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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.

Table 1
Summary of Treatments Employed to Evaluate the Disposition of Ethionamide in Guinea Pigs

Treatment	Formulation	Dose (mg/kg)	Route of Administration	No. of Animals (T_{min})	No. of Animals (T_{max})
IV	Solution	6	IV	8	4
Oral	Suspension	6	Oral	8	5
Insufflation	Dry powder (PPs)	6	Pulmonary (insufflation)	8	6
Passive inhalation	Dry powder (PPs)	[a]	Pulmonary (passive inhalation)	4	–

[a] 5 mg/kg, estimated from PK parameters (area under the curve, dose, and bioavailability) calculated for IV and insufflation groups, the drug load in the ETH-PPs (20%), the fine particle fraction in that particular batch ($FPF_{<0.52\ \mu m} = 27.37\%$ at 2 L/min using a Marple personal cascade impactor), the average weight of the animals (0.38 kg) in that treatment group. The number of animals in each treatment group was selected based on similar studies previously performed by our group.

Most importantly, it was calculated the FPF of the percent of nominal dose of PPs delivered at each port of the DPAG was less than or equal to an effective cut-off aerodynamic diameter of 3.5 μm ($FPF_{3.5}$) and 0.52 μm ($FPF_{0.52}$) as they are relevant to the nose and alveoli of guinea pigs, respectively.[21,22] The impactor was coupled to a vacuum pump calibrated at 2 L/min to mimic the breathing of a guinea pig. To calculate the dose delivered, approximately 60 mg of ETH PPs was deposited on top of the chamber propeller and the chamber was actuated for 5 min at a fixed speed and the powder dispersion aided with air from an external source (DPAG, Patent US 8,205,612 B2). After powder dispersion, the amount of ETH deposited in the stages of the impactor was determined by rinsing them thoroughly with methanol and the ETH concentration calculated by UV spectrophotometric analysis of the samples at 290 nm.

Pharmacokinetic Studies

Animals

Specific pathogen-free, male Dunkin-Hartley guinea pigs (Hilltop Lab Animals, Inc., Scottdale, PA) weighing 383.6 ± 28.4 g were housed individually in a constant temperature environment of 22°C on a 12 h light/dark cycle. Animals were allowed access to water and food *ad libitum*. The day before the study, each animal underwent cannulation of the right external jugular vein for continuous blood sampling and allowed to recover overnight. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill in accordance with "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985).

Treatments

Animals were divided into 4 groups receiving different ETH formulations (Table 1): solution, suspension, or dry powder, delivered by different routes (IV, oral, pulmonary). For IV administration, ETH drug substance was dissolved in pure ethanol and vortexed for ~1 min until completely dissolved. For oral administration, pure ETH was suspended in 1.25% carboxymethyl cellulose and distilled water by mortar and pestle followed by vortexing. ETH was administered intravenously to animals as a solution by the cannula implanted at the jugular and orally as a suspension to animals using an oral gavage needle. For the insufflation procedure, each animal was anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The animal was then intubated with the help of a laryngoscope and ETH-PPs were administered with a small dry powder insufflator (Penn Century, Wyndmoor, PA) directly into the animal's airways. The insufflator was removed and each animal was held in an upright position for 1-3 min. The animal was then placed at ~30° inclination with a heating pad underneath to prevent hypothermia. The animal's breathing was monitored continuously until recovery from anesthesia. Dosing of animals with ETH-PPs by passive inhalation was conducted by placing conscious animals in the ports of a custom-made nose-only DPAG and dispersing 360 mg of powder inside the chamber for 1 h so that animals would inhale powder only through the nose. After dosing, blood samples (0.3 mL) were collected from each animal into heparinized tubes at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, and 4 h. Sterile heparinized saline solution was used to replace the blood volume lost through sample collection. Plasma was separated and stored at −80°C until analysis.

Evaluation of Local Ethionamide Concentrations

After collection of the last blood sample, animals were anesthetized and euthanized by exsanguination. The amount of ETH remaining in the airways was determined by measuring ETH concentrations in bronchoalveolar lavage (BAL) fluid. The lungs of each animal were lavaged by instilling 5 mL of sterile saline and slowly withdrawing the fluid. An aliquot of the BAL fluid was placed in a microcentrifuge tube and immediately centrifuged for 10 min at 12,000 rpm. The BAL supernatant was separated from the pellet (i.e., cellular constituents) and both were stored at −80°C until later analysis. After BAL was performed, the trachea and lungs were resected and stored with the BAL components until analysis.

