Pharmacokinetics, Safety, and Tolerability of Oxfendazole in Healthy Volunteers: a Randomized, Placebo-Controlled First-in-Human Single-Dose Escalation Study

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Abbreviations: FIH, First-in-human; CNS, central nervous system; CDC, Centers for Disease
Control and Prevention; AE, adverse event; NOAEL, no-observed-adverse-effect-level; LLOQ,
lower limits of quantification; SPE, solid phase extraction; LC/MS, liquid chromatography mass
spectrometry;
Abstract

Cysticercosis is a parasitic disease that frequently involves the human central nervous system (CNS) and current treatment options are limited. Oxfendazole, a veterinary medicine belonging to the benzimidazole family of anthelmintic drugs, has demonstrated substantial activity against the tissue stages of *Taenia solium* and has potential to be developed as an effective therapy for neurocysticercosis. To accelerate the transition of oxfendazole from veterinary to human use, the pharmacokinetics, safety and tolerability of oxfendazole were evaluated in healthy volunteers in this Phase 1 first-in-human (FIH) study. Seventy subjects were randomly assigned to receive a single oral dose of oxfendazole (0.5, 1, 3, 7.5, 15, 30 or 60 mg/kg) or placebo and were followed for 14 days. Blood and urine samples were collected and the concentrations of oxfendazole were measured using a validated ultra-performance liquid chromatography mass spectrometry method. The pharmacokinetic parameters of oxfendazole were estimated using noncompartmental analysis. Oxfendazole was rapidly absorbed with mean plasma half-life ranging from 8.5 to 11 hours. The renal excretion of oxfendazole was minimal. Oxfendazole exhibited significant nonlinear pharmacokinetics with less than dose-proportional increases in exposure after single oral doses 0.5 mg/kg to 60 mg/kg. This nonlinearity of oxfendazole is likely due to the dose-dependent decrease in bioavailability that is caused by its low solubility. Oxfendazole was found to be well-tolerated in this study at different escalating doses without any serious adverse events (AEs) or deaths. There were no significant differences in the distribution of hematology, biochemistry or urine parameters between oxfendazole and placebo recipients.
INTRODUCTION

Cysticercosis is a parasitic tissue infection caused by larval cysts of the cestode *Taenia solium* (pig tapeworm). Whereas consumption of undercooked pork from a pig infected with *Taenia solium* can lead to pork tapeworm infection, ingestion of *Taenia solium* eggs from a person infected with pork tapeworm can lead to cysticercosis. These larval cysts can infect various human tissues, including muscle, eyes, skin, and most seriously, the CNS. Neurocysticercosis can cause a variety of neurologic symptoms including seizures, headaches and hydrocephalus (1). Neurocysticercosis is common in developing countries throughout Latin America, Indonesia, Africa and parts of India. WHO estimates that 2.56-8.30 million persons suffer from neurocysticercosis world-wide (2). In Mexico, Ecuador, and Brazil, the prevalence of neurocysticercosis found at autopsy exceeds 1% (3, 4). In Mexico and Peru, 12% of acute neurological hospital beds are occupied by patients with neurocysticercosis (5, 6). In addition, it has been reported that neurocysticercosis is the most important cause of acquired epilepsy in developing countries and probably in the world (7). In the US, neurocysticercosis is diagnosed relatively commonly in hospitals that treat large Hispanic populations, and the number of reported cases is increasing, probably as a result of widespread access to neuroimaging and of increased immigration of *Taenia solium*-infected individuals (8-10). In the US, cysticercosis represents a group of five parasitic diseases that have been targeted by the Centers for Disease Control and Prevention (CDC) for public health action (11).

For many years, therapeutic approaches to neurocysticercosis were limited to the use of steroids and surgery for the relief of intracranial hypertension. Praziquantel is the first antiparasitic drug that was identified to have the ability to kill intracerebral *Taenia solium* cysts (12, 13). Later, albendazole was found to be more effective, and it became widely used because of its efficacy...
For complicated infections, a combination of praziquantel, albendazole and steroids is recommended. However, both praziquantel and albendazole require prolonged duration of therapy, and complete cure is difficult to achieve. A course of one or two weeks of albendazole results in the destruction of 60 to 70% of brain cysts, but complete clearance of all brain cysts is obtained in less than 40% of cases. Because the current treatment for neurocysticercosis is far from ideal, there is a great unmet clinical need for an alternative treatment.

