Population Pharmacokinetic Model of Oxfendazole and Metabolites in Healthy Adults following Single Ascending Doses

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ABSTRACT Oxfendazole is a potent veterinary benzimidazole anthelmintic under transition to humans for the treatment of multiple parasitic infectious diseases. The first-in-human study evaluating the disposition of oxfendazole and its metabolites in healthy adults following single ascending oral doses from 0.5 to 60 mg/kg of body weight shows that oxfendazole pharmacokinetics is substantially nonlinear, which complicates correlating oxfendazole dose to exposure. To quantitatively capture the relation between oxfendazole dose and exposure, a population pharmacokinetic model for oxfendazole and its metabolites, oxfendazole sulfone and fenbendazole, in humans was developed using a nonlinear mixed-effect modeling approach. Our final model incorporated mechanistic characterization of dose-limited bioavailability as well as different oxfendazole metabolic processes and provided insight into the significance of presystemic metabolism in oxfendazole and metabolite disposition. Oxfendazole clinical pharmacokinetics was best described by a one-compartment model with nonlinear absorption and linear elimination. Oxfendazole apparent clearance and apparent volume of distribution were estimated to be 2.57 liters/h and 35.2 liters, respectively, at the lowest dose (0.5 mg/kg), indicating that oxfendazole is a low extraction drug with moderate distribution. The disposition of both metabolites was adequately characterized by a one-compartment model with formation rate-limited elimination. Fenbendazole formation from oxfendazole was primarily through systemic metabolism, while both presystemic and systemic metabolism were critical to the formation of oxfendazole sulfone. Our model adequately captured the concentration-time profiles of both oxfendazole and its two metabolites in healthy adults over a wide dose range. The model can be used to predict oxfendazole disposition under new dosing regimens to support dose optimization in humans.

KEYWORDS anthelmintic drugs, first-in-human, oxfendazole, pharmacometric modeling, population PK analysis

Trichuriasis, the infection of the large intestine by whipworm (*Trichuris trichiura*), and neurocysticercosis, the infection of brain tissue by larval cysts of pork tapeworm (*Taenia solium*), are two difficult-to-treat parasitic infections that affect more than 1 billion people worldwide (1, 2). First-line antiparasitic treatment for neurocysticercosis and trichuriasis is limited to albendazole and mebendazole (3, 4). However, both albendazole and mebendazole are far from ideal due to their variable efficacy, low cure rate (<50%) (3, 5), and short half-life (6). Therefore, there is a pressing clinical need to develop more effective anthelmintic drugs for the treatment of trichuriasis and neurocysticercosis in humans.

Oxfendazole is a broad-spectrum benzimidazole approved to treat lungworm and enteric helminths in cattle. Like other benzimidazoles, oxfendazole inhibits microtubule assembly by binding to tubulin, resulting in disruption of the cytoskeleton,

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subcellular organelle migration, and cell division (7). However, in contrast to albendazole and mebendazole, which inhibit helminthic and mammalian microtubule formation with similar potencies, oxfendazole inhibits helminthic microtubule formation more potently than the formation of mammalian tubulin (8, 9). Oxfendazole demonstrated antiparasitic efficacy superior to that of albendazole, evidenced in *Taenia solium*- and *Trichuris suis*-infected pig and in *Trichuris muris*-infected mouse models (10–16). These promising preclinical efficacy data indicated that oxfendazole has the potential to be an effective treatment for trichuriasis and neurocysticercosis in humans. In addition, oxfendazole displayed favorable safety profiles in various preclinical species, such as mouse, rat, dog, and cattle (17).

The pharmacokinetics of oxfendazole has been extensively investigated in several animal species, and the results show that after oxfendazole oral administration, the parent drug is the major moiety quantified in the plasma of most species, followed by two phase I metabolites, oxfendazole sulfone and fenbendazole (18). In horse, cattle, and sheep, following oral administration, percentages of oxfendazole dose excreted in urine and feces were 6.8 to 22% and 64 to 84.4%, respectively (18), indicating that oxfendazole is eliminated primarily through biliary excretion. Fecal metabolite profiles are not available. Oxfendazole was excreted in urine mostly as free amine (16 to 22%) or as 4'-hydroxylated oxfendazole (68 to 70%) present as sulfate or glucuronide conjugates (18). Enzyme(s) involved in oxfendazole metabolism in preclinical species as well as in human has yet to be elucidated. Most importantly, oxfendazole exposure and half-life was higher than or comparable to that of albendazole in dogs, sheep, and pigs (19–22).

