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ORIGINAL ARTICLE

Pharmacokinetic disposition of triclabendazole in cattle and sheep; discrimination of the order and the rate of the absorption process of its active metabolite triclabendazole sulfoxide

N. Mestorino · E. A. Formentini · M. F. Lucas · C. Fernandez · P. Modamio · E. Mariño Hernández · J. O. Errecalde

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Abstract A comparative pharmacokinetic study was conducted to determine the order and the rate of absorption of triclabendazole (TCBZ) in cattle and sheep. A commercial suspension of TCBZ (Biofasiolex, Biogénesis S.A., Argentina) was administered at a dose rate of 10 mg/kg by the oral route to six Holstein female calves and six Corriedale female sheep. The plasma concentration profiles of the metabolites triclabendazole sulfoxide (TCBZ-SO) and triclabendazole sulfone (TCBZ-SO_2) were analysed by means of the non-compartmental method. The order of the absorption process of the active metabolite, TCBZ-SO, was determined by construction of curves of cumulative absorbed fraction of the drug by means of the Wagner-Nelson method. The appearance of TCBZ-SO in plasma of cattle and sheep resembles the entry of a constant quantity of drug into the organism per unit time. This is explained by the reservoir effect of the rumen, which acts as a biological slow-release system for TCBZ-SO and its precursor TCBZ to the posterior digestive tract where they are absorbed. The plasma concentration profiles of TCBZ-SO in both species were well described by a one-compartment open model with zero-order process of absorption and first-order process of elimination. The values of $AUC_{0-\infty}$ and C_{max} of TCBZ-SO did not

N. Mestorino · M. F. Lucas · J. O. Errecalde
Cátedra de Farmacología, Farmacotécnia y Terapéutica, Facultad de Ciencias Veterinarias, Universidad Nacional de la Plata, La Plata, Argentina

E. A. Formentini (✉)
Cátedra de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, RP Kreder 2805, Esperanza 3080, Argentina
e-mail: eforment@fcv.unl.edu.ar

C. Fernandez · P. Modamio · E. M. Hernández
Unidad de Farmacia Clínica y Farmacoterapia. Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Barcelona, Barcelona, Spain

M. F. Lucas
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenas Aires, Argentina

differ between species, while other kinetic parameters except for λ_z had higher values in calves than in sheep. In the case of TCBZ-SO_2, t_{max} was the only parameter that did not differ between species, while other kinetic parameters except for λ_z had higher values in calves than in sheep.

Keywords Absorption · Cattle · Pharmacokinetics · Triclabendazole · Sheep

Abbreviations

$AUC_{0-\infty}$	area under the plasma drug concentration-time curve (from time zero to infinity)
BZDs	benzimidazolic agents
Cl_B	total body clearance
C_{max}	observed maximum peak concentration of the drug in plasma
MBZ	mebendazole
SPE	solid-phase extraction
t_{max}	observed time after drug administration at which peak plasma concentration occurs
TCBZ	triclabendazole
TCBZ-SO	triclabendazole sulfoxide
TCBZ-SO_2	triclabendazole sulfone

Introduction

Triclabendazole (TCBZ; 6-chloro-5-(2, 3-dichlorophenoxy)-2-methylthiobenzimidazole) is a member of the benzimidazole (BZD) group of anthelmintics and is highly effective against mature and immature stages of *Fasciola hepatica* and *Fasciola gigantica* in all ruminant species (Boray 1983; Turner et al. 1984; Bennett and Köhler 1987; Kinabo and Bogan 1988, Mottier et al. 2004).