Oxfendazole, [5-(phenylsulphonyl)-1H-benzimidazole-2-yl] carbamic acid methyl ester, a structure similar to that of albendazole, is a broad-spectrum anthelmintic which is currently marketed for use against lungworms and enteric helminths in beef livestock. Compared with albendazole, oxfendazole has demonstrated a longer plasma half-life in animals, suggesting that oxfendazole might maintain effective concentrations in the body for a longer period of time. In several preclinical studies conducted in pigs, oxfendazole demonstrated substantial activity against the tissue stages of *Taenia solium* (cysticercosis) even with only a single oral dose. Data comparing antiparasitic regimes in pigs demonstrates complete clearance of cysts in the pig carcass and partial clearance of brain cysts after only a single dose of oxfendazole, clearly superior to the effect of single dose albendazole or single dose praziquantel. These data suggest that oxfendazole has the potential to be used as an effective treatment for cysticercosis in humans. In addition to the encouraging efficacy data, oxfendazole has shown a favorable safety profile based on safety data accumulated in livestock and, more recently, in a series of formal and comprehensive safety and toxicology studies conducted in dog, rat and mouse. Based on its promising preclinical efficacy and safety profiles, as well as its potentially favorable pharmacokinetic profile in animals, oxfendazole
represents an attractive anthelmintic candidate for transition to human use for the treatment of neurocysticercosis and other helminthic infections. As part of the clinical development of oxfendazole, the pharmacokinetics, safety and tolerability of oxfendazole after single oral doses were evaluated in healthy volunteers in a phase 1 first-in-human (FIH) study. Here we are presenting the results from this FIH study.
MATERIALS AND METHODS

The study was carried out at the University of Iowa and was approved by the University of Iowa Institutional Review Board. Study activities were performed in accordance with the prevailing Declaration of Helsinki and good clinical practice guidelines. The study protocol was registered on ClinicalTrials.gov (NCT02234570). All subjects provided informed consent prior to any study-related procedures.

Subjects

Eligible participants were men or non-pregnant women 18-45 years of age who were in good health at the time of enrollment based on medical history, physical examination, electrocardiogram and screening blood hematology, blood chemistry, HIV, hepatitis B and C, serology and urine chemistry and, for women, pregnancy testing. Males were required to ensure use of condoms and spermicides for 4 months after study drug administration. Exclusion criteria included breastfeeding females, history of sensitivity to benzimidazole compounds, history of residence for ≥ 6 months in regions with endemic cysticercosis, use of chronic systemic medications, receipt of an experimental agent within 30 days of enrollment, history of chronic tobacco use, or alcohol consumption (greater than 7 alcoholic drinks per week) or any illicit drug use.

Study Design

This was a randomized, double-blinded, placebo-controlled, phase I study to evaluate the pharmacokinetics, safety and tolerability of single escalating doses of oxendazole in healthy subjects. Seventy subjects participated in this study in seven dose groups (0.5, 1, 3, 7.5, 15, 30 or 60 mg/kg). In each group, ten subjects were randomized in a 4:1 ratio such that eight subjects...
received oxfendazole and two subjects received placebo. The schematic diagram of the study
design is shown in Figure 1.
The starting dose of 0.5 mg/kg was selected based on the standard 10-fold safety margin from
the no-observed-adverse-effect-level (NOAEL) exposure from rat per regulatory guidance. Two
sentinel subjects (one randomized to receive oxfendazole and the other to receive placebo) were
dosed and monitored for 48 hours for adverse events (AEs) prior to enrolling the remaining
subjects in each group. Dose escalation proceeded after evaluation of the 2-week safety and
tolerability result from the preceding group. The final maximum dose (60 mg/kg) was selected
such that the anticipated human maximum observed plasma exposure did not exceed the NOAEL
exposures.
Pharmacokinetics assessment - blood samples for measurements of oxfendazole and its
metabolites were collected on study Day 1 prior to oral dosing, and at 1, 2, 4, 6, 8, 10 and 12
hours post-dosing and on Days 2 (between 24-32 hours), 3 (between 48-60 hours), 4, 6, 8 and 15.
Total urine collection was performed for quantification of oxfendazole and its metabolites prior
to dosing, and at 0-4, 4-8, 8-12, and 12-24 hours.
Safety assessment - on study Day 1, before administration of study drug, blood was drawn for
safety laboratory tests, and urine dipstick was collected for protein and glucose. Subjects were
assessed for AEs on Days 1 through 6 and on Days 8 and 15. AEs were graded as mild (Grade
1), moderate (Grade 2) or severe (Grade 3). In addition to study Day 1, blood for safety
laboratory tests was checked on Days 4, 8 and 15. Electrocardiograms were performed at
screening and on Days 4, 8 and 15.
Study Drug
Oxfendazole suspension Synanthic® was purchased from Animal Health International (Greeley, CO) in two concentrations 9.06% (w/v), which was used for the 0.5, 1.0, 3.0, and 7.5 mg/kg doses and 22.5% (w/v), which was used for the 15, 30 and 60 mg/kg doses. The placebo formulation consisted of polyethylene glycol, methyl paraben, and sterile water (SRI International). All study drugs (oxfendazole and placebo) were administered orally under fasting condition.

