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Pharmacokinetics of ABT-773, a new semi-synthetic ketolide in neutropenic lung-infected mice: a population approach

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Abstract

ABT-773 is an investigational ketolide antimicrobial agent with an in-vitro bactericidal activity against macrolide-susceptible and -resistant *Streptococcus pneumoniae*. The pharmacokinetics of this drug candidate were evaluated in lung-infected (108 CFU mL^{-1} starting inoculum) mice following a single dose (25, 50, 100 or 200 mg kg^{-1}) oral administration as a solution in 10% of 95% ethanol and 90% of 0.1 M pH 6.5 phosphate buffer solution. Serum ABT-773 concentrations were measured using a validated HPLC assay with fluorescence detection (excitation at 324 nm and emission at 364 nm). Population pharmacokinetic analysis was performed using the NONMEM computer program. Results from data analysis showed non-linear pharmacokinetics of ABT-773, noted by the increases in half-life (3.1 to 27.2 h) and AUC/dose (23.7 to 149 mg h^{-1} L^{-1} mg^{-1}), with doses from 25 to 200 mg kg^{-1}. A non-linear one-compartment model with parallel capacity-limited and linear first-order elimination best described the pharmacokinetics of ABT-773 in the mouse. The total volume of distribution was 0.316 L. The clearance for the linear first-order elimination was 0.0027 L h^{-1}. The Vm and Km were 0.0385 L h^{-1} and 0.141 mg L^{-1}, respectively, for the capacity-limited elimination.

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Introduction

Ketolides are a new chemical subclass of 14-membered ring macrolide antibiotics with a 3-keto group instead of a 3-OH group, which is semi-synthesized by oxidation of the 3-OH group. Through this transformation of the chemical structure ketolides obtain strong acid stability and potent in-vitro activity against the pathogens frequently associated with community-acquired respiratory tract infections, such as *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and the atypical pathogens (i.e. Legionella sp., *M. pneumoniae* and *C. pneumoniae*). In addition to this spectrum, a notable improvement in the microbiologic profile of these compounds has been observed for streptococci as these agents possess potent bactericidal effects against both macrolide-susceptible and macrolide-resistant *S. pneumoniae* (Bryskier et al 1996; Joly-Guillou et al 1997).

This study was conducted to determine the pharmacokinetic profile of ABT-773 after oral administration in infected mice. Data derived in this present study are intended to be used in a subsequent investigation that will evaluate the pharmacodynamic profile of this compound in the context of pneumococcal pneumonia.

Materials and Methods

Antimicrobial agents

ABT-773 (lot no. 31-500-AL) and ABT-267257 (lot no. A-267257, used as internal standard) were provided by Abbott Laboratories (Abbott Park, IL).

Animals

Specific pathogen-free outbred female ICR mice weighing 25 g (Harlan Sprague Dawley Inc., Indianapolis, IN) at an age of approximately 8 weeks were used. The mice underwent an acclimatization of a minimum of 7 days before the study. The mice were fed a standard laboratory rodent diet and allowed free access to drinking water. All care and the experiments described herein were approved by and performed in accordance with guidelines of the Hartford Hospital Institutional Animal Care and Use Committee.

The mice were group housed in cages with 5 mice per cage. The animal housing rooms had controlled environmental conditions with temperature and relative humidity of approximately 18–22°C and 40–70%, respectively, and artificial lighting, alternating on a 12-h light–dark cycle.

Animal infection

The mice were rendered transiently neutropenic by giving two intraperitoneal injections of cyclophosphamide (Cytoxan, Bristol-Myers Squibb, Princeton, NJ) at 150 mg kg^{-1} in a volume of 0.2 mL at 4 days and 1 day prior to the bacterial inoculation (Jolly-Guillou et al 1997; Andes & Craig 1998). *Streptococcus pneumoniae* suspension at a concentration of approximately 108 CFU mL^{-1} was inoculated to induce animal pneumonia. Briefly, the mice were induced into a semi-anaesthetized state with isofluorane (2% v/v in 100% oxygen carrier) inhalation prior to the bacterial inoculation. Then intratracheal inoculation was implemented by instilling 0.05 mL bacterial suspension to the mouth and completely blocking the nares of the animal, thus resulting in bacterial inhalation through the mouth to the lungs. The mice were allowed to fully recover from anaesthesia in an oxygen-enriched chamber after inoculation. Oral administration of ABT-773 was initiated 12 h post inoculation.

