PREDICTION OF HUMAN EQUIVALENT DOSE (HED) OF LEVOFLOXACIN (LEVO) IN NEW ZEALAND WHITE RABBITS (NZW) TO SUPPORT EFFICACY OF COMBINATION TREATMENT WITH NOVEL THERAPEUTICS AGAINST INHALATION ANTHRAX (IA)

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M. Beliveau[1], A.L. Menard[1], J.F. Marier[1], J.T. Troyer[2], E.K. Leffel[2]

[1]Pharsight Consulting Services, Pharsight - a Certara™ Company, Montréal, Canada [2]PharmAthene, Inc., Annapolis, MD, United States

ABSTRACT

Regulatory agencies have suggested that novel therapeutics for treatment of IA do not diminish the efficacy of the standard-of-care (SOC) antibiotics in combination therapy, which would be the likely treatment. The NZW is an accepted animal model for IA where the SOC LEVO is both well tolerated, efficacious and where benefits of combination therapy would therefore need to be demonstrated. However, for proper demonstration of the added benefit of the new drug, the administered dose of LEVO must not be supratherapeutic. Therefore, a HED of LEVO in NZW must be used. The objective of the current analysis was to determine pharmacokinetic (PK) parameters of LEVO in NZW and to determine the NZW dose equivalent to the HED of 7.14 mg/kg (500 mg QD) for IA. LEVO was administered to NZW by either PO or IV bolus administration and blood for PK analysis was collected at various times up to 48h post dose. Plasma samples were assayed for concentrations of LEVO using a validated LC/MS/MS method. Following calculation of PK parameters such as clearance (CL) and volume (Vss) by standard non-compartmental methods, the NZW HED was extrapolated from the clinical dose using the following equation:

Doserabbit(HED) = (HED/CLhuman) x CLrabbit

where the HED = 500 mg and the CLhuman was taken from the literature (175 mL/min). Following IV dosing, mean CLrabbit and Vss of LEVO were 17.7 mL/min/kg and 1619 mL/kg, respectively. Bioavailability in NZW was dose dependant and ranged from 37.9% to 79.7%. Using CLrabbit, CLhuman and the HED, the dose of LEVO in NZW corresponding to the HED was 50.5 mg/kg IV. If administered PO, the NZW HED would range from 54.0 mg/kg to 63.4 mg/kg. In conclusion, the above results suggest that LEVO IV dosing of 50.5 mg/kg or PO dosing of 54.0 to 63.4 mg/kg in NZW in combination with novel anthrax therapeutics are expected to allow sufficient demonstration of any added benefit of the novel drug.

INTRODUCTION

Anthrax is a disease caused by *Bacillus anthracis*[1], a spore-forming, gram positive bacterium that has potential use as a weapon of bioterror. *B. anthracis* has been categorized as a high-biothreat agent due to the perceived ease with which spores can be grown and then disseminated by aerosol[2,3].

Levofloxacin (Levaquin) is a broad spectrum antibiotic of the fluoroquinolone drug class.[4,5] Levofloxacin and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations.[6] Levaquin is used alone or in combination with other antibacterial drugs to treat certain bacterial infections including pneumonia[7], urinary tract infections[8,9], abdominal infections[10] and anthrax.

The human effective dose (HED) of levofloxacin for post-exposure to inhalation anthrax is 500 mg QD[11]. While levofloxacin is highly effective prior to the onset of disease symptoms, the drug only targets vegetative bacteria. Once antibiotic treatment is stopped, there is a concern that residual spores could germinate into vegetative cells. In the event of antibiotic therapy alone, insufficient exposure to vegetative cells may result in inadequate immune memory and suboptimal levels of toxin-neutralizing antibody production[12].

Regulatory agencies have suggested that novel therapeutics for treatment of IA do not diminish the efficacy of the standard-of-care (SOC) antibiotics in combination therapy, which would be the likely treatment. This information can ultimately be used to optimize efficacy studies in NZWR in order to evaluate the evaluate the effect of levofloxacin in combination with other therapies currently being considered for prophylaxis and therapy against inhalation anthrax.

