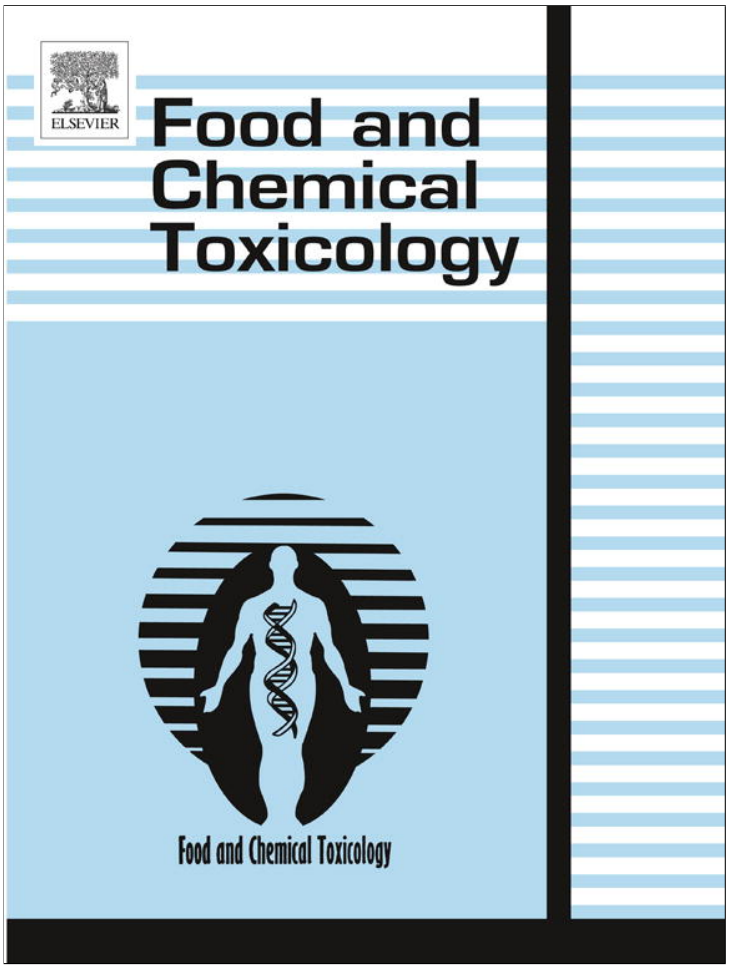

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Food and Chemical Toxicology 50 (2012) 3819–3825

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

A high oxfendazole dose to control porcine cysticercosis: Pharmacokinetics and tissue residue profiles

L. Moreno [a,*], M.T. Lopez-Urbina [b,g], C. Farias [a], G. Domingue [c], M. Donadeu [d], B. Dungu [d], H.H. García [e,f,g], L.A. Gomez-Puerta [b,g], C. Lanusse [a], A.E. González [b,g]

[a] Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Cent de la Provincia de Buenos Aires y Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET, Tandil, Argentina
[b] Preventive Veterinary Medicine Lab., School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru
[c] Express Microbiology Ltd., Mill Road Estate, Linlithgow, Scotland, UK
[d] Global Alliance for Livestock Veterinary Medicines, Pentlands Science Park, Edinburgh, Scotland, UK
[e] Department of Microbiology, School of Sciences and Center for Global Health – Tumbes;Universidad Peruana Cayetano Heredia, Lima, Peru
[f] Cysticercosis Unit, Instituto Nacional de Ciencias Neurológicas, Lima, Peru
[g] Cysticercosis Working Group

ARTICLE INFO

Article history:
Received 24 April 2012
Accepted 17 July 2012
Available online 26 July 2012

Keywords:
Oxfendazole
Pharmacokinetics
Tissue residues
Cysticercosis
Withdrawal time

ABSTRACT

Oxfendazole (OFZ) is efficacious for porcine cysticercosis at 30 mg/kg. OFZ is not registered to be used at this dose. The assessment of the OFZ and metabolites [(fenbendazole sulphone ($FBZSO_2$), fenbendazole (FBZ)] plasma pharmacokinetic and tissue residue profiles after its oral administration to pigs and the withdrawal period for human consumption were reported. Forty-eight pigs allocated into two groups received OFZ (30 mg/kg) orally as a commercial (CF) or as experimental formulation (SMF). Samples (blood, muscle, liver, kidney and fat) were collected over 30 days post-treatment and analyzed by HPLC. OFZ was the main compound recovered in plasma, followed by $FBZSO_2$ and low FBZ concentrations. OFZ $AUC_{0\text{-}LOQ}$ (209.9 ± 33.9 μg·h/ml) and C_{max} (5.40 ± 0.65 μg/ml) parameters for the CF tended to be higher than those for the SMF ($AUC_{0\text{-}LOQ}$: 159.4 ± 18.3 μg h/ml, C_{max}: 3.80 ± 0.35 μg/ml). The highest total residue (OFZ + $FBZSO_2$ + FBZ) concentrations were quantified in liver, followed by kidney, muscle and fat tissue. $FBZSO_2$ residue levels were the highest found in muscle (0.68 ± 0.39 μg/g) and fat (0.69 ± 0.39 μg/g). In liver and kidney the highest residues corresponded to FBZ (5.29 ± 4.36 μg/g) and OFZ (2.86 ± 0.75 μg/g), respectively. A withdrawal time of 17 days post-treatment was established before tissues are delivered for human consumption.

1. Introduction

Taenia solium taeniasis/cysticercosis continues to be a costly scourge for most developing countries (Garcia and Del Brutto, 2005). Control of *T. solium* has progressed along the past 10 years and several tools have been introduced including new diagnostics, and an effective pig vaccine (TSOL18, University of Melbourne) (Gonzalez et al., 2003; Flisser et al., 2004; Gonzalez et al., 2005). Antiparasitic therapy of the intermediate host is crucial to eliminate the reservoir. Even in the presence of a vaccine, treatment is required to cover pigs which are already infected at the time of vaccination and can become a source for new infections. Oxfendazole (OFZ), or [5-(phenylsulphinyl)-1H-benzimidazole-2-yl] carbamic acid methyl ester, was first identified and manufactured by Syntex Research (Palo Alto, CA), and shown to have anthelminthic properties against larval and adult forms of gastrointestinal (GI) nematodes and cestodes in various animal species. OFZ is the active sulphoxide metabolite of fenbendazole (FBZ), a broad-spectrum benzimidazole methycarbamate (BZD). BZD anthelmintics are extensively metabolized in all mammalian species studied. FBZ and OFZ are metabolically interconvertible and the sulphoxide undergoes a second, slower oxidative step which forms the inactive metabolite fenbendazole sulphone ($FBZSO_2$).