**Bioanalytical Methods**

The concentrations of oxfendazole, and its metabolites, namely fenbendazole and oxfendazole sulfone, in plasma and urine were determined using a validated ultra-performance liquid chromatography mass spectrometry (UPLC-MS) method. Albendazole was used as the internal standard. Oxfendazole and metabolites were extracted from plasma and urine samples using a solid phase extraction (SPE) procedure utilizing Bond Elut Plexa PCX cartridges (30 mg, 1mL volume, Agilent Technologies). Briefly, samples were spiked with the internal standard albendazole (final concentration was 200 ng/mL) then diluted with 500 µL of 4% H₃PO₄ in water, vortexed and loaded onto the SPE cartridge. The SPE cartridge was rinsed with 1 mL of 2% formic acid in water followed by 1 mL of methanol:acetonitrile (1:1, v/v). The analytes were eluted with 1 mL of 5% ammonium hydroxide in methanol:acetonitrile (1:1, v/v). Eluates were dried and the residue was reconstituted with 200 µl of the mobile phase. Reconstituted samples were sonicated for 5 minutes and transferred into LC vials. 2 µL of the supernatant was injected into the UPLC-MS system for analysis.

Quantitation was performed using a Waters Acquity UPLC system (Waters, Inc., Milford MA) interfaced to a Shimadzu LC-MS 2010A LC-MS (Shimadzu Inc., Columbia MD). The LC-MS was operated in positive ionization mode utilizing an electrospray interface. Separation was
achieved using a Waters Acquity UPLC BEH C18 column (2.1 x 100 mm, 1.7 µm, Waters Inc., Milford MA). The mobile phase consisted of an aqueous component (A) of water containing 0.1% formic acid and an organic component (B) of methanol with 0.1% formic acid, delivered at a total flow rate of 0.35 mL/min. The gradient elution started with 20% mobile phase B, then increased it to 85% in 7.5 min and increased it to 95% mobile phase B at 9 min, where it was held for 1 min. The system was equilibrated back to 20% mobile phase B for 2 min with a total run time of 12 min. The retention times for oxfendazole, fenbendazole, and oxfendazole sulfone were 4.2 min, 6.3 min and 4.4 min, respectively. The assay was linear from 2 to 1000 ng/ml for all analytes. Analysis of quality control samples at 6, 80 and 700 ng/mL (N=15) had inter-day coefficients of variation ranging from 3.1% to 7.9% for plasma samples and 2.2% to 8.0% for urine samples. Samples with analyte concentrations that exceeded the upper limit of quantitation were diluted and re-assayed. In addition, glucuronide and sulfate conjugates of oxfendazole in urine were analyzed by incubating urine samples with β-glucuronidase from *Escherichia coli* (100 units) or sulfatase from *Helix pomatia* (200 units) in the presence of D-saccharic acid and assaying free oxfendazole both before and after the incubation. Values that were less than the lower limits of quantification (LLOQ) for a given run were reported as zero. All samples were stored at −80°C. The storage stability was 4.5 months. All samples were analyzed within the validated storage stability.