The time to reach maximum ETH plasma concentrations (T_{max}) was calculated after completing the analysis of plasma concentration versus time profiles for animals in the 4 treatment groups. Then, in order to determine the corresponding ETH concentrations in lung tissues and BAL at T_{max}, another group of animals from each treatment received the respective ETH formulations and were euthanized at T_{max}. BAL was performed, and tissues collected as described above.

Determination of Ethionamide Concentration in Biological Samples

ETH concentrations in plasma, BAL, and tissue samples were determined using a validated HPLC method.[23] ETH concentrations in the standard curve ranged from 0.2 to 10 mg/mL and the absolute recovery of ETH was approximately 91%. The within-day precision (coefficient of variation [CV]) of quality control samples was 0.36%-6.39%, and the overall validation precision was 0.81%-4.66%.

Pharmacokinetic Analysis

ETH plasma concentration time profiles from 0 to 4 h following oral, IV, and pulmonary ETH administration for all animals were subjected to noncompartmental pharmacokinetic (PK) analysis using WinNonlin (Pharsight Corporation, Mountain View, CA). The following PK parameters were determined: area under the curve ($AUC_{(0\text{-}t)}$), apparent total body clearance (CL), mean residence time (MRT), elimination rate constant (K), and half-life ($t_{1/2}$). Maximum ETH concentrations (C_{max}) and time to achieve the maximum ETH concentration (T_{max}) were obtained from the nonfitted plasma versus time profiles for each individual animal. The following equation was used to calculate bioavailability:

$$F = \frac{AUC_{lung,\ oral}}{AUC_{IV}} \times \frac{D_{IV}}{D_{lung,\ oral}}$$

where $AUC_{lung,oral}$ and AUC_{IV} are the area under the curve after pulmonary, oral, or IV administration and D is the dose delivered (6 mg/kg) by each of these routes.

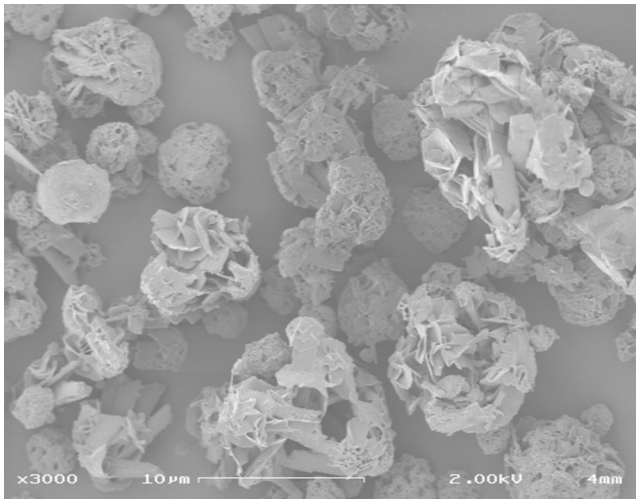

Figure 1. Scanning electron microscopy of spray-dried ETH PPs. Bar = 10 µm. This micrograph corresponds to PPs containing 50% ETH, but the morphology of PPs containing 20% ETH is similar and therefore not shown.