Different OFZ dose rates ranging between 2 and 10 mg/kg have been recommended in animals to control nematodes and cestodes. OFZ is highly efficacious for the treatment of porcine cysticercosis. It is well documented that OFZ (22.5% suspension, intended for use in cattle) when administered at a dose rate of 30 mg/kg, is effective for treatment of cysticercosis (Gonzalez et al., 1996; Gonzalez et al., 1997; Gonzalez et al., 1998). Several reasons made OFZ the drug of choice for the treatment of cysticercosis infected pigs. It is cheap, eliminates all cysts from the carcasses with a single dose (except for some residual cysts in the brain), is not associated with significant side effects, and the meat is useful for human consumption after treatment (Tsang et al., 1989; Gonzalez et al., 1996;

* Corresponding author. Tel.: +54 249 4439850.
E-mail address: lmoreno@vet.unicen.edu.ar (L. Moreno).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.07.023

Gonzalez et al., 1997; Gonzalez et al., 1998). As a consequence, OFZ has been used in several controlled trials and also in targeted and mass porcine chemotherapy programs in field conditions (Garcia et al., 2006; Assana et al., 2010). However, OFZ is not registered to be used at this high dose, neither for the cysticercosis treatment in pigs. One of the requirements for marketing authorizations for veterinary medicinal products is the availability of plasma pharmacokinetic (PK) and residue depletion profiles studies of the drug formulation in the species in which the new indication will be used. Pigs (and humans who could be also a target population for future uses of OFZ) are monogastric and thus pharmacokinetic parameters from ruminant hosts are poorly transferable. The goal of the present work was to study the OFZ and metabolites plasma disposition kinetics and residue profiles after its oral administration at 30 mg/kg to pigs using a 9.06% commercial formulation (Synanthic®, Pfizer, Mexico). A complementary goal of the current work included the comparison of the plasma PK of the standard commercial product (9.06%, Synanthic®, Pfizer, Mexico), with a locally formulated OFZ experimental preparation (22.5%) named as San Marcos Formulation (SMF). All together, the work reported here will contribute to: (a) establish a safe withdrawal period for human consumption of treated pig tissues; (b) for the purposes of medicinal product registration, and (c) provide a benchmark for the possible future development of improved formulations for pigs.

2. Materials and methods

2.1. Experimental animals and treatment

Forty-eight (48) healthy male or female (Peruvian local ecotypes breed) 3–5 months old pigs purchased from commercial farms in Lima (Peru) were used in the experiment. Animals were kept indoors with food and water supplied *ad libitum* during the whole experimental period. All animal procedures and management protocols were conducted with international quality standards following the (VICH GL9 (GCP), 2000). Pigs were weighted and sorted to be randomly allocated into two treatment groups of 24 pigs each which received a single oral dose of 30 mg/kg of OFZ. Group 1 received the drug as a commercial formulation (CF) (Synanthic® 9.06%, Pfizer, Mexico), and Group 2 received OFZ in a 22.5% experimental formulation locally developed (SMF) at the Department of Pharmacology of San Marcos University in Lima, Peru. After both treatments, blood samples were taken from all pigs by anterior vena cava venipuncture into heparinized tubes at: baseline (time 0) and 2, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72 and 96 h (day 4) and at 7 and 10 days post-treatment. Samples were immediately centrifuged and plasma separated and stored at −60 °C until assayed. Eight animals (4 from each group) were randomly assigned for sacrifice at each of six time points at 3, 5, 7, 10, 15 and 30 days post-treatment. Pigs were sacrificed using electro-stunning and total bleed out according to ethical animal euthanasia guidelines. Samples (near to 200 g) of muscle, liver, kidney and fat were collected from each treated animal. Tissue samples taken from untreated pigs were used as blank controls for development of the analytical methodology. Blank and experimental samples were frozen at –60 °C until assayed.

2.2. Chemicals

Pure reference standards (97–99% purity) of OFZ and its metabolites, FBZSO2 and FBZ were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). The OFZ commercial formulation (Synanthic® 9.06%) was kindly provided by Pfizer Lab, (Mexico). Acetonitrile solvent used during the extraction and drug analysis were HPLC grade and purchased from Sintorgan® S.A. (Buenos Aires, Argentina). Ammonium acetate (HPLC grade) was from Baker (Phillipsburg, NJ, USA). Water was double distilled and deionized using a water purification system (Simplicity®, Millipore, São Paulo, Brazil).

2.3. Plasma sample extraction

Plasma samples (1 mL) were spiked with albendazole sulphoxide (ABZSO) IS (30 μL, 20 μg/mL) and the molecules to be assayed (OFZ, FBZ, $FBZSO_2$). Drug molecules were extracted by addition of 2 mL of acetonitrile under a high speed vortexing shaker over 20 min. After sonication (15 min), samples were centrifuged (at 2000g for 20 min at 4 °C). The clear supernatant was transferred to a 5 mL glass tube and evaporated (40 °C) to dryness in a vacuum concentrator. The extract was reconstituted with 200 μL of mobile phase (acetonitrile:water, 27:73) and 50 μL was injected in the chromatographic system.

2.4. Tissue samples extraction

Experimental or spiked (OFZ, FBZ, and $FBZSO_2$) tissue samples were added of the IS albendazole (ABZ) and processed as follow. The muscle sample (1 g) was extracted by addition of 2 mL acetonitrile:dimethilsulphoxide (90:10). After shaking (vortexing 20 min), the sample was sonicated (30 min) and centrifuged (4000 rpm, 20 min, 4 °C). The clear supernatant was evaporated (40 °C) to dryness. The extract was reconstituted in 200 μL acetonitrile and after water addition (1.2 mL) was preceded to solid phase extraction (SPE). SPE was made using C18 cartridges previously conditioned with 1 mL of methanol, followed by 1 mL of water. The sample was applied and then sequentially washed with 3 mL of water, and 3 mL of methanol:water (1:4), dried with air for 5 min and eluted with 2 mL of methanol. The eluted volume was evaporated (40 °C) to dryness and the dry residue dissolved in 200 μL of mobile phase. An aliquot (50 μL) of this solution was injected in the chromatographic system. For liver tissue, an analytical portion (1 g) was extracted by addition of 5 mL acetonitrile:water (6:4). After vortexing (30 min), samples were centrifuged (2000g, 15 min, 10 °C), the clear supernatant transferred to a 50 mL plastic tube and 30 mL of HPLC water added. Then, a SPE procedure similar to that described for muscle samples was applied. The dry residue was dissolved (200/400 μL for calibration level 1 or 2) in mobile phase and 50 μL was injected in the HPLC. Kidney and fat samples were extracted in similar way. An aliquot of tissue (1 g) were extracted by addition of 2 mL acetonitrile:dimethilsulphoxide (90:10). After shaking (20 min), samples were sonicated 15 (for kidney) or 30 min (for fat) and centrifuged (4000 rpm) for 20 min at 4 (for kidney) or 15 °C (for fat). For fat samples, the supernatant was transferred to a 10 mL glass tube and hexane (5 mL) was added to clean the sample. The sample was shaked slowly for one hour and then centrifuged (2000 rpm, 10 min, 15 °C). The hexane layer was discarded and the cleaning process repeat again. The clear supernatant was transferred to a 5 mL glass tube and concentrated (200 μL) with nitrogen in a thermostatic bath at 60 °C. The sample was transferred to an eppendorf vial and centrifuged (13000 rpm, 20 min, 4 °C). Then, an aliquot of 50 μL was injected in the chromatographic system.