Considering the promising efficacy, safety, and pharmacokinetics of oxfendazole shown in preclinical models, together with the unmet clinical need for a more effective anthelmintic drug, the U.S. Food and Drug Administration (FDA) has granted fast-track designation to the Oxfendazole Development Group for the development of oxfendazole in the treatment of trichuriasis in humans. As part of the oxfendazole drug development process, oxfendazole safety and pharmacokinetics in healthy adults following single ascending oral doses (0.5, 1, 3, 7.5, 15, 30, and 60 mg/kg of body weight) were evaluated in the first-in-human (FIH) trial (ClinicalTrials.gov identifier NCT02234570), and we reported the results recently (2). This was the first comprehensive evaluation of oxfendazole and its metabolites in healthy adults. Our noncompartmental analysis (NCA) of the FIH data showed that oxfendazole has nonlinear pharmacokinetics, with the exposures increased less than dose proportionally (2). Oxfendazole was absorbed fast, with its peak concentrations reached within 2 h after dose, and has a consistent elimination half-life ($t_{1/2}$) (8.50 to 11.0 h) across all doses evaluated (2). In addition, oxfendazole was found to undergo minimal renal excretion and moderate metabolism to oxfendazole sulfone, the major metabolite, and fenbendazole, the minor metabolite, in humans (2).

To optimize oxfendazole dosing regimens in patients, it is important to characterize the relationship among oxfendazole dose, exposure, and response. However, correlating oxfendazole dose to exposure is not straightforward because of the drug's nonlinear pharmacokinetics. Population pharmacokinetic models mathematically describe the underlying system and represent highly valuable tools during drug development for optimal dose selection and clinical trial design. To facilitate oxfendazole dose optimization, in the current study, we performed a secondary analysis of the FIH data with the aim of developing a population pharmacokinetic model to quantitatively characterize the complex pharmacokinetics of oxfendazole and its metabolites in humans.

RESULTS

Clinical data. Dose-normalized concentration-time profiles of oxfendazole in different dose groups are presented in Fig. 1. Overall, oxfendazole dose-normalized exposure in plasma decreased with increasing dose from 0.5 to 60 mg/kg, indicating that oxfendazole exposure is less than dose-proportional, most likely because of oxfendazole solubility-limited absorption.

FIG 1 Dose-normalized oxfendazole concentration versus time following single oral dose of oxfendazole at 0.5, 1, 3, 7.5, 15, 30, and 60 mg/kg. For each dose group, the mean concentrations are provided.

Population pharmacokinetic modeling. (i) Structural model. The history of model development with key structural models is summarized in Table S1 in the supplemental material.

Oxfendazole pharmacokinetics was best described by a one-compartment model with first-order absorption and elimination. Because dose-limited bioavailability represents the most probable cause of oxfendazole nonlinear pharmacokinetics, we evaluated three bioavailability (F) equations.

$$F = \exp(-\theta_1 \cdot \text{dose}) \quad (1)$$

$$F = 1 - \theta_1 \cdot \text{dose} / (\theta_2 + \text{dose}) \quad (2)$$

$$\log(F) = \theta_1 - \theta_2 \cdot \log(\text{dose}) \quad (3)$$

Where F is the fraction of dose that was absorbed and reached the systemic circulation, dose is the individual dose in milligrams, and θ_1 and θ_2 are positive coefficients estimated by NONMEM.

Equation 1 was associated with extreme bias in all goodness-of-fit plots (Fig. S1). Equation 2 performed better than equation 1; however, there remained some underprediction at the population level (Fig. S2), leaving equation 3 as the best candidate. To further simplify the model, the relative oral bioavailability was assumed to be 100% (i.e., $F = 1$) at the lowest dose (i.e., 34.7 mg) by adjusting the bioavailability equation to $\log(F) = -\theta_1 \cdot \log(\text{dose}/34.7)$.

With respect to oxfendazole sulfone pharmacokinetics, initially, the model included only a systemic metabolic pathway from oxfendazole to oxfendazole sulfone. However, this model resulted in an unrealistically high value of the absorption rate constant (991 h^{-1}), bias in conditional weighted residual (CWRES) diagnostic plots (Fig. S3), and overprediction of oxfendazole sulfone concentration around the peak of the concentration-time profile (C_{max}). Consequently, a more mechanistic model was constructed by introducing the presystemic metabolic pathway (Fig. 2), which resulted in a better fit of oxfendazole sulfone pharmacokinetic profile and a more reasonable estimate of the absorption rate constant.