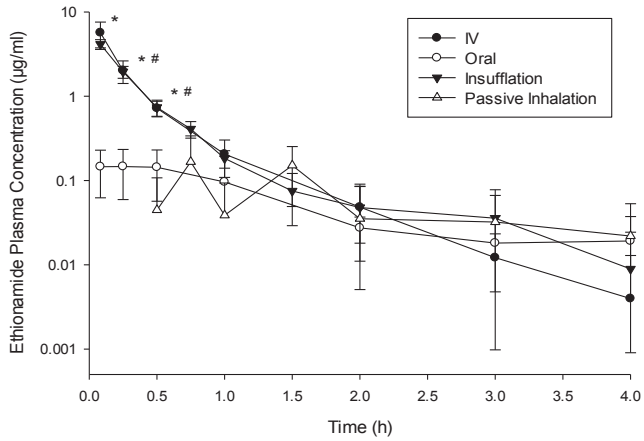

Figure 2. Average plasma concentration versus time curves (log scale) for all treatments administered by various routes and with different doses. Treatments include intravenous ethionamide United States Pharmacopeia (IV; 6 mg/kg), oral ethionamide United States Pharmacopeia (Oral; 6 mg/kg), ethionamide large porous particles (ETH-PPs; 6 mg/kg), and ethionamide large porous particles delivered by nose-only exposure (ETH-PPs; 5 mg/kg). *IV different from oral ($p < 0.05$); #Insufflation different from oral ($p < 0.05$) (average ± SD, $n = 4$-8).

Statistical Analysis

ETH plasma concentration versus time profiles, ETH concentrations in BAL and tissue data, as well as PK parameter of disposition were statistically analyzed using a one-way ANOVA and least-squares significant-differences multiple comparison method (i.e., Tukey's). A probability level of 5% ($p < 0.05$) was considered to be statistically significant.

Results

Physicochemical Characteristics of Ethionamide Porous Particles

Regardless of the %ETH in the formulation, all spray-dried ETH PPs were very porous spherical particle structures constructed of thin walled layers as shown by the scanning electron micrograph (Fig. 1). However, the MMAD, geometric standard deviation (GSD), and FPF as determined by impaction were different: MMAD and GSD of 50% ETH PPs was larger (4.94 and 1.53 µm, respectively) than those of 20% ETH PPs (4.01 and 1.07 µm, respectively), whereas the $FPF_{5.8}$ and $FPF_{3.3}$ were lower for the 50% ETH PPs (55.58% and 9.49%, respectively) than for the 20% ETH PPs (77.95% and 26.31%, respectively).

Performance of Ethionamide Porous Particles in a Dry Powder Aerosol Generator

Dispersion of PPs containing 20% ETH resulted in a 2-fold higher percentage of the nominal dose (18.35 ± 2.66%) delivered to the ports of the DPAG and was significantly higher ($p < 0.05$) than those resulting from the dispersion of PPs containing either 50% or 5% ETH (8.86 ± 1.17% and 9.42 ± 0.83%, respectively). Due to the low percentage of the dose delivered to the ports of the DPAG and the small drug content, the 5% ETH PP formulation was not considered further, as it was unlikely that their pulmonary administration to animals would yield therapeutic drug levels.

The emitted dose collected in the different stages of the Marple Personal Cascade Impactor attached to the port of the DPAG was 2-fold higher after dispersion of PPs containing 20% ETH (11.68%) compared to those containing 50% ETH (6.32%). The MMAD and GSD of 20% ETH PPs were smaller (2.89 and 2.41 µm, respectively) than that corresponding to 50% ETH PPs (4.85 and 2.66 µm, respectively). Most importantly, $FPF_{3.5}$ and $FPF_{0.52}$ were higher in the 20% ETH PPs (72.3% and 43.9%, respectively) than those of 50% ETH PPs (64.3% and 19.7%, respectively). This difference in $FPF_{3.5}$ and $FPF_{0.52}$ has important implications for administration of ETH PPs by passive inhalation studies in the guinea pig model as only particles in the 0.7-3.0 µm range could have a therapeutic effect.[21,22] Therefore, the 20% ETH large porous particles formulation was selected for subsequent animal studies.

Pharmacokinetic Studies

Figure 2 shows mean ETH plasma concentration versus time curves after dosing IV, oral, and pulmonary treatments (insufflation and nose-only exposure) to guinea pigs. As expected, after IV administration, ETH plasma concentrations at 0.083, 0.25, and 0.5 h were significantly higher ($p < 0.05$) than those receiving the same dose by oral administration. Remarkably, ETH plasma concentrations in animals dosed with ETH-PPs by insufflation at these times were similar ($p > 0.05$) to the IV dosed animals and significantly higher ($p > 0.05$) than orally dosed animals, indicating immediate absorption of ETH from the lungs. At 1 h postdosing, ETH plasma concentrations were similar for all treatment groups until the end of the study. ETH plasma concentrations in animals dosed by passive inhalation in the DPAG were similar to those of animals dosed orally throughout the study. The ETH dose received by animals dosed by passive inhalation was approximately 5 mg/kg. This dose was calculated using the drug load in the ETH-PPs (20%), the FPF in that particular batch ($FPF_{<0.52\ \mu m} = 27.37\%$ at 2 L/min using a Marple Personal Cascade Impactor), the average weight of the animals (0.38 kg) in that treatment group, the AUC obtained after passive inhalation of ETH PPs, and the pulmonary bioavailability obtained after insufflation.