Pharmacokinetics Analysis

The pharmacokinetic parameters of oxfendazole and metabolites in plasma and urine were calculated using non-compartmental analysis with Phoenix WinNonlin 8.0 (Certara, Princeton, NJ, USA). Only analyte plasma concentrations greater than the respective LLOQ for the assay were included in the pharmacokinetic analysis. The maximum observed plasma concentration
(C\text{\textsubscript{\text{max}}}) and time to \text{Cmax} (T\text{\textsubscript{\text{\text{max}}}}) were determined directly from the plasma concentration-time data. The area under the curve (AUC\text{\textsubscript{\text{0,-\infty}}}) was estimated using the linear-up log-down trapezoidal method from 0-\text{t\textsubscript{last}} and extrapolation from \text{t\textsubscript{last}} to infinity based on the observed concentration at the last time point divided by the terminal elimination rate constant (\lambda\text{\textsubscript{\text{e}}}). The half-life (t\textsubscript{1/2}) was calculated using the formula of 0.693/\lambda. Apparent clearance (CL/F) was calculated as dose/AUC\text{\textsubscript{0,-\infty}} and apparent volume of distribution (V\text{\textsubscript{\text{d}}}/F) was calculated as dose/(\lambda*AUC\text{\textsubscript{0,-\infty}}). In addition, the apparent fraction of unchanged oxfendazole recovered in urine (f\text{\textsubscript{e'}}) and renal clearance (CL\text{\textsubscript{R}}) were determined based on the urine data. Glucuronide- and sulfate-conjugated analyte urine concentrations were evaluated by subtracting evaluated free oxfendazole from total oxfendazole concentrations. Estimated concentrations below 0 were set to 0. The fraction of dose excreted through 24 hours was expressed as a percentage of dose.

**Statistical Analysis**

Descriptive statistics were performed for demographic parameters. Formal comparisons between dose groups were not made. Analyte plasma concentrations and pharmacokinetics parameters were summarized by and compared among dosing cohorts using descriptive statistics. The relationship between \text{C\text{\textsubscript{\text{max}}}}, AUC\text{\textsubscript{0,-\infty}} and dose, i.e., dose proportionality, were examined using the power model, i.e. \(P = a \times \text{Dose}^b\) where \(P\) represents the parameter and \(a\) and \(b\) are constants. A value of \(b\) of approximately 1 suggests linear pharmacokinetics.
RESULTS

There were total of 70 participants, and no subjects were discontinued or terminated early from the study. The study subjects were predominantly male (67/70, 96%) and Caucasians (57/70, 81%). As shown in Table 1, distributions of gender, ethnicity and race were similar when comparing oxfendazole (n=56) to placebo (n=14) recipients. The overall mean subject age was 25.6 (range: 18-45 years). Mean body weight for oxfendazole recipients was 82.0 kg (range: 60.0 kg to 128.0 kg). Mean body weight by dose group ranged from 75.3 kg for the 7.5 mg/kg dose group to 86.3 for the 30 mg/kg dose group.

The structure of oxfendazole and its metabolites, as well as the proposed metabolism pathways (Based on the metabolic scheme shown in the UN FAO document (24)) are shown in Figure 2. Mean oxfendazole plasma concentration-time profiles following single doses in human are presented in Figure 3. Oxfendazole pharmacokinetic parameters and pharmacokinetic exposures obtained from the non-compartmental analysis are summarized in Table 2. Following oral dose administration, the intestinal absorption of oxfendazole was fast, with its plasma concentrations reaching peak level within 2 hours. Mean terminal phase elimination half-life of oxfendazole ranged from 8.5 to 10.3 hours, on average, across all doses evaluated in the study. After single oral dose administration, oxfendazole Cmax increased from 944 ng/mL in 0.5 mg/kg dose group to 6770 ng/mL in 60 mg/kg dose group. As shown in Table 2 and Figure 4a, this increase in oxfendazole Cmax with the increase of dose is clearly less than dose-proportional, indicating the nonlinear pharmacokinetics of oxfendazole. In the dose schedule evaluated, Cmax plateaued at approximately 15 mg/kg. Similarly, with increase in dose, the AUC$_{0-\infty}$ of oxfendazole also increased in a less than dose-proportional manner (Table 2 and Figure 4b). Estimates of power model exponents were significantly different from the value of 1 that would be expected under
In addition to the parent drug, two Phase I metabolites of oxfendazole (oxfendazole sulfone and fenbendazole) were also evaluated. Oxfendazole sulfone plasma concentrations were found to be low across all seven dose groups, with the $C_{\text{max}}$ being between 5.6% (3 mg/kg group) and 7.1% (60 mg/kg group) of oxfendazole $C_{\text{max}}$. The fenbendazole plasma exposure was even lower, with the ratio of fenbendazole to oxfendazole being <0.4% in all dose groups for $C_{\text{max}}$ and <0.7% in all dose groups for AUC$_{0-\infty}$. The profiles of parent and metabolites look similar in all dose groups.

The mean ± SD plasma concentrations of oxfendazole and its Phase I metabolites following 15 mg/kg dose of oxfendazole are presented as an example (Figure 5). As shown in Figure 5, the terminal slope of oxfendazole sulfone appears to be in parallel with oxfendazole, indicating that oxfendazole sulfone undergoes formation-rate limited elimination. The elimination of fenbendazole is difficult to evaluate due to its very low concentration.