2.5. HPLC system and chromatography

Experimental and fortified plasma and tissue samples were analyzed for OFZ, FBZ, and $FBZSO_2$ by HPLC. Fifty (50) μL of sample were injected in a Shimadzu Chromatography system (Shimadzu Corporation, Kyoto, Japan). This was composed for a LC-20AT quaternary pump, an automatic sample injector (SIL-10AF), an ultraviolet visible spectophotometric detector (UV) (SPD-20A) set at a wavelength of 292 nm, a column oven (CTO-10AS vp) set at 30 °C, and a CBM-20A data integrator. Chromatograms were collected using the Class LC10 software (SPD-10A, Shimadzu Corporation, Kyoto, Japan). A C18 column (Kromasil®, Eka Chemicals AB, NY, USA) of 250 × 4.6 mm with 5 μm particle size was used for separation. Elution was carried out at a flow rate of 1.2 mL/min using acetonitrile:ammonium acetate buffer (0.025 M, pH 6.6) as mobile phase that was pumped with variable gradient during the run (17 min). The gradient changed from 27:73 to 50:50 in 5 min, then maintained for 7 min and modified to 27:73 in 1 min, in which was maintained during 4 min. The compounds were identified with the retention times of 97–98% pure reference standards.

2.6. Method validation

A full validation of the analytical procedures for each molecule (OFZ, FBZ and $FBZSO_2$) in the different pig matrixes (plasma, liver, muscle, kidney and fat) was performed following internationally recognized criteria (VICH GL 49 (MRK), 2009; Commission Decision 2002/657/EC). Stock and working solutions of a mix of the pure (97–99%) references standards (OFZ + FBZ + $FBZSO_2$) in methanol were prepared. The following parameters were determined: Selectivity, Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantification and Stability. Under the described chromatographic conditions, the mean retention times were: OFZ: 6.8 min; $FBZSO_2$: 8.8 min; FBZ: 14.2 min. The blank samples were free of interferences in the time regions of analytical interest. Standard calibration curves for OFZ, FBZ and $FBZSO_2$ in pig plasma, muscle, liver, kidney and fat tissue were obtained using the linear least squares regression procedure. The linearity was determined by the lack of fit test (GraphPad InStat, Version 3.00, GraphPad Software, San Diego, CA, US), giving determination coefficients near to 0.999. Molecules recoveries from matrixes were good near or >70%, except for kidney with mean values between 61.4% and 66.9%. The method exhibited a high degree of inter-day precision for the three molecules demonstrated by CV always <15% in both plasma and tissues. The accuracy was good, with mean relative error values <15% in most cases. The theoretical LOD was defined as the mean baseline noise/IS peak area ratio plus three standard deviations (SD). The limit of quantification (LOQ) was calculated as the lowest drug concentration ($n = 6$) on the standard curve that could be quantitated with precision not exceeding 20% and accuracy within 20% of nominal. Stability assays were based on analysis of blank samples of each matrix fortified with the three molecules at time 0 ($n = 3$), 7 ($n = 3$) and 30 ($n = 3$) days post-freezing; and after 3 freeze/thaw cycles ($n = 6$). The coefficients of variation after storing at

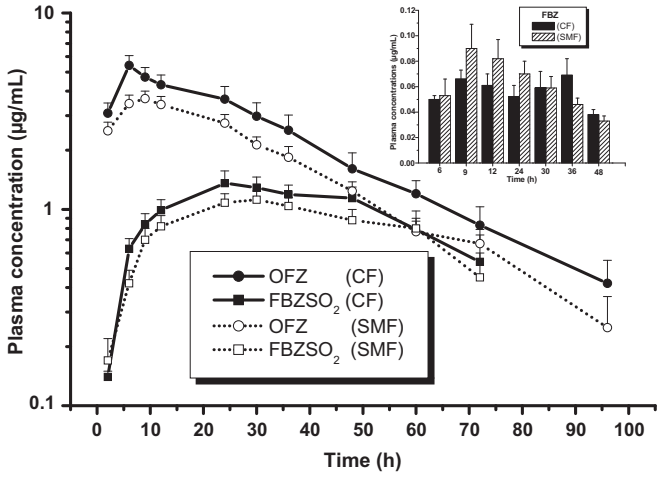

Fig. 1. Oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$) and fenbendazole (FBZ) mean (±SEM) plasma concentration vs. time profiles obtained after OFZ oral administration (30 mg/kg) to growing pigs fed *ad libitum* as two different formulations, Synanthic 9.06% commercial formulation (CF) and the 22.5% San Marcos experimental formulation (SMF).

−18 °C were always ⩽16.6%, indicating no significant degradation of molecules in these conditions. After 3 freeze/thaw cycles variations were ⩽20% except for FBZ in plasma with CV ⩽ 27.2%.

2.7. Pharmacokinetic analysis

The pharmacokinetic analysis of the plasma concentrations obtained after administration of a single oral dose (30 mg/kg) of both OFZ formulations to growing pigs fed *ad libitum* was carried out using the PK Solution software (Summit Research Services, Ashland, USA). Some pharmacokinetic parameters were estimated. The maximum peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration–time curve of each analyte. The area under the concentration time-curve (AUC) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (β). Statistical moment theory was applied to calculate the mean residence time (MRT) in plasma as follows: MRT = AUMC/AUC; where AUC is defined previously and AUMC is the area under the curve of the product of time and the plasma drug concentration vs. time from zero to infinity (Perrier and Mayersohn, 1982).