Local ETH concentrations in lungs, trachea, BAL pellet, and supernatant at the time of maximum concentration (T_{max}) and at the end of the study (T_{min}) are presented in Table 2. For ETH levels at T_{max}, 2 types of comparisons were performed (Table 2): (1) between fluids (plasma and BAL supernatant/pellet) and tissues within the same route of administration (denoted by a numerical superscript); and (2) between different routes of administration within the same fluid/tissue (denoted by a letter superscript).

Table 2
Ethionamide Concentration in Individual Components of BAL, Lung, and Trachea Tissues at Various Times After Dose Administration (average ± SD, $n = 4$-8)

Time/Treatment	C_{max} Plasma (μg/mL)	Trachea (μg/g)	BAL Supernatant (μg/mL)	BAL Pellet (μg/mL)	Lungs (μg/g)
T_{max}					
IV (0.083 h)	$5.85 \pm 1.61^{1,a}$	$2.69 \pm 0.59^{2,b}$	$0.75 \pm 0.37^{3,b}$	$0.04 \pm 0.03^{3,a,b}$	$4.08 \pm 0.62^{2,a,b}$
Oral (0.39 h)	$0.21 \pm 0.07^{1,b}$	$0.95 \pm 0.62^{1,b}$	$0.03 \pm 0.05^{2,b}$	$0.00 \pm 0.00^{2,b}$	$0.85 \pm 1.04^{1,b}$
ETH-PPs (insufflation, 0.083 h)	$3.37 \pm 0.87^{1,2,b}$	$23.29 \pm 9.88^{1,a}$	$9.15 \pm 6.15^{2,3,a}$	$0.47 \pm 0.47^{3,a}$	$18.50 \pm 15.72^{1,2,a}$
T_{min}					
IV	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Oral	0.01 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.04
ETH-PPs	0.01 ± 0.01	0.03 ± 0.04	0.00 ± 0.00	0.01 ± 0.02	0.02 ± 0.04

Comparisons between fluids (plasma and BAL supernatant/pellet) and tissues within the same route of administration: numeric superscripts show the relative ranks of values (starting from the highest values). When the means are not significantly different, the same numeric superscript is used.
[a,b]Comparisons between different routes of administration within the same fluid/tissue: when the means are not significantly different, the same letter superscript is used.

As expected in animals receiving ETH by the IV route, the highest concentrations were observed in plasma, with higher ETH concentrations in lungs and trachea (well perfused organs) than in BAL components. In contrast, animals dosed by insufflation exhibited the highest ETH concentrations in lungs and trachea which were 5- and 7-fold as those in plasma, followed by those in BAL supernatant, which were also 3-fold higher than those in plasma. Despite the large variability observed in ETH concentrations among trachea and lung tissues of animals receiving ETH by the oral route, these concentrations were significantly higher than those observed in BAL components and in plasma. Comparison of ETH concentrations in lung tissue and BAL pellet samples indicated that animals dosed by insufflation exhibited significantly higher concentrations than animals dosed orally. Likewise, significantly higher (>10-fold) ETH concentrations were observed in the trachea and BAL supernatant of animals dosed by the pulmonary route than by any other treatment. ETH levels in plasma and BAL were mostly undetectable in animals of all treatment groups at the end of the study period (T_{min}, Table 2).

The PK parameters that characterize the disposition of ETH after administration by different routes are reported in Table 3. As expected from the similarity in the plasma concentration versus time profiles, $AUC_{(0\text{-}t)}$, CL, MRT, and T_{max} were also similar ($p < 0.05$) between animals dosed IV and by insufflation, indicating similar disposition of ETH by both routes of administration. However C_{max} was significantly higher in IV dosed animals than in those receiving ETH by insufflation. AUC after oral administration of ETH was 6-7-fold smaller than that after IV or pulmonary administration. T_{max} indicated that, in the guinea pig, ETH was absorbed 5 times faster after pulmonary administration than after oral administration and was cleared (CL) significantly faster (6-fold) after oral administration than after IV or pulmonary administration. Most notably, ETH bioavailability after pulmonary administration was 85% with respect to IV administration, whereas the oral bioavailability was only 17%.