The efficacy of therapies against inhalation anthrax must be performed in well-characterized animal models, in compliance with the FDA Animal Rule[13]. Although the New Zealand White rabbit (NZW) is a widely accepted animal model for evaluating the efficacy of therapies against inhalation anthrax[14,15,16], limited information is currently available on the PK of levofloxacin following intravenous (IV) and oral (PO) dosing in NZW.

OBJECTIVE

The objective of this study was to determine pharmacokinetic (PK) parameters of LEVO in NZW and to determine the NZW dose equivalent to the HED of 7.14 mg/kg (500 mg QD) for inhalation anthrax (IA) in support of eventual preclinical efficacy studies for combination therapy.

METHODOLOGY

Study Design

• IV bolus doses (Levofloxacin Injection Solution [25 mg/mL] purchased from Akorn Incorporated) were administered via a marginal ear vein at 10 mg/kg to 10 rabbits.

• PO doses (Levofloxacin Oral Solution [25 mg/mL] purchased from Hi-Tech Pharmacal Co., Inc.) were administered via a stomach tube at 10, 25, 50 mg/kg (10 rabbits per dose).

• Serial blood samples (approximately 1 mL) for PK analyses were collected over 48 h and plasma samples were assayed using a validated LC/MS/MS method, with a lower limit of quantification of 0.100 µg/mL.

• PK analysis was performed using non-compartmental methods (WinNonlin® Enterprise v5.2, Pharsight, A Certara Company). AUC_{0-t} (Area under the levofloxacin concentration vs. time curve from time zero to the time of the last quantifiable concentration), $AUC_{0-\infty}$ (Area under the levofloxacin concentration vs. time curve extrapolated to infinity), C_{max} (Maximum observed concentration of levofloxacin measured after dosing), CL (Systemic clearance), $t_{1/2}$ (Terminal elimination half-life of levofloxacin) and %F (Bioavailable fraction) were estimated.[17] Concentration values of plasma levofloxacin that were reported as below the limit of quantitation (LOQ or BQL) were set to zero for PK and statistical analyses.

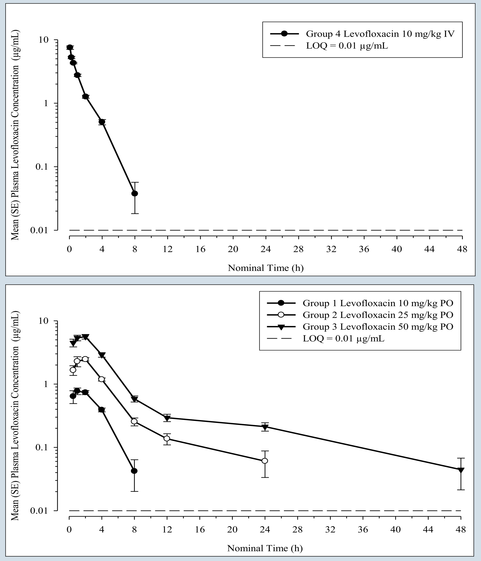
Human Equivalent Dose (HED) in Rabbits

The recommended human dose of levofloxacin (Levaquin) for the treatment of inhalation anthrax was 7.14 mg/kg (500 mg QD)[18]. The human clinical dose was extrapolated to NZW using the following equation [17]:

$$Dose_{rabbit}(HED) = (HED/CL_{human}) \times CL_{rabbit}$$

RESULTS

Figure 1: Mean (SE) Plasma Concentration-Time Profiles of Levofloxacin Following Intravenous and Oral Dosing



•Mean levofloxacin plasma concentration levels declined in an apparent mono (at lower dose) to bi-exponential (at higher dose) manner.

• Concentration levels dropped below the LLOQ (0.01 µg/mL) in a dose dependant manner, with BQL concentration generally appearing at 8 h at the lower dose, 24 h at the mid dose and 48 h at the higher dose level.

