallei* is transmitted through exposure to contaminated soil or water by the percutaneous or respiratory route. This pathogen is a category B potential bioterrorism agent and would likely be disseminated *via* the aerosol route if deployed in this capacity.[3,4]

Current treatment regimens for melioidosis consist of an intravenous (IV) intensive phase, followed by an eradication phase with an oral antibiotic.[1,5] The parenteral treatment phase consists of 40-mg/kg IV injection of ceftazidime every 8 h, followed by a continuous infusion of 3- to 5-mg/kg/h ceftazidime; or a 20-mg/kg IV injection of imipenem every 8 h. This phase continues for at least 10 days. The eradication phase consists of a combination therapy consisting of chloramphenicol (40 mg/kg per day in 4 divided

Abbreviations used: NC, NanoCluster; SSM, small-scale milling; LSM, large-scale milling; DLS, dynamic light scattering; DPI, dry powder inhaler; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; PXRD, powder X-ray diffraction; FSI, fast screening impactor; DUSA, dose unit sampling apparatus; ACI, Anderson cascade impactor.

Disclaimer: Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army or the Department of Defense.

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2016.07.029.

* *Correspondence to:* Mark M. Bailey (Telephone: 301-619-4499; Fax: 301-619-0347).
E-mail address: mark.m.bailey2.mil@mail.mil (M.M. Bailey).

http://dx.doi.org/10.1016/j.xphs.2016.07.029
0022-3549/Published by Elsevier Inc. on behalf of the American Pharmacists Association.

doses), doxycycline (4 mg/kg per day in 2 divided doses), and trimethoprim-sulfamethoxazole (10 mg/kg and 50 mg/kg per day, respectively, in 2 divided doses). Treatment is continued for at least 20 weeks. Additionally, studies have shown that prophylactic antibiotic treatment, when administered 24 h following bacterial challenge, can prevent infection in a murine model. Sivalingam et al.[6] demonstrated the high efficacy of oral doxycycline (60% survival) and co-trimoxazole (100% survival) after 20 days when mice were treated 24 h post–*B. pseudomallei* challenge. There is currently no effective vaccine against *B. pseudomallei*, underscoring the necessity of optimizing antibiotic therapy.

Delivering effective drugs *via* the respiratory route is ideal as it mirrors the inhalation exposure route and allows for direct deposition in high concentrations to the initial site of replication. Local antibiotic concentration in the lungs after inhalation can be many times higher than after IV administration and can achieve a faster onset of action.[7] Additionally, drugs can be selected that would have a long residence time in the lungs based on their physicochemical properties. This would reduce the number of treatments required, allow for the delivery of higher doses, eliminate the need for IV administration, and help to minimize systemic side effects.

To our knowledge, prophylactic treatment against an aerosolized *B. pseudomallei* challenge using a pulmonary antibiotic formulation has not been examined. Several antibiotics have been successfully formulated for pulmonary delivery to treat comorbid infection in cystic fibrosis patients and are currently in various phases of clinical development or are currently available to patients. Examples include tobramycin (TOBI® and BETHKIS®), aztreonam [Cayston®], levofloxacin [phase 3], vancomycin [phase 3], and gallium [phase 2].[8] Here, ceftazidime was engineered as a pulmonary formulation using the previously developed NanoCluster (NC) aerosol dry powder technology.[9] This technique produces a drug-nanoparticle colloid, which is thermodynamically driven into flocculates of drug nanoparticles. The aerodynamic sizes of these flocculates can be customized for optimal delivery into different regions of the lungs (e.g., alveolar, bronchial). This particle engineering technology has been successfully used to formulate locally acting and systemic therapeutics, as well as contrast agents for lung imaging applications.[10-20] Delivering dry powders to a small animal, such as a mouse, can be technically challenging and is prone to high levels of statistical error; thus, a nebulized liquid formulation was used in this study to estimate the pulmonary retention time of ceftazidime, which can be used in future studies to estimate appropriate dosing schedules of dry powder.