In addition to plasma, urine excretion of oxfendazole, oxfendazole sulfone and fenbendazole in urine were also evaluated. Figure 6 shows the time course of the cumulative amount of unchanged oxfendazole excreted in urine within 24 hours. The plot reached plateau at the last time point, indicating that most of the unchanged oxfendazole was excreted within 24 hours. The mean fraction of free oxfendazole excreted in urine (fe) was less than 1% of the dose across all 7 groups. The calculated renal clearance (CL$_R$) of oxfendazole is low, with the values ranging from 9.42 to 16.3 mL/h across different doses (Table 2). The urine excretion of oxfendazole sulfone is also low, although the concentrations ≥LLOQ were found in multiple participants in each dose group through the 48-60 hour spot urine collection. Fenbendazole concentrations in the urine were BLQ for the majority of subjects at all time points.
In addition to Phase I metabolites of oxfendazole, two Phase II metabolites, namely oxfendazole glucuronide and oxfendazole sulfate, were also detected in urine samples. As shown in Figure 7, both oxfendazole glucuronide and oxfendazole sulfate were recovered in urine, although the levels of both were found to be low.

The safety results are summarized in Table 3. In total, 16 unsolicited AEs were reported by ~14% of the subjects (9 in the study group and 1 placebo). Thirteen (~81%) were classified as mild and 3 (~19%) as moderate (Table 3). The moderate AEs included viral gastroenteritis, arthralgia and sore throat in one subject each in 1 mg/kg, 15 mg/kg and 60 mg/kg oxfendazole groups respectively. Of these moderate events, only the sore throat was determined to be related to the study drug. In total, 6 events (38% of all events) reported by 4 subjects (5 events in 3 oxfendazole recipients and 1 event in 1 placebo recipient) were considered related to the product with 3 subjects experiencing GI symptoms including one subject in each of the 30 mg/kg and 60 mg/kg groups and one subject in the placebo group. The subject in the 60 mg/kg group experienced 2 separate GI symptoms (diarrhea and flatulence) as well as sore throat (as mentioned above). The other drug related AE included a transient mild prolongation of the PR interval on Day 15 in a subject who received 3 mg/kg oxfendazole. The PR interval later returned to baseline spontaneously, without treatment. ECG changes were not observed in any other subject.

The difference between the proportion of subjects who experienced AEs in the placebo group, 1/14 (7.1%; CI 0.2-33.9), when compared to the oxfendazole recipients, 9/56 (16.1%; CI 7.6-28.3), was not statistically significant (p=0.6742; Fisher’s exact test). Furthermore, there were no significant differences in the AE incidence rates with increasing doses of oxfendazole. No severe or serious AEs or deaths were reported during the study period.
There were 40 subjects who experienced one or more abnormal clinical laboratory values, among which 14, 32 and 3 subjects experienced abnormal hematology, biochemistry and urine values respectively. There were no significant differences in the distribution of hematology, biochemistry or urine parameters when comparing oxendazole to placebo recipients (Fisher’s Exact p=0.1353, p=0.3810 and p= 0.4936 for hematology, biochemistry and urine parameters, respectively). Among the observed abnormal clinical laboratory values, 11 were moderate but clinically insignificant, including moderate grade leukocytosis or leukopenia, neutropenia, or eosinophilia, or changes in bicarbonate or AST. One subject had persistently prolonged APTT values. Hematology was consulted and diagnosed a factor XI or XII deficiency syndrome thought unrelated to study product and of long standing duration. All other assessments considered abnormal were mild or were outside of normal range but did not meet toxicity grading.
DISCUSSION

Cysticercosis is one of the parasitic diseases that has been targeted by the CDC as a priority for public health action due to the severity of the illness and large number of people infected. The infection of *Taenia solium* in brain (i.e. neurocysticercosis) is particularly serious because of the high morbidity associated with the infection, and cure rates are suboptimal (15). Albendazole, a compound belonging to the benzimidazole family of anthelmintic drugs, currently represents the treatment of choice for neurocysticercosis. Despite its extensive use in neurocysticercosis, albendazole is not ideal due to its varied and poor efficacy profiles (15). Oxfendazole, a veterinary medicine which shares structural similarities with albendazole, has demonstrated potent broad-spectrum anthelmintic activities in preclinical models, including substantial activity against the tissue stages of *Taenia solium* (20, 21) and has great potential to be developed as an effective therapy for cysticercosis including neurocysticercosis. To accelerate the transition of oxfendazole from veterinary use to human use, a series of clinical trials have been planned to evaluate the pharmacokinetics, safety and efficacy of oxfendazole in human. Here we report the results of oxfendazole pharmacokinetics and safety from the FIH study conducted in healthy volunteers.