•Peak levels generally occurred at the 5 min time point for the IV dose while they generally occurred at the 2 h time point for the PO dose.

•Although levels following IV dose were similar to the high dose administered PO, the biphasic nature of the decline was not as prominent following IV. This suggested that the second phase of elimination following PO might be more related to secondary absorption of the product, a phenomenon potentially linked to enterohepatic recirculation of the drug[19].

RESULTS

Table 1: Mean (CV%) Plasma PK Parameters of Levofloxacin following IV and PO Dosing

PK Parameters	IV Dosing	PO Dosing		
	10 mg/kg (N=10)	10 mg/kg (N=10)	25 mg/kg (N=10)	50 mg/kg (N=10)
AUC_{0-t} (µg.h/mL)	8.97 (15.2)	2.75 (31.2)	11.8 (27.0)	30.9 (22.7)
$AUC_{0-\infty}$ (µg.h/mL)	9.56 (13.0)	3.62 (19.6)	15.7 (60.8)	38.1 (22.7)
C_{max} (µg/mL)	7.66 (15.6)	0.941 (29.4)	2.79 (34.0)	6.14 (27.6)
$t_{1/2}$ (h)	1.32 (21.7)	2.06 (17.5)	14.8 (145.2)	27.4 (67.8)
%F	100	37.9	65.7	79.7
CL (mL/min/kg)	17.7 (13.8)	NA	NA	NA
HED (mg/kg)	50.5	NA	NA	**54.0 - 63.4**

AUC_{0-t} = Area under the levofloxacin concentration vs. time curve from time zero to the time of the last quantifiable concentration, $AUC_{0-\infty}$ = Area under the levofloxacin concentration vs. time curve extrapolated to infinity, C_{max} = Maximum observed concentration of levofloxacin measured after dosing, CL = Systemic clearance, $t_{1/2}$ = Terminal elimination half-life of levofloxacin, %F = Bioavailable fraction, HED = Human Equivalent Dose (HED) in Rabbits.

•The bioavailability (%F) of levofloxacin increased as a function of dose (from 37.9 to 79.7%).

•The observed CL of levofloxacin in NZW was approximately 7 times higher than the mean value reported in humans (2.5 mL/min/kg)[18]. This suggests that the relationship of CL between NZW and humans is not proportional to body weight. V_{ss} following IV dosing was 1.6 L/kg.

•The external dose of levofloxacin in NZW corresponding to the HED ($Dose_{rabbit(HED)}$) was 50.5 mg/kg IV or 162 mg for a typical 3.2-kg animal. This dose would result in similar AUC to levofloxacin as observed in humans treated with 500 mg QD doses (i.e., 47.6 µg.h/mL) [18].

•Oral administration of levofloxacin in NZW did not result in a F% value similar to that observed in humans (i.e., >99%) [19].

•Based on the %F value observed at the 50 mg/kg dose level (79.7%), the HED following oral administration in NZW would be 63.4 mg/kg or 203 mg for a typical 3.2-kg animal.

•Based on a linear relationship between observed AUC and %F in rabbits, the $Dose_{rabbit(HED)}$ was estimated at 54.0 mg/kg.

DISCUSSION

Traditional allometric scaling based on body surface area equations would have resulted in a IV dose of 22.1 mg/kg [20]. This value would have resulted in systemic exposure in NZW more than two-fold lower than those observed in humans treated at the human dose.

Results suggests that intravenous doses of 50.5 mg/kg or oral doses of 54.0 to 63.4 mg/kg in NZW will be expected to display systemic exposure to levofloxacin similar to that observed in humans treated with the human dose.

This information can be used to design optimal studies in NZW to assess the efficacy of levofloxacin in combination with other drugs, such as recombinant protective antigen (rPA) vaccine or humanized monoclonal antibodies that have a high affinity for *Bacillus anthracis* for either prophylaxis and therapeutic treatment against inhalation anthrax.

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