2.8. Withdrawal time calculation

The OFZ and metabolites ($FBZSO_2$, FBZ) residual concentrations measured in each edible tissue were analyzed, and a withdrawal time was estimated to be recommended after oral administration (30 mg/kg) of both OFZ formulations to pigs. The withdrawal time was calculated using the results of a linear regression analysis of the log residual concentrations vs. time of the elimination terminal phase. The withdrawal period was established at the time when the upper one-sided tolerance limit with a given confidence interval was below the MRL. If this time point did not make up a full day, the withdrawal period was rounded up to the next day. The calculations were done using the "Melk WTM 1.4" withdrawal-time calculation computer software (http://www.ema.europe.eu). The estimation of withdrawal periods by regression analysis using "Melk WTM 1.4" was applicable for liver and kidney, being inapplicable for muscle and fat tissues. The available muscle data did not agree with the assumption of homogeneity of variances of the $\log_c$–transformed data on each slaughter day. In the case of the fat tissue, there were few values of measured drug residual levels. As a consequence, the recommended "alternative approach" (EMEA/CVMP/036/95) was considered to estimate withdrawal periods for muscle and fat. This considers the withdrawal period at the time point when the drug residual concentrations in the tissue for all the experimental animals are below the MRL. The estimation of a safety span should be considered in order to compensate for the uncertainties of a biological variability. A safety span of 10% was used for muscle and fat in the current work.

2.9. Statistical analysis of the data

Pharmacokinetic parameters and residual concentration data are reported as arithmetic mean ± SEM. Student t were used to compare PK parameters between administrations. A value of $P < 0.05$ was considered statistically significant. Bioequivalence between the two formulations was determined following the FDA/CVM guidance document #35, 1996, released in Nov, 2006, comparing the kinetic parameters C_{max}, AUC0-LOQ and T_{max}. A value of $P < 0.05$ was considered statistically significant. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, CA, USA).

3. Results

3.1. Plasma disposition kinetics

After OFZ administration as both the CF and SMF formulations at the single oral dose of 30 mg/kg to growing pigs, OFZ, $FBZSO_2$ and FBZ were measured in plasma. The mean (±SEM) plasma concentration vs. time profiles of the three molecules for both treatments are shown in Fig. 1. OFZ was the main compound recovered in plasma between 2 and 96 h post-treatment. The inactive $FBZSO_2$ metabolite was recovered between 2 and 72 h post-treatment. Low FBZ concentrations were quantified from 6 to 48 h post-administration. The mean OFZ concentration profiles for the CF tended to be higher than those measured for the SMF. The mean (±SEM) pharmacokinetic parameters calculated for each molecule after both treatments are shown in Table 1. The pharmacokinetic study demonstrated that both the OFZ AUC and C_{max} values for the CF were higher than those obtained for the SMF. As it is shown in the Fig. 1 a trend towards increased OFZ plasma relative bioavailability of the CF is observed, therefore the statistical and bioequivalence study of the data was carried out to establish more precise conclusions on the comparison of both formulations. A 90% confidence interval was constructed based upon the observed estimate of the difference SMF/CF ratio, using the average log transformed values. The mean geometric ratios between the two formulations were 0.72 (SMF C_{max}/CF C_{max}) and 0.80 (SMF AUC_{0-LOQ}/CF AUC_{0-LOQ}) with 90% confidence intervals of 0.59–0.88 and 0.63–1.01, respectively. According to FDA/CVM guidance document #35 (1996), the limits of the ratio values for both parameters must be between 0.8 and 1.25 to stay bioequivalence between formulations, a condition that was not observed in the present study. Thus,

Table 1
Plasma pharmacokinetic parameters (mean ± SEM) for oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$) and fenbendazole (FBZ) obtained after its administration as a single oral dose (30 mg/kg) as two different formulations, Synanthic 9.06% commercial formulation (CF) and the 22.5% San Marcos experimental formulation (SMF), to growing pigs fed *ad libitum*.

Pharmacokinetic	OFZ		$FBZSO_2$		FBZ	
Parameter	CF	SMF	CF	SMF	CF	SMF
C_{max} (µg/mL)	5.40 ± 0.65	3.80 ± 0.35	1.44 ± 0.21	1.19 ± 0.12	0.07 ± 0.01	0.10 ± 0.01
T_{max} (h)	6.60 ± 0.60	8.10 ± 0.64	30.6 ± 2.3	30.6 ± 2.27	15.0 ± 2.9	13.2 ± 1.86
$AUC_{(0-\infty)}$(µg h/mL)	209.9 ± 33.9	159.4 ± 18.3	74.9 ± 13.8	60.6 ± 9.08	2.20 ± 0.50	2.7 ± 0.51
$T_{1/2\ el}$ (h)	21.6 ± 1.6	19.4 ± 2.5	10.3 ± 1.3	10.2 ± 1.96	18.6 ± 2.7	21.8 ± 5.83
MRT (h)	33.3 ± 1.9	34.3 ± 3.3	37.0 ± 2.5	36.7 ± 2.80	21.8 ± 2.5	24.3 ± 2.71

Peak plasma concentration; T_{max}, time to peak concentration; $AUC_{(0-\infty)}$, area under the concentration vs. time curve extrapolated to infinity; $T_{½el}$, Elimination half-life; MRT, mean residence time; SEM, standard error of the mean.

the assayed OFZ formulations did not reach the bioequivalence entirely after the administration at 30 mg/kg to pigs.

3.2. Tissue residue profiles

After OFZ administration of both formulations (CF and SMF) as a single oral dose (30 mg/kg) to growing pigs, residual concentrations of OFZ and/or its metabolites $FBZSO_2$ and FBZ were measured in all the studied tissues. The highest total residue (sum of OFZ + $FBZSO_2$ + FBZ) concentrations were quantified in liver, followed by kidney, muscle and fat tissue after treatment with both formulations (Fig. 2). In all tissues the highest drug residues were measured at 3 days post-treatment. The residual concentrations of drugs found in the different tissues at 3 days post-treatment after CF administration are shown in Fig. 3. The pattern of residue distribution differed between tissues. In muscle the highest residue level corresponded to $FBZSO_2$ (0.68 ± 0.39 μg/g) followed by OFZ (0.56 ± 0.45 μg/g) meanwhile only FBZ concentrations below LOQ were detected in two animals. In liver, FBZ residues were the highest (5.29 ± 4.36 μg/g), followed by similar OFZ (2.84 ± 1.24 μg/g) and $FBZSO_2$ (2.90 ± 2.61 μg/g) residues levels. In kidney OFZ residues were the highest (2.86 ± 0.75 μg/g), followed by $FBZSO_2$ (1.11 ± 1.01 μg/g) and FBZ (0.52 ± 0.47 μg/g). Finally, in fat the highest residue correspond to $FBZSO_2$ (0.69 ± 0.39 μg/g) followed by OFZ (0.30 ± 0.15 μg/g) and FBZ (0.04 ± 0.02 μg/g). The withdrawal times calculated according to the residue profiles found for each tissue after both OFZ treatments are reported in Table 2. A withdrawal time of 17 days post-treatment was recommended after a single oral administration (30 mg/kg) of either OFZ formulation (CF and SMF) to growing pigs fed *ad libitum*.