Fenbendazole pharmacokinetics was modeled using one-compartment distribution with first-order formation and elimination. When the model was first evaluated

FIG 2 Pharmacokinetic model structure for oxfendazole and metabolites. CL, clearance; FEN, fenbendazole; F , bioavailability; FM_1 , fraction of oxfendazole clearance attributed to metabolism to oxfendazole sulfone; FM_2 , fraction of oxfendazole clearance attributed to metabolism to fenbendazole; k_a , absorption rate constant; k_e , elimination rate constant; $k_{\text{gut, OXF-SO}_2}$, rate constant of presystemic metabolism of oxfendazole to oxfendazole sulfone; OXF, oxfendazole; OXF-SO₂, oxfendazole sulfone; V , volume of distribution.

without BLQ observations (i.e., observations below the limit of quantification), a clear underprediction of the fraction of BLQ data was noted (Fig. S4). This bias was mitigated after all BLQ observations were included in the final model using the M3 method (23).

As shown in Fig. 2, the final model for oxfendazole and metabolites consisted of one depot compartment with dose-limited bioavailability, $\log(F) = -\theta_1 \cdot \log(\text{dose}/\theta_2)$, one compartment for oxfendazole with volume of distribution, V_{OXF} , and one compartment each for oxfendazole sulfone and fenbendazole with fixed volume of distribution of 1 liter. From the depot compartment, the majority of bioavailable oxfendazole dose was absorbed following first-order absorption process (k_a), while a small part of the oxfendazole dose underwent presystemic metabolism to oxfendazole sulfone with a first-order metabolic rate constant ($k_{\text{gut, OXF-SO}_2}$). Oxfendazole was eliminated from the body, with total clearance of CL_{OXF} . Some fractions of oxfendazole clearance, labeled FM_1 and FM_2 , were attributed to systemic metabolism to oxfendazole sulfone and fenbendazole, respectively. Oxfendazole sulfone and fenbendazole were eliminated from the body with first-order elimination, $k_{e, \text{OXF-SO}_2}$ and $k_{e, \text{FEN}}$, respectively.

Oxfendazole and metabolite pharmacokinetics was characterized by the following differential equations.

$$\frac{dA_{\text{OXF,depot}}}{dt} = -(k_a + k_{\text{gut, OXF-SO}_2}) \cdot A_{\text{OXF,depot}} \quad (4)$$

$$\frac{dA_{\text{OXF,body}}}{dt} = k_a \cdot A_{\text{OXF,depot}} - \frac{CL_{\text{OXF}}}{V_{\text{OXF}}} \cdot A_{\text{OXF,body}} \quad (5)$$

$$\frac{dA_{\text{OXF-SO}_2}}{dt} = k_{\text{gut, OXF-SO}_2} \cdot \frac{A_{\text{OXF,depot}}}{0.952} + \frac{FM_1 \cdot CL_{\text{OXF}}}{V_{\text{OXF}}} \cdot \frac{A_{\text{OXF,body}}}{0.952} - k_{e, \text{OXF-SO}_2} \cdot A_{\text{OXF-SO}_2} \quad (6)$$

$$\frac{dA_{\text{FEN}}}{dt} = \frac{FM_2 \cdot CL_{\text{OXF}}}{V_{\text{OXF}}} \cdot \frac{A_{\text{OXF,body}}}{1.05} - k_{e, \text{FEN}} \cdot A_{\text{FEN}} \quad (7)$$

$A_{\text{OXF,depot}}$ is the amount of oxfendazole in the depot compartment, $A_{\text{OXF,body}}$ is the amount of oxfendazole in the body, $A_{\text{OXF-SO}_2}$ is the amount of oxfendazole sulfone, and A_{FEN} is the amount of fenbendazole.

Of note, at the beginning of model development, first-order conditional estimation (FOCE), one of the earliest and most common estimation algorithms in NONMEM, was

implemented. However, FOCE failed to reliably evaluate parameter precision. Therefore, newer algorithms, stochastic approximation expectation-maximization (SAEM) and Monte Carlo importance sampling parametric expectation-maximization (IMP), were applied. As model development proceeded, estimation failed with IMP. Ultimately, parameters in the final model were estimated using the SAEM algorithm.

(ii) Stochastic model. Attempts were made to estimate interindividual variability on all pharmacokinetic parameters. Interindividual variability in F , k_a , $k_{\text{gut,OXF-SO}_2}$, CL_{OXF} , V_{OXF} , FM_2 , $k_{e,\text{OXF-SO}_2}$, and $k_{e,\text{FEN}}$ were significant. Data for each analyte were best fitted by a combined proportional and additive residual error model.

(iii) Covariate model. Based on graphical investigation, the effect of age on $k_{e,\text{OXF-SO}_2}$, the effect of creatinine clearance on F , and the effect of serum creatinine on CL_{OXF} were evaluated by forward addition and backward elimination. No covariate significantly affected oxfendazole and metabolites pharmacokinetics.