Dosage form, doses and route of administration

ABT-773 was dissolved in 10% of 95% ethanol and 90% of 0.1 M pH 6.5 phosphate buffer solution (v/v) to achieve a concentration of 20 mg mL^{-1}. Oral administration of a single dose of ABT-773 at 25, 50, 100 or 200 mg kg^{-1} was initiated via oral gavage 12 h post inoculation.

Specimen collection and processing

Blood samples from 3 to 10 mice (mean: 6 mice) per each time point were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h post single-dose injection via cardiac puncture after euthanasia through CO_2 inhalation and cervical dislocation. After clotting, each sample was centrifuged at 6900 *g* for 10 min. The serum was then collected and frozen at −80°C until analysis.

HPLC assay

ABT-773 serum levels were measured by a validated reverse-phase HPLC method with fluorescence detection. The sample extraction method involved liquid-phase extraction. After addition of 100 μL of 0.5 M sodium carbonate and 50 μL of an internal standard to the mouse serum samples (200 μL), 1.2 mL of hexane/ethyl acetate (1:1, v/v) solution was added. Protein precipitation was achieved by vortexing followed by centrifugation at 3600 *g* for 6 min. The supernatants were transferred into labelled clean tubes and evaporated to dryness under a stream of nitrogen at 40°C. The residuals were then reconstituted with 100 μL of 0.05 M KH_2PO_4 solution containing acetonitrile (2:1, v/v) and 0.01 M HCl. Then 1.0 mL of hexane was added to each of the processed samples, and the samples were vortexed for 30 s and centrifuged at 3600 *g* for 6 min. The resultant aqueous solution was separated and transferred into WISP vials for injection onto the HPLC system.

The chromatography was done at ambient temperature on a reversed-phase Alltech Nucleosil 100 C18 column (10 μm, 4.6 mm × 25 cm, Phenomenex Co.) with an injection volume of 30 μL. The mobile phase, consisting of 0.01 M tetramethylammonium hydroxide in 0.05 M KH_2PO_4 (pH 6.0) solution/acetonitrile/methanol (100:90:10, v/v/v), was delivered via a Waters HPLC pump at a flow rate of 1.0 mL min^{-1}. ABT-773 and the internal standard were eluted at approximately 6.2 and 7.8 min, respectively. The fluorescence detection was performed at an excitation wavelength of 324 nm and an emission wavelength of 364 nm (Model 980, Applied Biosystems, Foster City, CA). An EZChrome Elite chromatography data system (Scientific Software, San Ramon, CA) was used for data acquisition.

The standard curve, which ranged from 0.05 to 2.0 $\mu g\ mL^{-1}$, was prepared by spiking blank mouse

serum with appropriate amounts of ABT-773 aqueous working solution. Independently of the standard samples, quality control samples with three different concentrations (0.1, 1.2 and 1.6 $\mu g\,mL^{-1}$) were prepared and analysed with the standard samples for validation. The relative peak height ratio was used for the construction of calibration curves. Samples over the standard curve range were diluted and re-assayed. The lower limit of quantitation was 0.05 $\mu g\,mL^{-1}$, with an accuracy as determined by relative deviation from the nominal value (% DEV) of 3.86%. The corresponding precision expressed as relative standard deviation (% RSD) was 2.25%. The inter- and intra-assay precision and accuracy for all the quality control samples were less than 6% RSD and 6% DEV, respectively.

Population pharmacokinetic analysis

A preliminary analysis was carried out using the non-compartmental approach (WinNonlin Pro 3.0, Pharsight Corporation, Mountain View, CA) to compute pharmacokinetic parameters from the average concentration values at each time point for each dosing regimen. Such an analysis allowed us to assess how the drug concentration increased in proportion to dose and to estimate the initial pharmacokinetic parameters that will be used in the population analysis. Individual data from all the dosing regimens were analysed using the population approach. The population pharmacokinetic analysis was performed using the NONMEM computer program version V, level 1.1, NM-TRAN version III level 1.0 and PREDPP Version IV level 1.0 (NONMEM Project Group, University of California at San Francisco, San Francisco, CA). One- and two-compartment structural models with first-order absorption and elimination were compared to fit the data using the first-order conditional estimation method. Additive, proportional and exponential error models were evaluated to describe the inter-individual variability in pharmacokinetic parameters and residual error in the model. To assess model fit, scatterplots were created which included predicted versus observed concentrations, residual and weighted residual versus time, and residual and weighted residual versus observed and predicted concentrations. The likelihood ratio test and Akaike's information criterion (AIC) were used for model discrimination; the significance level was set a priori at 0.005. Examination of the precision of parameter estimates, the inter-individual and residual variability and the residual plots was used as an aid for model comparison.

