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Pharmacokinetics of Ethionamide Delivered in Spray-Dried Microparticles to the Lungs of Guinea Pigs

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ABSTRACT

The use of ethionamide (ETH) in treating multidrug-resistant tuberculosis is limited by severe side effects. ETH disposition after pulmonary administration in spray-dried particles might minimize systemic exposure and side effects. To explore this hypothesis, spray-dried ETH particles were optimized for performance in a dry powder aerosol generator and exposure chamber. ETH particles were administered by the intravenous (IV), oral, or pulmonary routes to guinea pigs. ETH appearance in plasma, bronchoalveolar lavage, and lung tissues was measured and subjected to noncompartmental pharmacokinetic analysis. Dry powder aerosol generator dispersion of 20% ETH particles gave the highest dose at the exposure chamber ports and fine particle fraction of 72.3%. Pulmonary ETH was absorbed more rapidly and to a greater extent than orally administered drug. At T_{max}, ETH concentrations were significantly higher in plasma than lungs from IV dosing, whereas insufflation lung concentrations were 5-fold higher than in plasma. $AUC_{(0\text{-}t)}$ (area under the curve) and apparent total body clearance (CL) were similar after IV administration and insufflation. $AUC_{(0\text{-}t)}$ after oral administration was 6- to 7-fold smaller and CL was 6-fold faster. Notably, ETH bioavailability after pulmonary administration was significantly higher (85%) than after oral administration (17%). These results suggest that pulmonary ETH delivery would potentially enhance efficacy for tuberculosis treatment given the high lung concentrations and bioavailability.

Introduction

Tuberculosis (TB) continues to be the cause of most deaths worldwide due to a single organism, with some sources pairing it to the HIV[1] and others indicating that TB has surpassed HIV as a major killer.[2] The WHO estimated that in 2014 more than 9 million people developed TB, and 5% of those patients were infected with multidrug-resistant (MDR; resistance against isoniazid and rifampicin) bacterial strains.[3] Treatment of drug susceptible TB is difficult, requiring 6-9 months of large doses of antibiotics in combination; however, treatment of MDR-TB can extend up to 2 years and employs more complex, expensive, and poorly tolerated therapeutic regimens.[4] In 2001, data meta-analysis of 9153 MDR-TB individual patients treated with complex and long regimens in multiple centers reported treatment success in only 54% of these patients.[5] A successful regimen only appeared in 2010, when a short, standardized treatment regimen based on a fourth-generation fluoroquinolone combined with other second-line agents, known as the "Bangladesh Regimen," achieved a relapse-free cure of 87.9%.[6] Based on data from this and other similar

Abbreviations used: AUC, area under the curve; BAL, bronchoalveolar lavage; CL, apparent total body clearance; CV%, coefficient of variation; DPAG, dry powder aerosol generator; DPPC, 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine; ETH, ethionamide; FMO, Flavin-containing monooxygenases; FPF, fine particle fraction; GSD, geometric standard deviation; IV, intravenous; MDR, multidrug resistant; MIC, minimum inhibitory concentration; MMAD, mass median aerodynamic diameter; MRT, mean residence time; MTB, *Mycobacterium tuberculosis*; PK, pharmacokinetic; PPs, porous particles; PRO, prothionamide; TB, tuberculosis.

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studies, in May 2016, the WHO announced new recommendations for a 9- to 11-month shortened treatment regimen for selected MDR-TB patients.[7] This regimen has 2 phases: in the first, intensive phase patients receive a combination of kanamycin, moxifloxacin, prothionamide, clofazimine, and pyrazinamide with high-dose isoniazid for 4-6 months. In the second, continuation phase patients receive moxifloxacin, clofazimine, pyrazinamide, and ethambutol for 5 months. However, this shorter regimen is still associated with significant side effects, with 71% of the patients in the Bangladesh Regimen reporting nausea and vomiting, 12% reporting negative neurological side effects, and 6% reporting other miscellaneous side effects, with several patients experiencing more than one of these undesired side effects.[6]

An alternative approach to decrease the incidence and extent of the side effects to this short regimen is to deliver some of these drugs directly to the lungs to achieve high local drug concentration for extended durations. Such an approach has the possibility of accelerating the onset of drug action, decreasing the dose to achieve the therapeutic effect, which in turn would reduce systemic side effects. Also, it may be possible to exchange some of these drugs with more potent or longer half-lived analogs, which would decrease the dosing frequency. For instance, in the Bangladesh Regimen, prothionamide (PRO) could be replaced by ethionamide (ETH).