(iv) Model evaluation. Final parameter estimates are presented in Table 1. All pharmacokinetic parameters, interindividual variability, and residual variability were estimated with good precision. The percent relative standard error (%RSE) was below 30% for all pharmacokinetic parameters and below 40% for all but one interindividual variability terms and residual variability terms. Calculated shrinkage of interindividual variability and residual variability was no more than 25% (Table 1). Shrinkage of less than 20 to 30% suggested that diagnostic plots are informative and reliable for model evaluation (24). The condition number (ratio of the greatest to the smallest eigenvalue) of the final model was 81.69. A condition number of less than 1,000 indicated that the final model was not ill-conditioned or overparameterized (25).

To evaluate model fitting, model estimation of key pharmacokinetic parameters of oxfendazole, including maximum concentration (C_{max}), area under the concentration-time curve to infinity (AUC_{inf}), apparent volume of distribution (V_z/F), apparent clearance (CL/F), and elimination half-life ($t_{1/2}$), were compared with NCA results (Fig. 3). Overall, model predicted values and NCA estimated values were in close agreement.

Goodness-of-fit plots, including scatterplots of observed concentration versus population predicted concentration, observed concentration versus individual predicted concentration, CWRES versus time, and CWRES versus population predicted concentration for all analytes, are presented in Fig. 4. Goodness-of-fit plot for individual analyte are provided in Fig. S5 and S6. As shown in Fig. 4A and B, most points distributed evenly around the identity line without any specific pattern. Similarly, Fig. 4C and D show the normal distribution of CWRES with respect to concentration and time, except for some bias in fenbendazole CWRES at initial time points. This is not unexpected, since there was a high level of BLQ data for fenbendazole in the absorption phase, while CWRES was calculated only for quantifiable concentrations. To better evaluate model performance in capturing BLQ data, the distribution of normalized prediction distribution error (NPDE) versus time and population predicted concentration was assessed (Fig. S7). All fenbendazole concentrations, both quantifiable and BLQ, scattered evenly around the zero line without any noticeable bias, and most data points were within the 95% confidence band. In general, the final structural model was appropriate for oxfendazole and metabolites pharmacokinetics without major systemic bias.

Figure 5 illustrates the time course of the mean observed concentrations and population predicted concentrations of oxfendazole and metabolites in each dose group. There was good agreement between model prediction and observed values, except for the over-prediction of fenbendazole concentration in the lowest dose group (i.e., 0.5 mg/kg). The lack of fit in fenbendazole concentrations at 0.5 mg/kg can be explained by the scarcity of the data. Indeed, there were only 7 quantifiable fenbendazole concentrations, among which 5 belonged to the same subject.

Prediction-corrected visual predictive check of oxfendazole and metabolites are presented in Fig. 6, and prediction-corrected visual predictive checks for each analyte stratified by dose are presented in Fig. S8 to S11. As shown in Fig. 6A to C, the observed prediction-corrected concentrations scattered evenly around the simulated

TABLE 1 Final estimates of oxfendazole and metabolite pharmacokinetic parameters, interindividual variability, and residual variability

Parameter	Definition	Estimate	%RSE	%Shrinkage
θ_1	$\log(F) = -\theta_1 \log(\text{dose}/\theta_2)$; F , bioavailability	0.541	2.1	
θ_2 (mg)	One component for F estimation	34.7 FIX		
k_a (h^{-1})	Absorption rate constant	1.59	10.6	
CL_{OXF} (liters/h)	Oxfendazole total clearance	2.57	6.9	
V_{OXF} (liters)	Oxfendazole vol of distribution	35.2	6.1	
$k_{\text{gut,OXF-SO}_2}$ (h^{-1})	Presystemic metabolism rate constant	0.00134	9.7	
FM_1	Fraction of oxfendazole clearance attributed to metabolism to oxfendazole sulfone	0.00420	7.6	
$k_{\text{e,OXF-SO}_2}$ (h^{-1})	Oxfendazole sulfone elimination rate constant	0.105	5.1	
FM_2	Fraction of oxfendazole clearance attributed to metabolism to fenbendazole	0.000162	13.2	
$k_{\text{e,FEN}}$ (h^{-1})	Fenbendazole elimination rate constant	0.0942	12.7	
ω_{F1}^2	Variance of interindividual variability on F	0.172	16.9	3.1
ω_{ka}^2	Variance of interindividual variability on k_a	0.521	8.3	10.3
ω_{CLOXF}^2	Variance of interindividual variability on CL_{OXF}	0.0184	3.2	24.9
ω_{VOXF}^2	Variance of interindividual variability on V_{OXF}	0.0344	9.3	16.3
$\omega_{\text{kut,OXF-SO}_2}^2$	Variance of interindividual variability on $k_{\text{gut,OXF-SO}_2}$	0.637	15.6	6.6
$\omega_{\text{ke,OXF-SO}_2}^2$	Variance of interindividual variability on $k_{\text{e,OXF-SO}_2}$	0.0707	16.4	5.1
ω_{FM2}^2	Variance of interindividual variability on FM_2	0.527	23.7	9.4
$\omega_{\text{ke,FEN}}^2$	Variance of interindividual variability on $k_{\text{e,FEN}}$	0.493	34.1	18.0
$\sigma_{\text{prop,OXF}}^2$	Variance of oxfendazole proportional error	0.0255	7.4	11.8
$\sigma_{\text{add,OXF}}^2$	Variance of oxfendazole additive error	2.89	31.7	11.8
$\sigma_{\text{prop,OXF-SO}_2}^2$	Variance of oxfendazole sulfone proportional error	0.0208	8.8	12.0
$\sigma_{\text{add,OXF-SO}_2}^2$	Variance of oxfendazole sulfone additive error	4.46	32.9	12.0
$\sigma_{\text{prop,FEN}}^2$	Variance of fenbendazole proportional error	0.102	6.4	7.8
$\sigma_{\text{add,FEN}}^2$	Variance of fenbendazole additive error	0.640	8.5	7.8