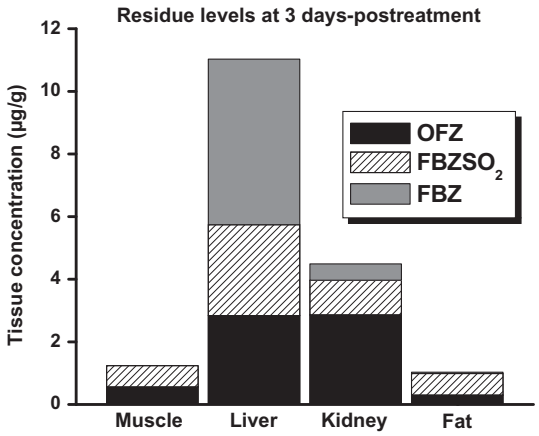

Fig. 3. Comparative residual concentrations profiles of oxfendazole (OFZ), fenbendazole sulphone ($FBZSO_2$) and fenbendazole (FBZ) measured in muscle, liver, kidney and fat at 3 days post-administration of OFZ orally at 30 mg/kg to pigs.

4. Discussion

Plasma concentration profiles reflect those attained for the different fluid/tissues where target parasite may be located. Consequently, its characterization contributes to understanding the relationship between drug concentration and clinical efficacy, which is useful to optimize parasite control. The OFZ plasma PK behavior in sheep (Marriner and Bogan, 1981) cattle (Prichard et al., 1985), goats (Hennessy et al., 1993) and horses (Sánchez Bruni

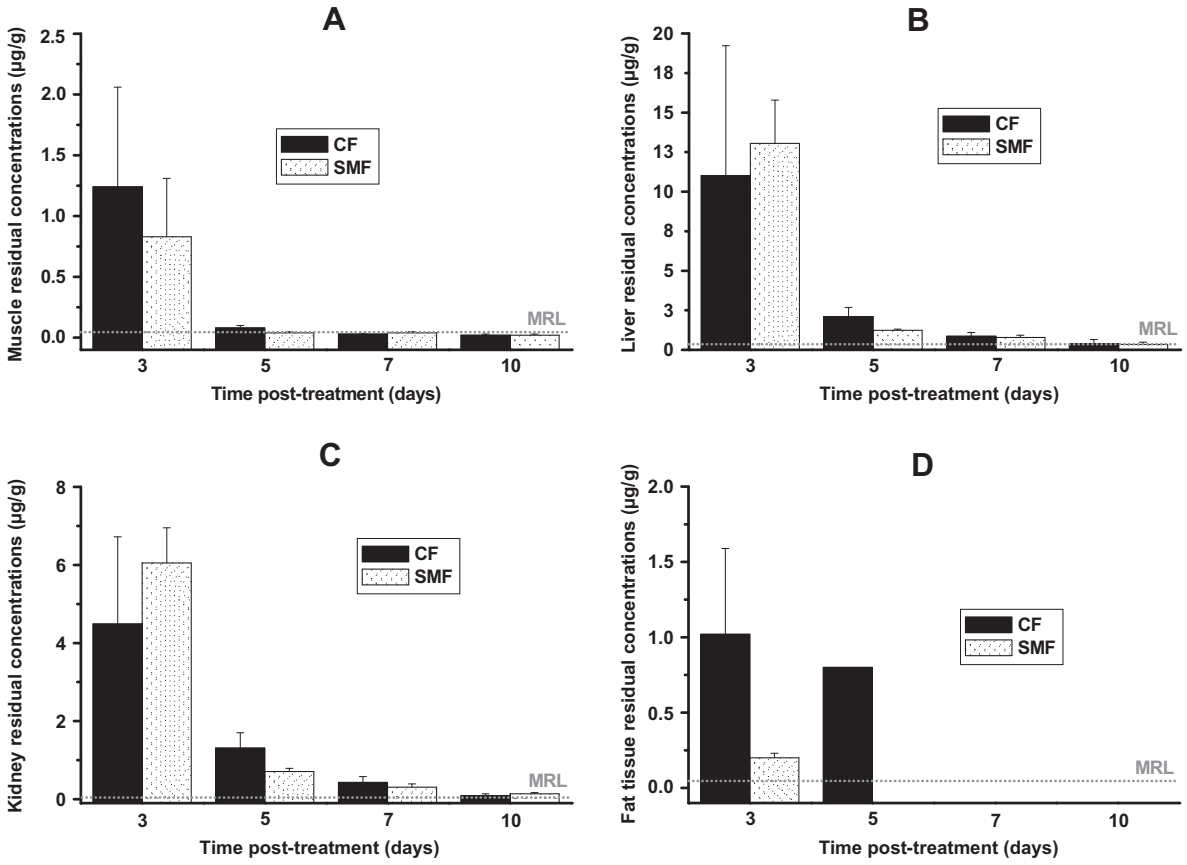

Fig. 2. Total (oxfendazole (OFZ) + fenbendazole sulphone ($FBZSO_2$) + fenbendazole (FBZ)) mean (±SEM) residue concentrations quantified in muscle (A), liver (B), kidney (C) and fat tissue (D) at each time interval after OFZ administration by oral route (30 mg/kg) to growing pigs fed *ad libitum* as two different formulations, Synanthic 9.06% commercial formulation (CF) and the 22.5% San Marcos experimental formulation (SMF). MRL: Maximum residue limits established for OFZ by the Council Regulation (EEC) N°2377/90 (EMEA/MRL/888/03).

Table 2
Estimated withdrawal times for each tissue after oxfendazole (OFZ) administration as a single oral dose (30 mg/kg) in two different formulations, Synanthic 9.06% commercial formulation (CF) and a 22.5% San Marcos experimental formulation (SMF), to growing pigs fed *ad libitum*.

Pig tissue	Withdrawal time (days)	
	CF	SMF
Muscle	17	11
Liver	16	17
Kidney	16	17
Fat tissue	8	6
Estimated withdrawal time	17	17

Note: The estimation of the reported withdrawal times was done considering the MRL values established for OFZ by the Council Regulation (EEC) No. 2377/90 (sum of extractable residues which may be oxidized to oxfendazole sulphone in porcine – 50 μg/kg in muscle, fat, kidney; 500 μg/kg in liver) (EMEA/MRL/888/03)

et al., 2005), animal species in which OFZ was first indicated, has been extensively investigated. However, no information on OFZ disposition kinetics in pigs, the target species for cysticercosis control purposes, is available. This study demonstrated that a single oral dose of 30 mg/k of OFZ in pigs quickly reaches very high plasma levels and systemic exposure, and is associated with measurable plasma levels of FBZ, all conditions which should result in a high parasiticidal efficacy.