TCBZ parent drug is not detected in plasma after its oral administration, indicating that it is completely removed from portal blood by the liver following absorption, providing evidence of first-pass hepatic metabolism (Hennessy et al. 1987). TCBZ is oxidized to form the sulfoxide and sulfone metabolites triclabendazole sulfoxide (TCBZ-SO) and triclabendazole sulfone (TCBZ-SO_2), respectively (Bogan et al. 1985; Hennessy et al. 1987). Hydroxylation of TCBZ occurs at the 4-position of the dichlorophenoxy ring, forming the corresponding hydroxylated metabolites hydroxy-TCBZ (OH-TCBZ), hydroxy-TCBZ-SO (OH-TCBZ-SO) and hydroxy-TCBZ-SO_2 (OH-TCBZ-SO_2). Extremely low amounts of TCBZ were detected in bile; the major metabolites identified being TCBZ-SO, TCBZ-SO_2 and the hydroxy metabolites. Hydroxylated TCBZ metabolites have not been detected in plasma, indicating that they do not exchange with the plasma pool, TCBZ-SO (potent flukicidal agent) and TCBZ-SO_2 being the only metabolites found in plasma (Hennessy et al. 1987).

As demonstrated for other benzimidazolic agents (BZDs), TCBZ exerts its action by binding to the β-tubulin of flukes. Immunocytochemical studies, using an anti-tubulin antibody, showed that tubulin organization was disrupted in the tegument of triclabendazole-susceptible flukes (Robinson et al. 2002). This is a rather slow mode of action. As a consequence, the effectiveness of TCBZ and TCBZ-SO is conditioned by the time during which the parasites are exposed to the active principles. Therefore, the success of therapy resides in the maintenance of active concentrations of the drugs at the site of *Fasciola hepatica* for as long as possible (Prichard et al. 1978; Hennessy 1994).

In this sense, the rumen plays an important role as physiological reservoir of drugs. It acts as a biological slow-release system for TCBZ and TCBZ-SO towards the posterior digestive tract where these drugs are absorbed.

Triclabendazole sulfoxide circulates in plasma 90–95% bound to albumin (Hennessy et al. 1987), its binding affinity being greater than values reported for other BZDS, whose percentage binding to plasma proteins does not exceed 50% (Hennessy et al. 1985). This high percentage binding to plasma albumin probably contributes to protect the molecule from biotransformation/elimination, resulting in its long persistence in the body (Sanyal 1995).

In this study a commercial formulation of TCBZ as suspension was administered to bovines and ovines by the oral route, and a comparative pharmacokinetic study was carried out using the TCBZ-SO and TCBZ-SO_2 plasma concentration data obtained in order to establish differences and/or similarities in the kinetic disposition of these metabolites in both species, with particular attention on the order and rate of the absorption process.

Materials and methods

Experimental animal treatment and sampling procedures

Six Holstein female calves weighting 150 ± 20.6 kg and six Corriedale female sheep weighting 52.5 ± 9 kg, healthy and parasite free, were used. Before and during the study the animals were freely grazed on high-quality Lucerne pasture, and water was provided *ad libitum*.

Following acclimatization to the experimental conditions for two weeks, triclabenzadole was administered by oral route as 5% (sheep) and 10% (calves) commercial suspensions (Biofasiolex 5% and Biofasiolex T10, respectively, Biogénesis, S.A., Argentina) at a dose rate of 10 mg/kg. Blood samples were drawn from the right jugular vein at the following times: in calves, prior to drug administration and 2, 4, 8, 12, 18, 24, 36, 48, 72, 96 and 120 h; in sheep, the sample at 96 h was omitted. Blood samples were drawn into heparinized tubes. Plasma separated by centrifugation was frozen at −20°C until analysis.

Analytical procedures

TCBZ-SO and TCBZ-SO_2 were extracted from the plasma matrix by means of a modification of the method reported by Sanyal (1994). The detection and quantification of the sulfoxide and sulfone metabolites were performed by reversed-phase high-performance liquid chromatography using a sensitive method reported by Hennesy et al. (1987).

Reagents

Pure standards of TCBZ-SO and TCBZ-SO_2 were supplied by Australian Government Analytical Laboratories. Mebendazole (MBZ) used as internal standard was supplied by Sigma Aldrich (SIGMA ALDRICH®, Saint Louis, USA). Solid-phase extraction (SPE) of the plasma was done using C_{18} Strata cartridge (Phenomenex, Torrance, CA, USA).