50th percentile for all analytes. Additionally, for the most part, the 5th and 95th percentiles of the prediction-corrected observations were within the 95% confidence interval (CI) of the corresponding simulated percentiles. The observed 5th percentile for fenbendazole could not be calculated, because the fraction of BLQ was higher than 5%. To address this limitation, we compared the observed fraction of BLQ versus the simulated fraction of BLQ (Fig. 6D), since it is a more appropriate way to assess model predictive performance in the case of high BLQ level. As shown in Fig. 6D, the observed fraction of BLQ was within the 90% CI of the predicted BLQ fraction, indicating that the model predictive performance was acceptable.

DISCUSSION

Following the administration of single ascending doses of oxfendazole in healthy adults, substantial nonlinear pharmacokinetics was observed. Specifically, oxfendazole dose-normalized exposure (i.e., AUC_{inf}) decreased with increasing dose from 0.5 to 60 mg/kg, indicating that oxfendazole pharmacokinetics was less than dose proportional. As noted earlier, the source of the nonlinear pharmacokinetics of oxfendazole is likely from the absorption process. Oxfendazole is a biopharmaceutics classification system (BCS) class II drug with low solubility and high permeability (18). As the dose increases, the fraction of oxfendazole dose solubilized and absorbed decreases, leading to a decrease in bioavailability and, subsequently, a less than dose-proportional increase in oxfendazole exposures with an increase in doses. To characterize this mechanism, dose-limited bioavailability was incorporated in our population pharmacokinetic model, which successfully captured the nonlinear pharmacokinetics of oxfendazole. Solubility-limited bioavailability was not unique for oxfendazole. When albendazole, another benzimidazole anthelmintic, was administered to healthy volunteers at 400, 800, and 1,200 mg, the dose-normalized exposure of albendazole sulfoxide, the main moiety detected in plasma following albendazole oral administration, was lower at higher doses (26). Whether oxfendazole is a substrate of any influx transporters remains unknown, so the contribution of transporter saturation at high oxfendazole dose to oxfendazole nonlinear pharmacokinetics cannot be excluded.

At the lowest dose evaluated (i.e., 0.5 mg/kg), oxfendazole's apparent clearance was 2.57 liters/h, and the apparent volume of distribution was 35.2 liters (Table 1). Because

FIG 3 Oxfendazole major pharmacokinetic parameters following single ascending doses (0.5 to 60 mg/kg) obtained by noncompartmental analysis (black) and by population pharmacokinetic model fitting (gray). For each dose group, data are presented as means and standard deviations ($N=8$).

oxfendazole total clearance was much lower than the hepatic blood flow of 87 liters/h in human (27), oxfendazole can be classified as a low extraction ratio drug. The oxfendazole volume of distribution is between the human plasma volume (3 liters) and the total body water volume (42 liters) (27), suggesting that oxfendazole distribution is moderate. The interindividual variability in oxfendazole clearance and volume of distribution was low (less than 20%) (Table 1), which was expected given that all subjects were healthy and oxfendazole was dosed by body weight. Variability in the absorption rate constant was a little high ($\sim 70\%$) (Table 1), possibly due to the delayed absorption in a small number of subjects. While the median T_{max} was approximately 2 h, 6 out of 56 subjects in the study had prolonged T_{max} values ranging from 4 to 10 h. To capture the great difference in absorption window in a small fraction of subjects, a mixture model with one subpopulation experiencing a lower absorption rate than the other and an absorption model with a transit compartment (28, 29) were tested. The former model failed to converge, while the latter resulted in even higher estimates of interindividual variability in absorption. As the study population was quite homogenous, individual baseline characteristics cannot explain the differences in absorption rate. In addition, all subjects received oxfendazole after an overnight fast and were asked to remain fasted for 2 h after drug administration, so there was little chance for food-drug or drug-drug interaction. However, administration of drug on an empty stomach can reveal random interindividual variability in gastric motility. In contrast to the intense and continuous gastric motility in the fed state, the gastrointestinal tract in the fasted state stays in the quiescence phase, with some intermittent irregular contractions and sometimes short periods of intense contractile movement to empty the stomach of any residual solid (30). Where and for how long the drug is in the gastrointestinal tract