In this study, single oxfendazole doses up to 60 mg/kg were found to be well-tolerated without any serious AEs or deaths. There was no statistically significant difference between the treatment group and the placebo in the proportion of patients experiencing AEs. There may have been a minor trend toward more GI symptoms in the 30 and 60 mg/kg oxfendazole group. However, this finding may be associated with the polyethylene glycol in the formulation, since these two groups received higher volumes of study product. Similarly, there were no significant differences in the distribution of hematology, biochemistry or urine parameters between oxfendazole and...
placebo recipients or trends to suggest that an increasing dose of oxfendazole was associated with laboratory abnormalities.

Oxfendazole exhibited significant nonlinear pharmacokinetics with less than dose-proportional increases in exposure after single oral doses of 0.5 mg/kg to 15 mg/kg. The exposure of oxfendazole appeared to reach plateau and was not dependent on the dose given following the doses of 15 mg/kg or higher. Despite the fact that oxfendazole exposure increased in a less than dose-proportional manner, the terminal half-lives remained similar across all doses, indicating that source of the nonlinearity is from its absorption process, rather than its elimination phase.

Accordingly, calculated apparent clearance (CL/F) varied greatly among groups, and differences were very likely due to decreased bioavailability (i.e. absorption process) with increases in doses rather than changes in real clearance of the drug (i.e. elimination process). Similar to albendazole and other benzimidazole compounds, oxfendazole is a Biopharmaceutics Classification System (BCS) class II drug (i.e. drug with low solubility and high permeability). Therefore, the observed oxfendazole nonlinear pharmacokinetics was very likely due to its low water solubility. With increase in dose, the fraction of dose that was solubilized in gastrointestinal fluid and available to be absorbed may be decreased, resulting in decreased bioavailability. At higher doses, the amount of oxfendazole absorbed may have reached its limit and consequently was no longer dependent on the dose given; this potentially could explain why oxfendazole exposure reached plateau in those three high dose groups (i.e. 15 mg/kg, 30 mg/kg, and 60 mg/kg).

The phenomenon of low solubility mediated nonlinear pharmacokinetics is not unique to oxfendazole. Albendazole demonstrated similar nonlinear pharmacokinetics behavior (25). It’s worth pointing out that the solubility of oxfendazole (44.12 µg/ml), albeit low, is still much higher than several other benzimidazole compounds such as albendazole (26.58 µg/ml) and...
fenbendazole (1.60 µg/ml) (26). Consistent with the solubility difference, a comparative pharmacokinetics of oxfendazole, albendazole, and fenbendazole conducted in dogs showed that oxfendazole has much higher pharmacokinetic exposure than that of albendazole and fenbendazole following oral administration at the same dose (18). Similarly, in a human study the albendazole sulfoxide Cmax following a 400 mg dose of albendazole (equivalent to 6 mg/kg dose for 70 kg man) was reported to be only 147.6 ng/mL (27), which is much lower than the oxfendazole Cmax of 2440 ng/mL observed in the 3 mg/kg dose group in our study. There is no report on the EC50 of albendazole and oxfendazole on Taenia solium tissue infection. If the anti-parasite activity of albendazole and oxfendazole is similar, then this difference in plasma concentration between these two drugs may be of clinical importance; the higher oxfendazole concentrations achieved in plasma may lead to higher tissue levels and potentially greater effect against the larval forms of Taenia solium and other tissue helminths.

In addition to having a better solubility profile, another potential advantage of oxfendazole compared to albendazole is that oxfendazole has fewer sources of variability in its absorption phase. Our metabolism data showed that oxfendazole underwent mild metabolism; the parent drug remained the main moiety detected in human plasma. In contrast, it has been reported that albendazole is rapidly metabolized to its active metabolite albendazole sulfoxide, with little detection of the parent drug. Therefore, besides is poor solubility, the extensive first-pass metabolism of albendazole adds another source of variability in its absorption phase. The variable albendazole efficacy observed in the clinic could be caused by its highly variable absorption process and subsequently variable pharmacokinetic exposure (28).