The most commonly used BZD compounds in pigs are oxibendazole and FBZ. Both are usually indicated for 3 to 10 consecutive days in-feed medication (1.6–3 mg/kg). At a higher single oral dose (5–10 mg/kg), FBZ exhibits a high efficacy against most important GI nematodes in pigs, except *Trichuris suis* (Biehl, 1986). The OFZ plasma disposition kinetics administered at 30 mg/kg as a single oral dose to pigs is reported for the first time in the present work. None of the animals involved in the current trial showed any adverse events/side effects during the study. After its oral administration, OFZ was rapidly absorbed from the GI tract. A high concentration (close to 3 μg/mL) was measured as early as 2 h post-treatment. On the other hand, the early appearance of the sulphone ($FBZSO_2$) metabolite in the bloodstream, confirmed the rapid OFZ liver microsomal biotransformation. Low FBZ concentrations were recovered between 6 and 48 h post-treatment. OFZ is metabolized either irreversibly to $FBZSO_2$ in a cytochrome P-450-mediated sulphonation, or reversibly by microbial reduction to FBZ in the GI tract (Beretta et al., 1987; Murray et al., 1992; Virkel et al. 2002).

Reduction by the GI microflora plays an important role in the metabolism of a number of drugs, particularly those containing nitro and sulphoxide groups (Lanusse and Prichard, 1993). The OFZ reduction to FBZ has been shown to occur in the ruminal fluid of sheep and cattle (Beretta et al. 1987; Virkel et al. 2002). Such a phenomenon correlates with the FBZ detection in plasma following OFZ administration in the current experiment, which probably occurs in the pig large intestine, where an important microbial activity is present since is the main site of cellulose degradation in this species. In terms of binding to tubulin (the main BZD mode of action), FBZ is more potent than OFZ, and $FBZSO_2$ is an inactive metabolite (Lacey 1990; Lubega and Prichard, 1991). The main mechanism of entry of BZD anthelmintics into parasites is passing through its external surface after a passive diffusion process which determines the amount of drug accumulation (Mottier et al., 2006). The sulfide FBZ is more lipophilic (higher octanol–water partition coefficient $-\log P$-) and thus, more soluble in the lipoidal membranes of the helminth parasites than their respective sulphoxide metabolite (OFZ) (Mottier et al., 2003). As a consequence, the systemic FBZ concentration observed after OFZ administration surely accounts for the high efficacy against porcine cysticercosis reported for OFZ (Gonzalez et al., 1996; Gonzalez et al., 1997; Gonzalez et al., 1998).

OFZ plasma concentration profiles in pigs were higher than those observed in sheep. In fact, after OFZ oral administration to sheep, lower plasma C_{max} and AUC values (0.39 μg/mL and 23.5 μg h/mL, respectively) (Lanusse et al., 1995) than that observed in pig in the present work (5.4 μg/mL and 209.9 μg h/mL for CF treatment) were reported. These differences may be determined by the increased OFZ dose used in pigs (30 mg/kg) compared to sheep (5 mg/kg). A previous work demonstrated that increasing the ABZ dose in sheep was clearly associated with enhanced plasma ABZ metabolites exposure (Alvarez et al., 2012). Most interesting, the enhanced systemic drug exposure achieved after treatments at the highest ABZ dose, correlated with significant increment in drug efficacy against a resistant *H. contortus* strain (Barrère et al., 2012). The enhancement in the OFZ/FBZ plasma exposure after a single "high" dose of OFZ in pigs, may account for parasites being exposed to toxic drug concentrations for extended periods of time.

Slight differences in the disposition kinetics of anthelmintically active metabolites among BZD compounds may results in important differences in efficacy, especially against those parasites located in tissues, where the presence of high drug concentrations for an extended period of time is usually crucial to achieving optimal clinical efficacy (Lanusse et al., 1995). ABZ was shown to be an effective drug for treatment of porcine cysticercosis. However, multiple-dose schedules are required, severe side-effects have been observed and the cysts are not completely reabsorbed after treatment (Gonzalez et al., 1995). After ABZ oral administration to pigs (10 mg/kg), ABZ parent drug was not detected in plasma and the ABZ-suphoxide (ABZSO) metabolite was the only active compound found in the bloodstream (Alvarez et al., 1996). Additionally, while ABZSO was depleted from plasma relatively quickly reaching non-detectable concentrations at 30 h post-ABZ administration, in the present work OFZ was found in plasma for up to 96 h (CF formulation). This extended OFZ plasma exposure correlated with a longer residence in plasma ($T_{1/2\ el}$: 21.6 h; MRT: 33.3 h) than that reported for ABZSO ($T_{1/2\ el}$: 3.5 h; MRT: 7.1 h) (Alvarez et al., 1996). Similar differences between OFZ and ABZ PK behavior have been described in ruminants (Lanusse et al., 1995), being associated to differences in the biotransformation pattern between these two molecules. ABZ, an aliphatic substituted BZD, is sufficiently polar when oxidized to be largely excreted in urine rather than undergo further conjugation and secretion in bile (Hennessy, 1989). Meanwhile, OFZ and FBZ are aromatic BZD compounds that require more extensive metabolism than aliphatic derivatives to achieve sufficient polarity for excretion (Hennessy et al., 1993). The aromatic substitution may slow the oxidation of the sulfur atom (FBZ, OFZ) (Hennessy, 1989). Such a phenomenon may have accounted for the significantly longer residence time and elimination half-life found after OFZ oral administration in pigs, compared to that found after ABZ oral administration (Alvarez et al., 1996). These differences may explain the observed improved efficacy of OFZ compared to ABZ used in the treatment of cysticercosis-infected pigs (Gonzalez et al., 1995).