FIG 4 Goodness-of-fit plots for the final model of oxfendazole and metabolites. (A) Observed concentration versus population predicted concentration. (B) Observed concentration versus individual predicted concentration. (C) Conditional weighted residuals versus time. (D) Conditional weighted residuals versus population predicted concentration. The solid line represents the identity line (A and B) or zero line (C and D).

greatly influences drug absorption, because each gastrointestinal segment has distinct physiological characteristics. For example, gastrointestinal pH is most acidic in the stomach and gradually becomes neutral distally from the small intestine to the large intestine (30). Oxfendazole was predicted to be ionized at a pH below 3.03 (ADMET Predictor, SimulationPlus); thus, the longer the drug is in the stomach, the larger the fraction of solubilized drug.

In addition to the dose-limited bioavailability of oxfendazole, another key feature in the current model is the presystemic metabolism of oxfendazole to oxfendazole sulfone, which was uncovered based on population pharmacokinetic modeling without any *a priori* information specific to the mechanisms of oxfendazole metabolism. The enzyme(s) that is responsible for metabolizing oxfendazole to oxfendazole sulfone in mammals is unknown. Fortunately, information was available for the metabolism of albendazole to albendazole sulfoxide (31) and metabolism of fenbendazole to

FIG 5 Time course of population predicted concentrations (solid lines) versus observed concentrations (symbols) of oxfendazole, oxfendazole sulfone, and fenbendazole. In each dose group, the observed concentration is presented as means $\pm$ standard deviations.

FIG 6 Prediction-corrected visual predictive check for oxfendazole (A), oxfendazole sulfone (B), and fenbendazole (C and D). In panels A to C, the 5th, 50th, and 95th percentiles of the observed concentrations could only be calculated when the fraction of BLQ data were smaller than the percentile in question.

oxfendazole (32), which are very similar to the metabolism of oxfendazole to oxfendazole sulfone. Using human microsomes and recombinant enzymes, Rawden et al. showed that albendazole was metabolized to albendazole sulfoxide by flavin-containing monooxygenase (FMO) 3 and several cytochrome P450 (CYP) enzymes, principally CYP3A4 (31). In humans, albendazole presystemic metabolism is so extensive that albendazole sulfoxide, the active metabolite, is the major analyte detected in serum, whereas albendazole concentrations are mostly undetectable (33). Other studies using immunoinhibition and chemical inhibition of rat microsomal activity suggested the involvement of FMO and CYP3A in fenbendazole metabolism (32). Since FMO expression is ubiquitous (34–36) and CYP3A is highly expressed in the gastrointestinal tract (37, 38), the importance of gut metabolism in oxfendazole sulfone formation from oxfendazole is plausible. More studies on mechanisms of oxfendazole metabolism (e.

g., *in vitro* testing with recombinant enzymes and/or enzyme-specific inhibitors) are warranted to validate the role of presystemic metabolism in oxfendazole and oxfendazole sulfone disposition.

Despite the high degree of BLQ in fenbendazole data, the model could capture the fenbendazole concentration-time profile reasonably well in most dose groups. Inadequate quantifiable fenbendazole concentrations in the 0.5-mg/kg dose group rendered the evaluation of model performance at this dose level challenging. Moreover, the high degree of BLQ data also resulted in high estimation of interindividual variability in fenbendazole formation rate (70.2%) and elimination rate (72.6%) (Table 1). To obtain more robust data, analysis of fenbendazole concentrations following the administration of low oral oxfendazole doses with a more sensitive assay is warranted.

Based on the typical value of oxfendazole clearance (2.57 liters/h) and volume of distribution (35.2 liters), the oxfendazole elimination rate constant is estimated to be 0.073 h^{-1} . The oxfendazole sulfone elimination rate constant (0.105 h^{-1}) and fenbendazole elimination rate constant (0.0942 h^{-1}) were slightly higher than the oxfendazole elimination rate constant, meaning that oxfendazole sulfone and fenbendazole pharmacokinetics are formation rate limited. An FM_1 of 0.0042 and FM_2 of 0.000162 (Table 1) suggested that less than 1% of oxfendazole was metabolized to fenbendazole and oxfendazole sulfone. This was in line with preclinical pharmacokinetic studies showing that oxfendazole was excreted primarily through biliary excretion, and the majority of oxfendazole excreted was under the form of either free amine or 4'-hydroxylated oxfendazole present as sulfate or glucuronide conjugates (18).