As noted earlier, we found that oxfendazole remains the main moiety detected in human plasma following oral administration of oxfendazole, with little oxfendazole metabolized to the sulfone.
derivative. The metabolite profile of oxfendazole following its oral administration to healthy human subjects appears to be similar to that found in dog (18) and pig (29), with the parent compound predominating (87% in dog and 73% in pig) and the sulfone metabolite present at significantly lower levels (13% in dog and 26% in pig). In contrast, the predominant oxfendazole related moiety reported in a study conducted in rat was the inactive sulfone metabolite, comprising 71% of the oxfendazole related species and oxfendazole itself comprising only 29% of the moieties measured (30). These data suggest that dog and pig may represent suitable preclinical models for oxfendazole toxicity and efficacy. In fact, dog has been used especially in cardiovascular safety studies (23) and extensive pharmacology study has been conducted in *Taenia solium* cysticercosis in pig (20, 21).

The present study evaluated the pharmacokinetics of oxfendazole in healthy adults following single oral doses administered at fasting condition. For the treatment of certain parasitic diseases, oxfendazole may be used in patients multiple times at a relatively low doses rather than a very high single dose. In this case, oxfendazole pharmacokinetics following multiple dose regimens needs to evaluated. The information of food effect on oxfendazole disposition could also be valuable since the exposure of albendazole has been found to be significantly increased (Cmax increased approximately 5-fold) when it was given under fed condition (31). The evaluation of the pharmacokinetics of oxfendazole following multiple dose regimens as well as the food effect on oxfendazole disposition is currently underway in an ongoing clinical trial (Clinical Trial registration number NCT03035760).

Evaluation of oxfendazole efficacy will require treatment studies in disease-endemic regions, in which elevated levels of parasitic diseases such as cysticercosis still persist. Efficacy studies in human patients infected with cysticercosis and other parasite diseases such as soil transmitted...
helminthiasis will provide valuable information regarding the therapeutic potential for oxfendazole use in human parasitic infections. The first efficacy study of oxfendazole in human patients is currently being planned (Clinical Trial registration numbers NCT03435718; NCT02636803).
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Conflict of Interests:

Ellen Codd, John Horton, Robert H Gilman, Hector H Garcia and Armando E Gonzalez are members of the Oxfendazole Development Group (https://oxfendazoledevelopmentgroup.org/)
References


**Figure Legends**

**Figure 1.** Schematic diagram of study design

**Figure 2.** Structures of oxfendazole and its metabolites, as well as the proposed elimination pathways of oxfendazole in human. (Based on the metabolic scheme shown in the UN FAO document (24))

**Figure 3.** Mean oxfendazole plasma concentration-time profiles in human following single escalating doses. Within each dose group mean value at each time point is shown.

**Figure 4.** Dose-normalized oxfendazole plasma exposure (i.e. Cmax/dose and AUC/dose) vs doses. Mean ± SD values are shown.

**Figure 5.** Plasma concentrations of oxfendazole, and its metabolites, namely fenbendazole, and oxfendazole sulfone, in human following 15 mg/kg dose of oxfendazole. Mean ± SD values are shown.

**Figure 6.** Time course of the mean cumulative amount of unchanged free oxfendazole excreted in urine in human following single escalating doses.

**Figure 7.** Cumulative amount of free and total oxfendazole (Mean ± SD) excreted in urine within 24 hours in each dose
Table 1. Demographic characteristics of the subjects participating OXF FIH study

<table>
<thead>
<tr>
<th>Demographic</th>
<th>OXF group (N=56)</th>
<th>Placebo Group (N=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.3 ± 7.1</td>
<td>26.9 ± 7.2</td>
</tr>
<tr>
<td>Sex, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>54 (96.4%)</td>
<td>13 (92.9%)</td>
</tr>
<tr>
<td>Female</td>
<td>2 (3.6%)</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.0 ± 13.5</td>
<td>89.3 ± 12.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.1 ± 8.1</td>
<td>182.0 ± 7.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0 ± 3.5</td>
<td>27.0 ± 3.6</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.950 ± 0.132</td>
<td>1.01 ± 0.12</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min/1.73 m²)</td>
<td>118 ± 19</td>
<td>112 ± 11</td>
</tr>
</tbody>
</table>