The use of 9.06% OFZ CF for pigs in field conditions is less practical than the more concentrated formulations (requiring smaller volumes per animal thus decreasing the risks for choking and the chances of aspiration pneumonia). Since the 22.5% commercial formulation was not available, we produced and tested the concentrated locally prepared SMF. The time period in which OFZ/metabolites were quantified in plasma of pigs treated with both formulations was similar. Beyond that the mean C_{max} and AUC values for OFZ after CF administration were higher than the found after SMF treatment; the high individual variation in these PK parameters did not permit obtaining statistically significant differences

between treatments (Student t test, $P > 0.05$). Furthermore, the study showed similar values for other pharmacokinetic parameters calculated for OFZ, $FBZSO_2$ and FBZ after administration of both formulations. However, the bioequivalence analysis of the obtained data was undertaken to establish more precise conclusions on the comparison of both formulations. Although the work reported here was carried out under standardized experimental conditions (homogeneous group of pigs of the same breed and similar age), it fails to represent an ideal situation for bioequivalence study since it was performed using a parallel instead of a cross-over design (FDA/CVM guidance document #35, 1996, released in Nov, 2006). According to the results obtained in the current study, bioequivalence between the two OFZ formulations could not be demonstrated. Although these results may have been influenced by the limited power of the selected parallel experimental design, the magnitude of the differences observed on the OFZ systemic exposure between the CF and SMF (Fig. 1), seems to indicate some influence of drug formulation on the GI absorption. BZD anthelmintics are poorly soluble in water and are mainly administered as drug suspensions, paste or granules (Lanusse and Prichard, 1993). The dissolution of drug particles at the acidic pH found in the stomach is a critical factor limiting the GI absorption of OFZ in pigs. Therefore, different issues related to the quality of the manufacturing procedures (micronized, particle size/surface/crystal structure of the active substance, type of excipients, etc.) and the differences of drug concentration in the product may have accounted for bioequivalence differences observed between CF and SMF formulations.

After OFZ administration (both CF and SMF formulations) to growing pigs, residual concentrations of OFZ and its metabolites $FBZSO_2$ and FBZ were measured in all the assayed tissues. The total residue profiles (OFZ + $FBZSO_2$ + FBZ) were similar for both formulations, being the highest concentrations profile quantified in liver, followed by kidney, muscle and fat tissue (Fig. 2). The highest total residues was found at 3 days post-treatment declining until the lowest residue level being measured at 10 days post-administration in muscle, liver and kidney. In fat tissue, the total residue was measured at 3 days post-treatment for both formulations, and at 5 days after the CF administration. Considering the official MRL for OFZ (EMEA/MRL/888/03), a withdrawal time was estimated to each tissue after each treatment (Table 2). After the OFZ CF treatment, the longest estimated withdrawal time corresponded to muscle, meanwhile after SMF administration the extended was for kidney. However, since these time periods were similar, the withdrawal times established after OFZ oral administration at 30 mg/kg to pig for both formulations were the same, 17 days. After FBZ administration by the oral route to pig at 5 mg/kg, no FBZ was detected in tissue samples collected (kidney, liver and muscle), whereas minimum amounts of metabolites below MRL were found in liver only at 4 days post- administration (Szprengier-Juszkiewicz et al., 2002). These results did not allow calculating a precise withdrawal time and a 3 day period was advised. In the current work, after OFZ oral administration the concentrations measured in the different tissues were much higher and correlated with the high efficacy found against porcine cysticercosis with the treatment at 30 mg/kg dose rate.

In conclusion, high OFZ concentrations over a long period of time complemented with the recovery of the anthelmintically active compound FBZ in the bloodstream are relevant pharmacokinetic data obtained after OFZ administrations at 30 mg/kg, which correlates with the adequate clinical efficacy obtained in pigs infected with cysticercosis. According to the tissue residue profiles described here, a withdrawal time of 17 days must be allowed before treated animal can be derived for human consumption when OFZ is used as a therapeutic tool for control porcine cysticercosis at 30 mg/kg.

Finally, there is a great potential for OFZ to be of use in treating tissue helminths in humans. Work is in progress to confirm its safety and PK in humans in a Phase I study. Although the use of OFZ has been greatly neglected mostly because of poor commercial perspectives, results obtained in this study have contributed to characterizing it as a therapeutic tool of great value in the control of cysticercosis in pigs and humans.

Funding: This work was funded by the Bill and Melinda Gates Foundation through grants number 1016506 and 23981 and also funds from the Department for International Development (DFID) UK. HG is a Wellcome Trust Senior Research Fellow in Public Health. The funders had no role in study design; data collection, analysis, or interpretation; in writing the report, or in the decision to submit the article for publication.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We are grateful to Rosa Perales, Eloy Gonzales, and all the personnel of the UNMSM School of Veterinary Medicine of the Universidad Nacional Mayor de San Marcos in Lima, Peru, for their collaboration on study performance, to the staff of the School of Veterinary Science of the Universidad Nacional del Centro in Tandil, Argentina, for Laboratory analyses, and to Gianfranco Arroyo, DVM, Ph.D., for his help in organizing the study data and operational support. We also acknowledge the gift of 9.06% oxfendazole, Synanthic®, from Pfizer Mexico.

References

Alvarez, L., Saumell, C., Sanchez, S.F., Lanusse, C.E., 1996. Plasma disposition kinetics of albendazole metabolites in pigs fed different diets. Res. Vet. Sci. 60, 152–156.

Alvarez, L., Suárez, G., Ceballos, L., Moreno, L., Lanusse, C., 2012. Dose-dependent systemic exposure of albendazole metabolites in lambs. J. Vet. Pharmacol. Therap.. http://dx.doi.org/10.1111/j.1365-2885.2011.01326.x.

Assana, E., Kyngdon, C.T., Gauci, C.G., Geerts, S., Dorny, P., De Deken, R., Anderson, G.A., Zoli, A.P., Lightowlers, M.W., 2010. Elimination of *Taenia solium* transmission to pigs in a field trial of the TSOL18 vaccine in Cameroon. Int. J. Parasitol. 40, 515–519.

Barrère, V., Alvarez, L., Suarez, G., Ceballos, L., Moreno, L., Lanusse, C., Prichard, R., 2012. Relationship between increased albendazole systemic exposure and changes in single nucleotide polymorphisms on the beta-tubulin isotype 1 encoding gene in *Haemonchus contortus*. Vet. Parasitol.. http://dx.doi.org/10.1016/j.vetpar.2011.11.068.

Beretta, C., Fadini, L., Malvisi, J., Montsissa, C., 1987. In vitro febantel transformation by sheep and cattle ruminal fluids and metabolism by hepatic subcellular fractions from different animal species. Biochem. Pharmacol. 36, 3107–3114.

Biehl, L.G., 1986. Anthelmintics for swine. Vet. Clin. North Am. Food Anim. Pract. 2, 481–487.

Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:221:0008:0036:EN:PDF>.

Council Regulation (EEC) No. 2377/90 (EMEA/MRL/888/03-FINAL) June, 2004. European Medicines Agency. Veterinary Medicines and Inspections. Committee for Veterinary Medical Products. Oxfendazole. Summary Report (4). <http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015330.pdf>.

EMEA/CVMP/036/95: Note for Guidance: Committee for Medicinal Veterinary products. Approach towards Harmonisation of Withdrawal Periods (CVMP adopted April 96). http://www.ema.europe.eu.