ETH was first used in the mid-1950s for TB treatment and in 1963 it was evaluated with the objective to prevent resistance to isoniazid or to treat isoniazid-resistant MTB. In the United States, ETH is one of the 10 drugs approved by the Food and Drug Administration to treat TB.[8] ETH is on the WHO's List of Essential Medicines and it is classified as an "oral bacteriostatic second-line agent," Group 4 of drugs to treat MDR-TB.[9] The 2011 Update of the Guidelines for the Programmatic management of MDR-TB strongly recommends the use of ETH or PRO, as the association of their use with cure was higher than that for cycloserine and para-aminosalicylate sodium.[10] It is frequently added to drug regimens around the world because it is the only drug in Group 4 that has bactericidal activity against MTB.[11] However, bactericidal ETH serum concentrations are hard to achieve because of poor tolerability by patients.[12] Therefore, the use of ETH decreased when PRO, a better tolerated analog, became available.[11] Several studies comparing the efficacy and tolerability of ETH and PRO have determined that both compounds are equally effective in treating TB, but that PRO was much better tolerated by the patients than ETH.[13,14] PRO is an analog of ETH, in which the ethyl group is substituted by a propyl molecule at the alpha position. ETH is 2-fold more potent (minimum inhibitory concentration [MIC] = 0.25 μg/mL) than PRO (MIC = 0.5 μg/mL) against *Mycobacterium tuberculosis* (MTB) strain H37Rv and has a longer half-life.[15,16] In humans, administration of the same dose (250 mg) of these compounds results in a maximum plasma concentration that is about 1.8 times higher for ETH than for PRO.[16] The objective of this study was to evaluate the disposition of ETH after pulmonary administration of spray-dried microparticle porous particles (PPs) to guinea pigs and contrast it with that after intravenous (IV) and oral administration. Powder formulations consisting of PPs deliver drug to the lung periphery more efficiently than other powder formulations, avoiding natural clearance mechanisms in the respiratory tract.[17] We postulate that delivery of ETH by the pulmonary route will increase its efficacy and decrease its toxicity, which can bring it back to the forefront of TB treatments.

Materials and Methods

ETH and L-leucine were obtained from Spectrum Chemicals & Laboratory Products (Gardena, CA). 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) was purchased from Genzyme Pharmaceuticals (Liestal, Switzerland). Carboxymethyl cellulose (molecular weight = 90,000 kDa) was purchased from Sigma-Aldrich (St. Louis, MO). ETH was a yellow powder soluble in ethanol and very sparingly soluble in water. Ethanol United States Pharmacopeia grade and acetonitrile were purchased from Pharmco Products, Inc. (Brookfield, CT). Water from a Millipore Corporation (Billerica, MA) Milli-Q water purification system was used. All other chemicals and reagents used were of pharmaceutical or analytical grade.

Formulation of Ethionamide Porous Particles

ETH PPs were manufactured as described previously[18,19] by spray drying (LabPlant, Model SD-06) water/ethanol solution containing different proportions of ETH, L-leucine, and DPPC. In order to increase the residence time of a standing aerosol cloud of ETH PPs in a dispersion chamber, 3 different formulations of PPs were prepared with 3 different compositions of ETH (50%, 20%, and 5%), leucine, and DPPC as follows (50:40:10 wt/wt, 20:70:10 wt/wt, and 5:90:5 wt/wt).

Characterization of Ethionamide Porous Particles

The dry particles were viewed using scanning electron microscopy. An LEO 982 field emission scanning electron microscope (Carl Zeiss, Inc., Thornwood, NY) was operated at 2 kV with a filament current of about 0.5 mA. Powder samples were prepared by deposition on a double-coated carbon conductive tape tab (Ted Pella, Inc., Redding, CA) mounted on a pin mount and dusted. The sample was then coated with a platinum/palladium layer with a 208HR Sputter Coater (Cressington Scientific Instruments, Inc., Watford, UK), operated for 60 s at a sputtering current of 40 mA.

An 8-stage Andersen nonviable 1ACFM cascade impactor (Copley Scientific Limited, Nottingham, UK) was used to determine the mass median aerodynamic diameter (MMAD) and the fine particle fraction (FPF) of the total dose of powder less than or equal to an effective cut-off aerodynamic diameter of 5.8 μm ($FPF_{5.8}$) and 3.3 μm ($FPF_{3.3}$) relevant to humans and laboratory animals, respectively.

Performance of Ethionamide Porous Particles in a Dry Powder Aerosol Generator

A custom-made nose-only exposure chamber and dry powder aerosol generator (DPAG, Patent US 8,205,612 B2) was developed with the purpose of generating and delivering dry powder aerosol from PP formulations to guinea pigs. The percentage of the nominal dose of ETH PPs delivered at each port of the DPAG was evaluated, by a method described previously,[20] as follows: approximately 50 mg of ETH PPs (nominal dose) was loaded in the main chamber of the DPAG and cotton balls of similar sizes were snuggly placed to cover each port and the DPAG was actuated for 5 min to aerosolize the ETH PPs. The cotton balls were then carefully taken out of the ports and each cotton ball was placed in a beaker, where ETH was extracted into 10 mL of methanol and the concentration determined by UV spectrophotometry from a standard curve constructed using known amounts of ETH PPs. The percent of nominal dose of PPs delivered at each port was determined dividing the amount of ETH PPs deposited at each port (calculated by correcting with the ETH content in each formulation) by the nominal dose loaded in the chamber of the DPAG.

The aerodynamic performance of ETH PPs in the DPAG was evaluated as a means of estimating the respirable dose that would be delivered to each animal in the chamber. A Marple Personal Cascade Impactor (Series 290; Westech Instruments, Inc., Marietta, GA) was employed to determine the MMAD and FPF delivered to each port of the chamber following dispersion of ETH PPs.