Discussion

The global spread of strains of MTB with resistance to the most effective anti-TB drugs (rifampicin and isoniazid), defined as MDR-TB, has been a challenging hurdle to the global TB control. Furthermore, the increasing incidence of extensively (resistance to more than the 2 first-line drugs, rifampicin and isoniazid) and totally drug-resistant TB is a global health emergency. The therapeutic regimen for the management of patients with MDR-TB usually consists of 4 or more drugs and is based on the susceptibility of the isolated bacteria. In general, these regimens follow national treatment guidelines in line with WHO-recommended standards but their composition is influenced by the efficacy of the drugs, the treatment strategy, possible adverse effects, and the cost of treatment.[24] Thus, even though ETH is more potent and has a longer half-life, the inclusion of PRO in these regimens is favored over ETH with the purpose of reducing the severe adverse effects of the treatment.[25] Because the main adverse effects associated with the use of ETH are gastrointestinal, it is postulated that administration of ETH by the pulmonary route would decrease its toxicity and increase its efficacy because higher local drug concentration could be achieved at the site of MTB infection.

Previous studies have demonstrated that PPs are more efficiently delivered to the lung periphery (the major site of MTB infection) than smaller solid particles with the same mass, because their site of deposition in the lungs is determined by their aerodynamic rather than their geometric diameter.[26] In this study, ETH PPs were manufactured with same procedure (spray drying) and materials (leucine and DPPC) that we have used successfully with other anti-TB drugs (rifampicin, capreomycin, and PA-824).[27-30] The previous studies demonstrated that the antimicrobial activity of these antibiotics was not affected by the process of spray drying. This determination was not performed for ETH PPs because similar conditions were used for their manufacture. However, because the drug was recovered intact following delivery and there was no evidence of degradation during *in vitro* characterization (dose and aerodynamic particle size distribution measurement), there is no reason to believe that its chemical composition was affected by spray drying. Therefore, it is unlikely that its activity was affected by spray drying but this requires confirmation by determining the minimum inhibitory concentration for MTB.

In this study, 3 different formulations of ETH PPs were prepared by varying the percentages of ETH (5%-50%), leucine (40%-90%), and DPPC (5%-10%) to maximize the respirable fraction (FPF) that could

Table 3
Pharmacokinetic Parameters Obtained by Noncompartmental Analysis After Administration of the Various Treatments of Ethionamide by the Different Routes (mean ± SD, $n = 6$-8)

Parameter	Treatments		
	IV (6 mg/kg)	Oral (6 mg/kg)	ETH-PPs (6 mg/kg)
$AUC_{(0\text{-}t)}$ (μg·h/mL)	1.60 ± 0.39^{1}	0.21 ± 0.13^{2}	1.34 ± 0.12^{1}
CL (mL/h/kg)	3.93 ± 0.95^{2}	26.46 ± 12.81^{1}	4.40 ± 0.38^{2}
K (h^{-1})	1.45 ± 0.27	1.10 ± 0.55	1.77 ± 1.41
$t_{1/2}$ (h)	0.49 ± 0.08	0.79 ± 0.40	0.75 ± 0.57
MRT (h)	0.41 ± 0.07^{2}	0.92 ± 0.25^{1}	0.43 ± 0.15^{2}
C_{max} (μg/mL)	5.03 ± 0.90^{1}	0.19 ± 0.08^{3}	3.71 ± 0.60^{2}
T_{max} (h)	0.08 ± 0.00^{2}	0.39 ± 0.21^{1}	0.08 ± 0.00^{2}
$F_{(0\text{-}\infty)}$	1.00 ± 0.00^{1}	0.17 ± 0.12^{3}	0.85 ± 0.07^{2}

Numeric superscripts show the relative ranks of values (starting from the highest values). When the means are not significantly different, the same superscript is used.
$AUC_{(0\text{-}t)}$, area under the curve, C_{max}, maximum concentration; CL, clearance; $F_{(0\text{-}\infty)}$, bioavailability; K, elimination rate constant; $t_{1/2}$, half-life; T_{max}, time to reach C_{max}.