Plasma extraction

Plasma (1 ml) was spiked with 0.5 μg MBZ internal standard and protein precipitated with 1 ml of acetone. After centrifugation, the supernatant was diluted with 5 ml of water and applied to a C_{18} Strata cartridge that had been preconditioned with 5 ml each of methanol

and water. Following washing with 10 ml water, the compounds were collected in 2.5 ml methanol and evaporated under a nitrogen stream. The residue was reconstituted with 200 μl of mobile phase and then a 50 μl aliquot was injected directly into the HPLC system.

Chromatographic analysis

The chromatographic system consisted of an isocratic pump (Gilson Inc. 307, GILSON®, Middleton, USA), an automatic injector (Gilson Inc. 234, GILSON®, Middleton, USA) and a UV-VIS detector (Gilson Inc. 155, GILSON®, Middleton, USA) set at a wavelength of 292 nm. An octadecylsilane column (Luna C_{18}, 4.6 mm×150 mm, 5 μm; Phenomenex) was eluted with a mixture of 0.025 mol/L sodium acetate and acetonitrile (50:50, v/v) at a flow rate of 1.2 ml/min. Metabolites in plasma were identified by comparison with the retention times of the reference standards. The precision of the extraction procedure and chromatography was evaluated by processing as replicates on six different occasions aliquots of pooled plasma samples containing known amounts of TCBZ-SO and TCBZ-SO_2.

Preparation of the standard curve

Stock solutions of TCBZ-SO, TCBZ-SO_2 and MBZ were prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/ml. From the stock solutions, working solutions between 0.03 and 2.20 μg per 20 μl (external standard) were prepared in DMSO. Plasma samples were spiked with the external standards at seven different concentrations of 0.03–2.20 μg and 0.5 μg of the internal standard MBZ was added to each vial. The spiked plasma samples were extracted by SPE and dried under a nitrogen stream. The residue was reconstituted with 200 μl of mobile phase and then a 50 μl aliquot was injected directly into the HPLC system to enable calibration curves to be prepared over seven different ranges of the two compounds. The concentrations in the cleaned up plasma extracts were determined by comparing the detector response for each component in the sample with that of the corresponding peak in the standard mixture.

The analytical method was validated prior to the start of the study. The limit of detection (LOD) of TCBZ-SO or TCBZ-SO_2 was established with HPLC analysis of blank cattle or sheep plasma fortified with the standard, measuring the baseline noise at the retention time of the peak. The mean baseline noise at the peak retention time plus three standard deviations was defined as the detection limit. The mean baseline noise plus ten standard deviations was defined as the limit of quantification. The limits of quantification (LOQ) of the assay were 0.03 μg/ml for both TCBZ-SO and TCBZ-SO_2. The mean overall recovery rates were 90.94% for TCBZ-SO and 79.87% for TCBZ-SO_2, and the between-day coefficients of variation (CV) were 4.12% and 5.89%, respectively.

Pharmacokinetic analysis of TCBZ-SO

Discrimination of the order of the absorption process

Because TCBZ-SO is formed by oxidation of its parent molecule TCBZ, the relevant reference is not to the absorption process of TCBZ-SO but rather to the appearance rate of the metabolite in the general circulation. Given the complexity of this process, for convenience we will refer to it as the "apparent absorption process".

The pharmacokinetic study of TCBZ-SO and TCBZ-SO_2 assumed linear kinetics for both molecules; that is, in each case the total clearance value (Cl_B) behaves independently

of the plasma concentration (C_p), and the area under the plasma concentration-time curve (AUC) is proportional to the quantity of drug present in the organism.