Results

The mean serum concentration–time profiles of ABT-773 following oral administration of 25, 50, 100 or 200 mg kg^{-1} to mice are shown in Figure 1. At low dose (25 mg kg^{-1}), 12 h after administration, ABT-773 concentrations were below the limit of quantitation in the majority of the animals. ABT-773 was absorbed rapidly in mice and the peak concentration achieved at approximately 1–2 h after dosing. The dose-normalized AUCs (AUC/D) in the animals ranged from 23.7, 61.4, 106 and 149 mg*h^{-1} L^{-1} mg^{-1} for doses of 25, 50, 100 and 200 mg kg^{-1}, respectively. For the elimination from serum after 25, 50, 100 and 200 mg kg^{-1} dosing, the MRTs (mean residence time) were 5.5, 18.2, 25 and 52.8 h, respectively, and the corresponding half-lives were 3.1, 13.5, 17.6 and 37.2 h, respectively. The total clearances were 0.0420, 0.0160, 0.0094 and 0.0067 L h^{-1}, respectively. A total of 258 concentrations were used to compute population parameters. A one-compartment model with first-order absorption was chosen as the structural model based on the likelihood ratio test. Evaluation of the AIC indicated that the exponential error model best describes the inter-individual variability in pharmacokinetic parameters and an additive error model best describes the residual error in the model. The exploratory analysis showed dose dependence in pharmacokinetic parameters, suggesting non-linear kinetics. Accordingly, a non-linear one-compartment model with parallel capacity-limited and linear first-order elimination was used to fit the data

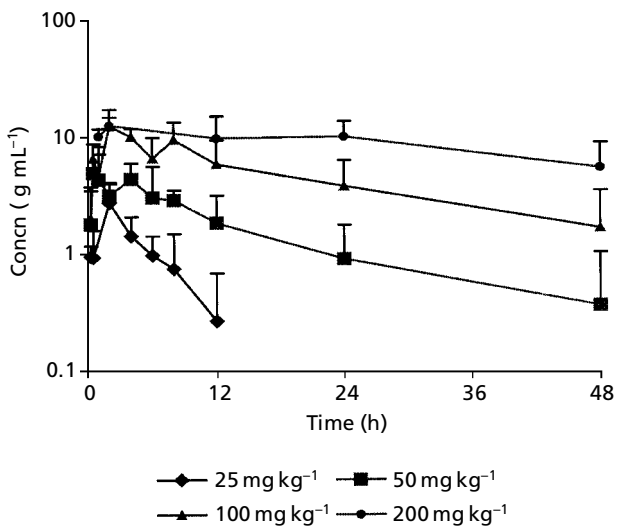

Figure 1 Mean serum concentration–time profiles of ABT-773 following oral administration of 25, 50, 100 or 200 mg kg^{-1} in mice with pneumococcal lung infection.

Table 1 Parameter estimates of ABT-773 using the final non-linear one-compartment model with parallel capacity-limited and linear first-order elimination.

Parameter	Parameter estimate	Relative standard error (% RSE)	95% confidence interval
CL (L h^{-1})	0.0027	40.7	0.00048–0.0049
V (L)	0.316	6.58	0.275–0.357
Ka (L h^{-1})	1.78	23.3	0.969–2.59
Vm (L h^{-1})	0.0385	24.1	0.0203–0.0567
Km (mg L^{-1})	0.141	58.9	–[a]
Inter-individual variability (CV)			
CL (L h^{-1})	83.7%	69.9	–[a]
V (L)	42.9%	45.1	4.9–80.9
Ka (L h^{-1})	82.1%	85.4	–[a]
Vm (L h^{-1})	32.2%	65.4	–[a]
Km (mg L^{-1})	0.0268%	10112	–[a]
Residual error, σ	0.254 mg L^{-1}	94.7	–[a]

CL, clearance; V, volume; Ka, absorpotion rate constant; Vm, maximum rate of elimination; Km, Michaelis constant.
[a] The parameter is poorly estimated. The 95% confidence interval estimated from the standard error includes zero.