be delivered to the alveoli of guinea pigs using the DPAG. Leucine is a nonpolar amino acid that has weak surfactant properties and low aqueous solubility, whereas DPPC is a phospholipid that also has surfactant properties. The use of leucine in the formulation of inhalable particles has been reported to increase significantly the respirable fraction, as it enhances the flowability and dispersibility of the dry powder.[31] Inclusion of DPPC (up to 60%) in PP formulation has shown to decrease particle density, but this decrease is accompanied by an increase in particle size.[32] However, at 10% or less DPPC content with respect to leucine, no increase in particle size was observed.[33] Thus, while formulating the ETH PPs, the maximum proportions of ETH and DPPC that were considered were 50% and 10%, respectively.

Evaluation of the performance of the 3 ETH PP formulations in the DPAG determined that dispersion of the PPs containing 20% ETH achieved the highest percentage (18.35%) of the nominal dose delivered at the ports of the DAPG and the highest respirable fraction relevant to guinea pigs ($FPF_{0.52}$). It is likely that the highest respirable fraction in that powder resulted in the highest percentage of the nominal dose delivered at the ports of the DAPG. The higher respirable fraction in the 20% ETH PPs is probably due to the higher percentage of leucine in this formulation (70%), compared to the percentage in the 50% ETH PP formulation (40%).[34,35]

The formulation containing 20% ETH was selected for use in animal studies despite the higher drug content observed for the formulation containing 50% ETH. This may be explained by estimates of the fraction of the nominal dose delivered to the port (18.35%) and the respirable fraction corresponding to guinea pigs ($FPF_{0.52} = 43.9\%$) predicting that animals would inhale more than 4-fold more ETH with the 20% formulation than with the 50% formulation. Therefore, the immediate absorption of ETH after pulmonary administration of ETH PPs ($T_{max} = 0.089$ h or 5 min) may be attributed to the large FPF of this optimized formulation being deposited directly in the alveoli of animals. Even though very rapid absorption of several compounds has been reported after their pulmonary administration, ETH concentrations in plasma after pulmonary administration were remarkably similar to those after IV administration. This may be explained by the larger surface area for absorption in the alveolar region together with the small molecular weight of ETH and partition coefficient (log *P*) of 0.705 allowing it to cross the thin barrier from the alveoli to the capillary blood supply very rapidly. In contrast, the absorption of ETH from the gastrointestinal tract of the guinea pig appears to be slightly delayed ($T_{max} = 0.39$ h), limited, and variable. Despite limited reports of rapid and complete ETH absorption when administered to humans,[36] a larger number of studies describe the pattern of ETH absorption as "erratic" and that delayed absorption is typically common.[23,25,37] The T_{max} in healthy volunteers is reported to be between 1.02 and 2.6 h depending on the formulation (film or enteric-coated tablet) and coadministration of food or antacids.[23,38-40]

The disposition of ETH in the guinea pig after oral administration has been published in a small number of papers of the seminal work of Dickinson and Mitchison, where large doses (10-320 mg/kg) were administered,[41-44] but there are no reports of pulmonary delivery of ETH powders to guinea pigs or humans. The dose of ETH in this study (6 mg/kg) was selected for direct comparison with the PK studies using oral administration performed in volunteers by Auclair et al.[23] and Zhu et al.,[38] which indicated that this dose appeared to be the minimum required to achieve serum concentrations above MIC = 0.25 μg/mL.[15] In this study, while IV and pulmonary administration of this dose did achieve plasma concentrations significantly above MIC (C_{max} of 5.85 and 3.37 μg/mL, respectively), oral administration did not do so at any time during the study ($C_{max} = 0.21$ μg/mL). These differences can be noted when comparing the C_{max} of early studies in guinea pigs and those from humans. In guinea pigs, C_{max} of <1.0 and 5 μg/mL was recorded for doses of 10 and 40 mg/kg, respectively,[41,42] whereas for healthy volunteers the C_{max} recorded for a dose of approximately 6 mg/kg is between 2.16 and 2.3 μg/mL, respectively.[23,25,38,39] This may result from a species difference in enzyme expression and/or activity and, consequently, metabolism. In man, ETH is extensively metabolized in the liver, where 6 metabolites have been isolated to date.[39] The first step in ETH metabolism is transformation to sulfoxide metabolites, which have antimicrobial activity, by Flavin-containing monooxygenases (FMOs).[25] From the 5 functional FMOs, the isoforms 1, 2, and 3 are implicated in the metabolism of ETH: FMO1 is primarily expressed in the liver, kidney, and intestine; FMO2 is the major isoform in the lungs; and FMO3 is the primary FMO in the liver.[45] There are no reports of expression and localization of FMOs in the guinea pig but it is plausible that they would be located in the same organs as in man. If so, they would have different activities as has been reported with other liver enzymes.[46] Therefore, the lower ETH concentrations in plasma after oral administration in the guinea pig may be due to its erratic absorption and extensive metabolism in intestine and liver.