In conclusion, we have successfully developed, for the first time, a comprehensive population pharmacokinetic model that adequately captures the concentration-time profiles of both oxfendazole and its two metabolites in healthy adults over a wide oral dose range. Our final model incorporated mechanistic characterization of dose-limited bioavailability as well as different oxfendazole metabolic processes, providing insight into the significance of presystemic metabolism in oxfendazole and metabolite disposition. The model can be used to simulate oxfendazole and metabolite exposure under different dosing regimens, an important step to optimizing oxfendazole dosing regimens.

MATERIALS AND METHODS

Clinical data. The data used for population pharmacokinetic model development were from an oxfendazole FIH study that we reported recently (2). The key information of this FIH study is briefly summarized below.

This was a randomized, double-blind, placebo-controlled, single-ascending-dose study with 7 groups administered single oral oxfendazole doses of 0.5, 1, 3, 7.5, 15, 30, and 60 mg/kg. In each group, 10 healthy adults from 18 to 45 years of age were randomized at a 1:4 ratio to receive either placebo or a single oral oxfendazole dose in the fasted state (2). Subject's demographics at baseline were published recently (2) and are provided in Table S2 in the supplemental material. Most subjects were males. Subjects' weight, height, body mass index (BMI), serum creatinine, and creatinine clearance were within normal ranges. Baseline characteristics were similar among dose groups.

For pharmacokinetic assessment, blood samples were collected at predetermined time points at pre-dose and at 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 120, 168, and 336 h postdose (2). Oxfendazole and metabolites in biological samples were simultaneously quantified by a validated ultraperformance liquid chromatography-mass spectrometry (UPLC-MS) method with a lower limit of quantification (LLOQ) of 2 ng/ml and linear range of 2 to 1,000 ng/ml. Intraday and interday accuracy were in the range of 90.4 to 108.3%, and the coefficient of variation was less than 7.9% for all analytes. Samples above the limit of quantification were diluted with blank plasma and reanalyzed. All samples were analyzed within the validated stability window.

In total, 2,352 concentrations were collected for oxfendazole, oxfendazole sulfone, and fenbendazole in plasma (784 concentrations for each analyte). Among the measured plasma concentrations, the number of BLQ concentrations were 150 (19.1%), 173 (22.1%), and 401 (51.1%) for oxfendazole, oxfendazole sulfone, and fenbendazole, respectively. Since oxfendazole and metabolite had minimal urinary excretion (2), urine data were not included in our population pharmacokinetic analysis.

Population pharmacokinetic modeling. Nonlinear mixed-effect modeling was performed in NONMEM 7.4 (Icon Development Solutions, Ellicott City, MD) with user-defined subroutine ADVAN13. Various estimation methods, including FOCE with interaction, IMP with interaction, and SAEM with interaction, were attempted to estimate the population mean of pharmacokinetic parameters, interindividual

variability, and residual variability. Modeling was performed on a parallel system with 16 CPUs, and MU referencing was implemented to enhance modeling efficiency. The PsN 4.8.0 (Uppsala Pharmacometrics Group) interface with Pirana 2.9.9 was used to perform prediction-corrected visual predictive check. SigmaPlot 13.0 (Systat Software, San Jose, CA) and RStudio 4.0.2 (RStudio, PBC, Boston, MA) were employed for data handling and graphical analysis.

Structural model. The structural model was explored sequentially for the parent drug and each metabolite, but pharmacokinetic parameters were fitted simultaneously.

(i) Modeling oxfendazole data. Different compartmental models (i.e., one-compartment and two-compartment) were explored. To capture oxfendazole at less than dose-proportional exposure, several equations correlating oxfendazole bioavailability to dose were evaluated. The rates of absorption and elimination were assumed to be first order.

Among the three evaluated equations, equation 3 best fit oxfendazole nonlinear pharmacokinetics. To reduce model complexity, F was fixed at 1 at the lowest dose of 34.7 mg (corresponding to a subject weighing 69.4 kg in the 0.5-mg/kg dose group).

(ii) Simultaneous modeling of oxfendazole and oxfendazole sulfone data. To the final structural model of oxfendazole, one compartment was added for oxfendazole sulfone. To make the model identifiable, oxfendazole sulfone volume of distribution was fixed at 1 liter. To adjust for the difference in molecular weight between oxfendazole and oxfendazole sulfone, the amount of oxfendazole sulfone was multiplied by 0.952, which is the molecular weight ratio between oxfendazole and oxfendazole sulfone. In the first attempt, the fraction of oxfendazole clearance through systemic metabolism to oxfendazole sulfone was characterized by FM_1 . During model development, a second model with the addition of pre-systemic metabolism was found to better capture oxfendazole sulfone pharmacokinetics.