BMI, body mass index
## Table 2. Summary of oxfendazole pharmacokinetics parameters.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0.5</th>
<th>1</th>
<th>3</th>
<th>7.5</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$(h)</td>
<td>2.00 (2.00 – 2.13)</td>
<td>1.98 (0.98 – 5.95)</td>
<td>2.00 (1.00 – 2.03)</td>
<td>2.00 (0.98 – 4.00)</td>
<td>2.00 (1.95 – 9.53)</td>
<td>1.98 (1.90 – 5.95)</td>
<td>1.99 (1.35 – 7.92)</td>
</tr>
<tr>
<td>$C_{\text{max}}$(ng/mL)</td>
<td>944 ± 278</td>
<td>1160 ± 321</td>
<td>2440 ± 598</td>
<td>4780 ± 1260</td>
<td>6250 ± 1390</td>
<td>5300 ± 1690</td>
<td>6770 ± 2200</td>
</tr>
<tr>
<td>$C_{\text{max}}$/Dose ([ng/mL]/[mg/kg])</td>
<td>1890 ± 555</td>
<td>1160 ± 321</td>
<td>812 ± 199</td>
<td>638 ± 168</td>
<td>417 ± 93</td>
<td>177 ± 56</td>
<td>113 ± 37</td>
</tr>
<tr>
<td>$AUC_{\text{int}}$(h*ng/mL)</td>
<td>11700 ± 5730</td>
<td>13100 ± 8210</td>
<td>30800 ± 6310</td>
<td>73700 ± 31900</td>
<td>99500 ± 24400</td>
<td>78300 ± 28300</td>
<td>109000 ± 44900</td>
</tr>
<tr>
<td>$AUC_{\text{int}}$/Dose ([h*ng/mL]/[mg/kg])</td>
<td>23400 ± 11500</td>
<td>13100 ± 3210</td>
<td>10300 ± 2100</td>
<td>9830 ± 4200</td>
<td>660 ± 1630</td>
<td>2610 ± 944</td>
<td>1810 ± 749</td>
</tr>
<tr>
<td>$V_{z}/F$(mL/kg)</td>
<td>577 ± 122</td>
<td>935 ± 499</td>
<td>1440 ± 604</td>
<td>1410 ± 332</td>
<td>2170 ± 893</td>
<td>5420 ± 2220</td>
<td>8760 ± 4940</td>
</tr>
<tr>
<td>$CL/F$(mL/h/kg)</td>
<td>42.8 ± 20.4</td>
<td>76.2 ± 48.5</td>
<td>97.4 ± 20.2</td>
<td>102 ± 32</td>
<td>151 ± 36</td>
<td>383 ± 142</td>
<td>552 ± 275</td>
</tr>
<tr>
<td>$t_{1/2}$(h)</td>
<td>9.07 ± 0.02</td>
<td>8.50 ± 2.22</td>
<td>10.3 ± 3.8</td>
<td>9.60 ± 3.43</td>
<td>9.97 ± 2.22</td>
<td>9.82 ± 3.46</td>
<td>11.0 ± 5.2</td>
</tr>
<tr>
<td>$Fe'$(%)</td>
<td>0.415 ± 2.27</td>
<td>0.287 ± 1.51</td>
<td>0.153 ± 0.443</td>
<td>0.154 ± 1.12</td>
<td>0.109 ± 0.406</td>
<td>0.0504 ± 0.160</td>
<td>0.0218 ± 0.121</td>
</tr>
<tr>
<td>$CL_{R}$(mL/h)</td>
<td>12.7 ± 6.3</td>
<td>15.0 ± 7.1</td>
<td>12.3 ± 4.4</td>
<td>9.42 ± 6.77</td>
<td>12.1 ± 6.5</td>
<td>16.3 ± 3.3</td>
<td>10.7 ± 9.9</td>
</tr>
</tbody>
</table>

*Median (Min – Max)  

$C_{\text{max}}$, The maximum observed plasma concentration; $T_{\text{max}}$, the time to $C_{\text{max}}$; $AUC$, the area under the curve; $V_{z}/F$, apparent volume of distribution; $CL/F$, apparent clearance; $t_{1/2}$, half-life; $Fe'$, the apparent fraction of unchanged drug (i.e. oxfendazole) recovered in urine; $CL_{R}$, renal clearance.
Table 3. Summary of Adverse Events (AEs)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>N (%) Subjects</th>
<th>N (%) Subjects with Particular AE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥1 AE</td>
<td>≥1 AE Related to Study Drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GI symptoms</td>
<td>Prolonged PR Interval</td>
</tr>
<tr>
<td>Placebo</td>
<td>14</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>0.5</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>1 (12.5)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>7.5</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>1 (12.5)</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>60</td>
<td>8</td>
<td>1 (12.5)</td>
<td>1 (12.5)</td>
</tr>
</tbody>
</table>