In addition to the immediate absorption and higher plasma concentrations due to lack of ETH metabolism in the lungs, there are 3 additional advantages of delivering ETH by the pulmonary route: (1) the achievement of higher local concentrations at the site of MTB infection; (2) smaller variability in the overall disposition; and (3) the similarity of its corresponding PK parameters ($AUC_{(0\text{-}t)}$, CL, MRT) to those after IV administration.

ETH concentrations in the trachea, BAL, and lungs of animals dosed by insufflation were several fold higher at T_{max} than those after oral administration and MIC. Thus, more drugs would be available to kill MTB at the site of infection. Local ETH concentrations were not determined in animals dosed by passive inhalation, but the resulting plasma concentrations were similar to those after oral administration. These lower concentrations may be explained by the breathing parameters of guinea pigs, rather than limited absorption and extensive metabolism such as in the oral route. The dose that guinea pigs can inhale by passive administration was limited to the amount that the animal could breathe through its nose and the fraction of the dose that could be inhaled by the nose, which is limited by the cut-off diameter of particles.[47]

There was considerable interanimal variability in the calculation of PK parameters for the different treatments, but the CV% was significantly smaller after pulmonary administration (CV% = 8%-16%) than after oral administration (CV% = 42%-62%). This fairly larger variability in PK parameters after oral administration of ETH has been also observed in humans (healthy and TB infected).[23,38] Nevertheless, pulmonary delivery of ETH resulted in a 7-fold larger $AUC_{(0\text{-}t)}$ and the drug being cleared 6-fold less after oral delivery. Notably, the similarity of $AUC_{(0\text{-}t)}$ resulted in a much higher bioavailability (85%) than that after oral administration (17%). Thus, with the higher local concentrations achieved in the lungs and the higher bioavailability after pulmonary administration compared to those obtained orally, it may be possible to reduce the dose of ETH required for the treatment of MDR-TB.

Efficacy studies with inhaled ETH alone or in combination are required to confirm its effectiveness in treating MDR-TB. However, the results of this study have the potential to inform existing treatment regimens. A possible application of the findings of this study could be that the formulation and route of administration for ETH could be switched from a tablet and suspension administered orally to a dry powder formulation administered by inhalation in currently used regimens such as the "Bangladesh Regimen." Pulmonary administration of ETH is likely to decrease significantly

or even eliminate the high frequency of gastrointestinal side effects caused by the oral administration of ETH. Pulmonary administration can also avoid the first pass metabolism of ETH, which is suspected to contribute to the large intrasubject variability observed after oral administration to patients[23,38] that we also observed in guinea pigs. Finally, pulmonary administration of ETH is likely to achieve bactericidal levels at the primary site of infection, with a potentially reduced dose. However, more detailed PK studies are required to evaluate the use of reduced doses by inhalation, or the dosing frequency to achieve the desired effect. Nevertheless, pulmonary delivery of ETH can be a prime candidate for use in individualized MDR-TB regimens,[9] whenever these are possible.

Conclusion

The results of this study suggest that administration of ETH by the pulmonary route may enhance its efficacy as indicated by the higher concentrations achieved in the lungs, trachea, and BAL of guinea pigs and also by the significantly higher bioavailability. Thus, it is plausible that the higher bioavailability may allow administration of smaller doses to achieve the same effects observed by other routes of administration, which in turn could decrease the incidence of adverse effects. The advantages of this approach would allow reconsideration of the role of ETH in TB therapy.

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