(iii) Simultaneous modeling of oxfendazole, oxfendazole sulfone, and fenbendazole data (final model). To the model of oxfendazole and oxfendazole sulfone with both presystemic and systemic metabolism, one compartment was added for fenbendazole. Fenbendazole volume of distribution was fixed at 1 liter, and the systemic metabolism of oxfendazole to fenbendazole was characterized by FM_2 . The amount of fenbendazole was multiplied by 1.05, the molecular weight ratio of oxfendazole to fenbendazole, to account for the difference in molecular weight between parent and metabolite. A noticeable feature of fenbendazole data is the high level (more than 50%) of BLQ observations. Therefore, the M3 method, with which all BLQ data would be retained (23), was used for population parameter estimation.

Stochastic model. (i) Interindividual variability. Interindividual variability was evaluated using an exponential model.

$$P_i = TVP \cdot \exp(\eta_i) \quad (8)$$

where TVP represents the population mean of a pharmacokinetics parameter, P_i represents the individual estimate of the pharmacokinetics parameter, and η_i represents interindividual variability, which is assumed to have a normal distribution with a mean of 0 and variance of ω^2 .

(ii) Residual variability. Proportional error model, additive error model, and combined additive and proportional error model were evaluated to model residual variability. The combined and additive error model has the general form

$$C_{ij} = \bar{C}_{ij} \cdot (1 + \epsilon_{1ij}) + \epsilon_{2ij} \quad (9)$$

where $\bar{C}_{ij}$ represents the predicted concentration of individual i at time j , C_{ij} represents the observed concentration, ϵ_{1ij} represents the proportional error, and ϵ_{2ij} is the additive error. ϵ_{1ij} and ϵ_{2ij} were assumed to be normally distributed with a mean of 0 and variance of σ_1^2 and σ_2^2 , respectively. For proportional error model and additive error model, ϵ_{2ij} and ϵ_{1ij} , respectively, were fixed at 0.

Covariate model. Covariates including weight, BMI, height, age, serum creatinine, and creatinine clearance were first explored by visual inspection of interindividual variability versus covariate plots. Potential covariates identified during exploratory visualization were then assessed using stepwise forward addition followed by backward elimination. For forward addition, a decrease in objective function value (OFV) of more than 6.63 is considered significant improvement in model performance at $\alpha = 0.01$ and 1 degree of freedom. For backward elimination, an increase in OFV of more than 10.83 is considered significant at $\alpha = 0.001$. Sex effect was not evaluated, because most subjects were males (54 males versus 2 females in the oxfendazole group). Since all covariates were continuous variables, covariates were normalized to the population mean values and modeled using the general equation

$$TVP_i = TVP \cdot (\text{cov}_i / \text{cov}_m)^\theta \quad (10)$$

where TVP_i represents the individual pharmacokinetic parameter, TVP is the population mean pharmacokinetic parameter, cov_i is the individual covariate, cov_m is the population mean of the covariate, and θ is the covariate effect.

Model evaluation. Model selection was based on feasibility and precision of parameter estimates and goodness-of-fit plots, including scatterplots of (i) population predicted concentration versus observed concentration, (ii) individual predicted concentration versus observed concentration, (iii) CWRES versus population predicted concentration, and (iv) CWRES versus time. For a good model, all data points will distribute evenly around the identity line in the first two plots and around the 0 line in the latter two plots without any bias. Because CWRES are not calculated for BLQ observations and the

level of BLQ was high in our data set, model validation was enforced by normalized prediction distribution error (NPDE) analysis (39). In NPDE analysis, NPDE was calculated for all observations, including BLQ data, and scatterplots of NPDE versus time and population predicted concentration were investigated. For a good model, NPDE values will scatter evenly around the zero line without any bias, and most NPDE values should be within the range of -1.96 to 1.96 , corresponding to the 95% confidence interval of a normal distribution with a mean of 0 and variance of 1. To compare competing nested models, a decrease in OFV of at least 3.84 is considered significant improvement in model performance at $\alpha = 0.05$. For nonnested models, the model with smaller Akaike information criterion (AIC) was chosen.

Model evaluation was complemented by comparing the model-estimated pharmacokinetic parameters with NCA results reported previously (2). As this is a population pharmacokinetic model of different dose levels, predictive performance was assessed via prediction-corrected visual predictive check (40) with 1,000 simulations.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 3.1 MB.

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