FDA/CVM guidance document #35 of 1996, and released in November, 2006. <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052363.pdf}.

Flisser, A., Gauci, C.G., Zoli, A., Martinez-Oca, J., Garza-Rodriguez, A., Dominguez-Alpizar, J.L., Maravilla, P., Rodriguez-Canul, R., Avila, G., Aguilar-Vega, L., Kyngdon, C., Geerts, S., Lightowlers, M.W., 2004. Induction of protection against porcine cysticercosis by vaccination with recombinant oncosphere antigens. Infect. Immun. 72, 5292–5297.

Garcia, H.H., Gonzalez, A.E., Gilman, R.H., Moulton, L.H., Verastegui, M., Rodriguez, S., Gavidia, C., Tsang, V.C., 2006. Combined human and porcine mass chemotherapy for the control of *T. Solium*. C.V. Am. J. Trop. Med. Hyg. 74, 850–855.

Garcia, H.H., Del Brutto, O.H., 2005. For the cysticercosis working group in Peru neurocysticercosis: updated concepts about an old disease. Lancet Neurol. 4, 653–661.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, second ed. Marcel Dekker, New York.

Gonzalez, A.E., Garcia, H.H., Gilman, R.H., Gavidia, C.M., Tsang, V.C., Bernal, T., Falcon, N., Romero, M., Lopez-Urbina, M.T., 1996. Effective single-dose treatment of porcine cysticercosis with oxfendazole. Am. J. Trop. Med. Hyg. 54, 391–394.

Gonzalez, A.E., Garcia, H.H., Gilman, R.H., Lopez, M.T., Gavidia, C.M., McDonald, J., Pilcher, J.B., Tsang, V.C., 1995. Treatment of porcine cysticercosis with albendazole. Am. J. Trop. Med. Hyg. 53, 571–574.

Gonzalez, A.E., Falcon, N., Gavidia, C., Garcia, H.H., Tsang, V.C., Bernal, T., Romero, M., Gilman, R.H., 1998. Time-response curve of oxfendazole in the treatment of swine cysticercosis. Am. J. Trop. Med. Hyg. 59, 832–836.

Gonzalez, A.E., Garcia, H.H., Gilman, R.H., Gilman, R.H., Tsang, V.C., 2003. Control of *Taenia Solium*. Acta Trop. 87, 103–109.

Gonzalez, A.E., Falcon, N., Gavidia, C., Garcia, H.H., Tsang, V.C., Bernal, T., Romero, M., Gilman, R.H., 1997. Treatment of porcine cysticercosis with oxfendazole: a dose-response trial. Vet. Rec. 141, 420–422.

Gonzalez, A.E., Gauci, C., Barber, D., Gilman, R.H., Tsang, V.C.W., Verastegui, M., Lightowlers, M., 2005. For The Cysticercosis Working Group in Peru, Vaccination of pigs to control human neurocysticercosis. Am. J. Trop. Med. Hyg.72, 837–839.

Hennessy, D., 1989. Exploiting physical and chemical characteristics of anthelmintic drugs to improve efficiency, In: Veterinary Therapeutics. Australian College of Veterinary Scientists, Indooroopilly, Australia, 1–26.

Hennessy, D.R., Sangster, N.C., Steel, J.W., Collins, G.H., 1993. Comparative pharmacokinetic behaviour of albendazole in sheep and goats. Int. J. Parasitol. 23, 321–325.

Lacey, E., 1990. Mode of action of benzlmidazoles. Parasitol. Today 6, 112–115.

Lanusse, C.E., Gascon, L.H., Prichard, R.K., 1995. Comparative plasma disposition kinetics of albendazole, fenbendazole, oxfendazole and their metabolites in adult sheep. J. Vet. Pharmacol. Therap. 18, 196–203.

Lanusse, C., Prichard, R., 1993. Clinical pharmacokinetics and metabolism of benzimidazole anthelmintics in ruminants. Drug Metab. Rev. 25, 235–279.

Lubega, G., Prichard, R., 1991. Interaction of benzimidazole anthelmintics with *Haemonchus contortus* tubulin: binding affinity and anthelmintic efficacy. Exp. Parasitol. 73, 203–209.

Marriner, S.E., Bogan, J.A., 1981. Pharmacokinetics of oxfendazole in sheep. Am. J. Vet. Res. 42, 1143–1145.

Mottier, L., Alvarez, L., Ceballos, L., Lanusse, C., 2006. Drug transport mechanism in helminth parasites: passive diffusion of benzimidazole anthelmintics. Exp. Parasitol. 113, 49–57.

Mottier, L., Alvarez, L., Pis, M., Lanusse, C., 2003. Transtegumental diffusion of benzimidazole anthelmintics into *Moniezia benedeni*: correlation with their octanol-water partition coefficients. Exp. Parasitol. 103, 1–7.

Murray, M., Hudson, A.M., Yassa, V., 1992. Hepatic microsomal metabolism of the anthelmintic benzimidazole fenbendazole: enhanced inhibition of cytochrome P450 reactions by oxidized metabolites of the drug. Chem. Res. Toxicol. 5, 60–66.

Perrier, D., Mayersohn, M., 1982. Non-compartmental determination of the steady-state volume of distribution for any mode of administration. J. Pharm. Sci. 71, 372–373.

Prichard, R., Hennessy, D., Steel, J., Lacey, E., 1985. Metabolite concentrations in plasma following treatment of cattle with five anthelmiintics. Res. Vet. Sci. 39, 113–178.

Sánchez Bruni, S.F., Saumell, C., Moreno, L., Alvarez, L., Fusé, L., Fiel, C., Mckellar, Q.A., Lanusse, C.E., 2005. Changes to oxfendazole chiral kinetics and anthelmintic efficacy induced by piperonyl butoxide in horses. Equine Vet. J. 37, 257–262.

Szprengier-Juszkiewics, T., Semeniuk, S., Wlodarczyk, B., 2002. Plasma kinetics and tissue residues of fenbendazole following oral administration to pigs. Bull. Vet. Inst. Pulawy 46, 119–125.

Tsang, V.C., Brand, J.A., Boyer, A.E., 1989. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). J. Infect. Dis. 159, 50–59.

VICH GL9 (GCP), June 2000. Good Clinical Practice Recommended for Implementation at Step 7 of the VICH Process on 15 June 2000 by the VICH Steering Committee. <http://www.vichsec.org/pdf/2000/Gl09_st7.pdf9>.

Virkel, G., Lifschitz, A., Pis, A., Lanusse, C., 2002. In vitro ruminal biotransformation of benzimidazole sulphoxide anthelmintics: enantioselective sulphoreduction in sheep and cattle. J. Vet. Pharmacol. Ther. 25, 15–23.