Experimental Section

Materials

Ceftazidime pentahydrate was purchased from Bosche Scientific LLC, New Brunswick, NJ. Ethanol, acetone, n-propanol, and tert-butanol were purchased through Fisher Scientific, Fair Lawn, NJ. Double-distilled water was used throughout the study, provided by an EASYpure® RODI (Barnstead International, Model #D13321, Dubuque, Iowa). For pharmacokinetic studies, ceftazidime pentahydrate (USP) and cephalexin hydrate (USP) were purchased from Sigma Aldrich, St. Louis, MO.

Formulation and Characterization

NC Dry Powder Formulation

Small-Scale Wet Milling. Ceftazidime NC suspensions were prepared using a wet milling technique on a small scale, as described previously with modifications.[21,22] Briefly, 100 mg of the as-received drug powder was suspended in 4 mL of selected antisolvent in a 20-mL scintillation vial containing 7 mL of YTZ® grinding media (0.5 mm, Tosoh Corporation, Tokyo, Japan). Then the suspension was milled for 6 h under a stirring speed of 1200 rpm using a round magnetic Stir Bar (Komet 15 [K15], 9 × 15 mm, Variomag Magnetic Stirrers). The selected antisolvents for NC formulation were ethanol, acetone, tert-butanol, and n-propanol. Samples were collected at different time intervals and evaluated for the particle size. After milling, the suspension was collected in 20-mL antistatic vials, flash-frozen using liquid nitrogen, and lyophilized for ~36 h at a temperature of −72°C and under vacuum of ~150 millitorr (VirTis Freezemobile-12XL, The Virtis Company, NY). Lyophilized NC powder was stored under desiccant at room temperature for further use.

Large-Scale Milling (LSM). Ceftazidime NC suspensions were prepared in tert-butanol as an antisolvent using a Netzsch MiniCer Media Mill (NETZSCH Fine Particle Technology, LLC, Exton, PA). The as-received drug (1 g) was added to 250 mL of tert-butanol and decanted into the mill. The instrument was operated using YTZ® grinding media (0.2 mm, Tosoh Corporation, Tokyo, Japan) with an agitation speed of 2772 rpm. The drug particles and the media were suspended in tert-butanol inside the mill chamber and attrition was achieved by a ceramic agitator inside the mill. The suspension was continuously circulated for 15 min. The milled product was then collected in 20-mL antistatic vials, flash-frozen using liquid nitrogen, and lyophilized for 72 h at a temperature of −72°C and under vacuum of ~150 millitorr (VirTis Freezemobile-12XL, The Virtis Company, NY).

Dynamic Light Scattering

Particle size of NC suspensions was determined by dynamic light scattering (Brookhaven Instruments Corporation, ZetaPALS, Holtsville, NY). NC particle size was measured by diluting 100 μL of the milled suspension to 2 mL of antisolvent (matched to antisolvent used during preparation). To measure the size of the nanoparticle, the diluted sample was sonicated with a microtip probe sonicator for 30 s at an amplitude of 20% (Fisher Scientific, Sonic Dismembrator, Pittsburgh, PA).

Water Solubility and Water Content Measurement

The equilibrium solubility experiment in water was performed by equilibrating an excess amount of NC dry powders and the as-received drug powder in glass vials with 3 mL of water (saturated solution of the powder in water) and was shaken for 48 h at 37°C and 100 rpm. Next, the suspension was filtered and the drug concentration in the supernatant was analyzed by UV spectrophotometry. Water content of all samples and as-received drug was measured using the Karl Fisher method (756 KF Coulometer; Metrohm Ion Analysis, Herisau, Switzerland).

Powder Flow Characteristics

Bulk and tap densities of all samples and as-received drug were estimated using the tap density test approach as described in the US Pharmacopeia.[23]

Differential Scanning Calorimetry (DSC)

Thermograms for all samples and as-received drug were measured using DSC (Q100 Universal V4.3A; TA Instruments, New Castle, DE). Approximately 2-3 mg of lyophilized dry mass was sealed and placed in an aluminum pan and heated at a scan rate of 10°C/min over a temperature range of 25°C-400°C. An inert atmosphere was maintained by purging with nitrogen at 50 mL/min.