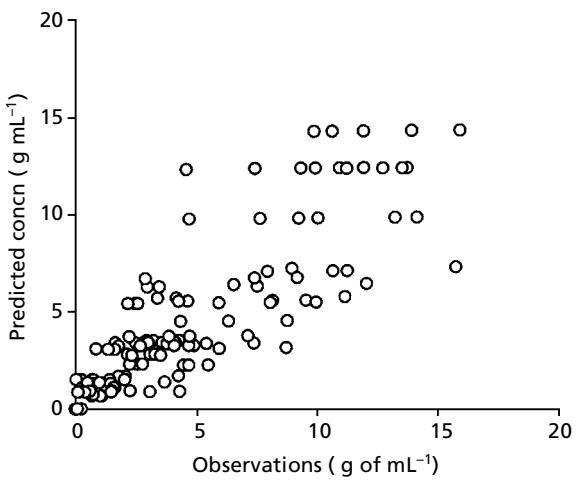

Figure 2 Population predicted concentrations versus observed concentrations for the final model.

and significant improved model fitting was achieved with this model. The final pharmacokinetic parameter estimates are shown in Table 1.

The population pharmacokinetic analysis was quite robust, as is evident from the plot of the observed versus the population predicted concentration of ABT-773 in the infected animals (Figure 2). The overall r^2 was in excess of 0.7, indicating that the fit of the model to the data was reasonably good. No significant bias pattern was observed with the plot of the predicted concentration versus the weighted residual, as shown in Figure 3.

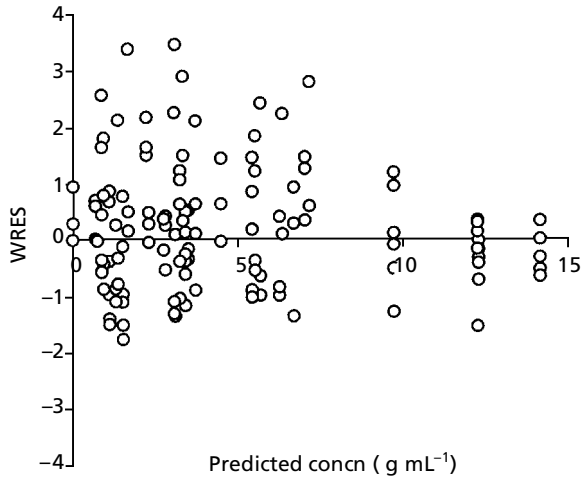

Figure 3 Weighted residuals versus population predicted concentrations for the final model.

Discussion

ABT-773, a ketolide antibiotic with potent bactericidal effects against the spectrum of pathogens responsible for community-acquired respiratory tract infections, including both macrolide-susceptible and -resistant *S. pneumoniae*, is currently under clinical development (Willey et al 2000; Zhanel et al 2000). During this developmental process a variety of factors influence the final dosage regimen selection to be used in clinical trials. Among these factors is the complete delineation of the pharmacodynamic profile of the compound of interest. While a pharmacodynamic investigation of a new compound is often initiated using in-vitro studies, it is now common practice to gather in-vivo data concerning the pharmacodynamic profile using either a murine thigh or a pneumonia model (Andes & Craig 1998; Nicolau et al 1999, 2000). Because of the use of these in-vivo data in antimicrobial drug development it has become critical that the pharmacokinetic profile of the study compound should be well delineated in the model of interest since drug disposition together with microbiologic activity will define the agent's pharmacodynamic profile.

The current study was undertaken to define the pharmacokinetic profile of ABT-773 in the murine pneumococcal pneumonia model, a model that will be

utilized for the pharmacodynamic profiling of the compound. As a result of this investigation the pharmacokinetic profile of orally administered ABT-773 has been well characterized in the murine pneumonia model over a wide range of doses. These studies reveal that ABT-773 exhibits non-linear pharmacokinetics, noted by the increases in half-life, MRT and AUC/dose, with the studied doses. Andes & Craig (2000) also reported that ABT-773 showed non-linear kinetics with doses from 1.5 to 24 mg kg^{-1} in a murine thigh infection model. In a first-time human study, ABT-733 exhibited non-linear pharmacokinetics with doses from 100 to 1200 mg (Gustavson et al 2001). Our observations are in accordance with these reports. Guan et al (1999) studied the in-vitro and in-vivo metabolism of ABT-773 and showed that the excretion of ABT-773 was primarily via hepatic metabolism, with small fractions in urine, and the major metabolic pathway was oxidation. The latter report provides a metabolic rationale for the observed non-linear pharmacokinetic disposition of ABT-773.

Conclusion

In conclusion, this report provides valuable and previously unavailable information regarding the non-linear pharmacokinetic profile of ABT-773, a novel ketolide antibiotic in a well-defined murine pneumonia model. Based on these data, subsequent studies can de designed to fully evaluate the pharmacodynamic profile of this investigational compound.

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