In order to study the effect of the rumen on the order and the rate of appearance of TCBZ-SO in the systemic circulation, cumulative curves of the absorbed/formed fraction of TCBZ-SO were constructed according the method reported by Wagner and Nelson (1963) as follows:

$$f_{\mathrm{abs}\,0-t} = X_{\mathrm{a}\,0-t}/X_{\mathrm{a}\,0-\infty}$$

where $f_{\mathrm{abs}0\text{-}t}$ is the cumulated absorbed/formed fraction of TCBZ-SO, $X_{\mathrm{a}\ 0\text{-}t}$ is the quantity of drug entering the organism from time zero to a determined time t calculated as

$$X_{\mathrm{a}\,0-t} = C_{\mathrm{p}(t)} + (\lambda_z \mathrm{AUC}_{0-t})$$

where $C_{\mathrm{p}(t)}$ is the plasma concentration given at any time, λ_z is the first-order rate constant of elimination estimated from the terminal phase, $\mathrm{AUC}_{0\text{-}t}$ is the area under the plasma concentration-time curve from time zero to any time estimated by the trapezoidal method; and $X_{\mathrm{a}0\text{-}\infty}$ is the quantity of drug entering the organism from time zero to infinity calculated as

$$X_{\mathrm{a}\,0-\infty} = \lambda_z \mathrm{AUC}_{0-\infty}$$

where $\mathrm{AUC}_{0\text{-}\infty}$ is the area under the plasma concentration-time curve from time zero to infinity calculated by the trapezoidal method. Subsequently, individual curves of the fraction of TCBZ-SO remaining unabsorbed/unformed (f_{rem}) of TCBZ-SO vs time at the site of apparent absorption as described by Wagner and Nelson (1963) were constructed as follows:

$$f_{\mathrm{rem}\,0-t} = 1 - f_{\mathrm{abs}\,0-t}$$

The estimated mean values of the fraction of TCBZ-SO remaining unabsorbed/unformed were fitted by

$$C = A - Bx$$

This equation can be associated with a zero-order process in which C is the fraction of TCBZ-SO remaining unabsorbed/unformed in the site of apparent absorption, A is the total quantity of drug in the site of apparent absorption at time zero expressed as unity, B is equivalent to the zero-order constant or k_0, and x is the time.

Non-compartmental analysis

In a second analysis, the pharmacokinetics of TCBZ-SO was studied by analysing the C_p data by the non-compartmental method. The individual pharmacokinetic parameters were calculated and expressed as mean (X) and standard deviation (SD). The AUC was calculated by the trapezoidal rule method reported by Baggot (1977) and extrapolated to infinity by dividing the last measured plasma concentration by the value of λ_z. The mean residence time (MRT) was calculated as follows:

$$\mathrm{MRT} = \mathrm{AUMC}_{(0-\infty)}/\mathrm{AUC}_{(0-\infty)}$$

where $\mathrm{AUMC}_{(0\text{-}\infty)}$ is the total area under the first moment curve calculated as reported by Baggot (1977). As long as the order of the appearance of TCBZ-SO in plasma is better described as a zero-order process, the mean absorption time (MAT) can be calculated as

$$\mathrm{MAT} = t_{\mathrm{abs}}/2$$

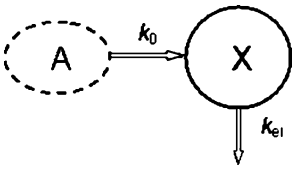

Fig. 1 Graphical representation of a one-compartmental open model with first-order elimination and zero-order absorption processes, where A is the drug present at the absorption site, k_0 is the apparent zero-order absorption rate constant, X is the drug present in the central compartment, and k_{el} is the apparent first-order elimination rate constant

where t_{abs} is the observed time corresponding to the end of the absorption process that precedes the beginning of the disposition phase. The maximum plasma concentration (C_{max}) and t_{abs} correspond to the observed values in the experimental data. The half-life of disposition ($t_{1/2(d)}$) was calculated by the relationship

$$t_{1/2(d)} = (\ln 2)/\lambda_z$$

where ln is the natural logarithm. Other pharmacokinetic parameters were calculated by classic methods using the non-compartmental method.

Compartmental analysis

The compartmental analysis was performed by fitting the individual plasma concentration profiles to a one-compartment open model with first-order process of elimination and zero-order process of absorption:

$$C_p = \frac{k_0}{V_d \times \lambda_z}\left(1 - e^{-\lambda_z t_{abs}}\right)e^{-\lambda_z t}$$

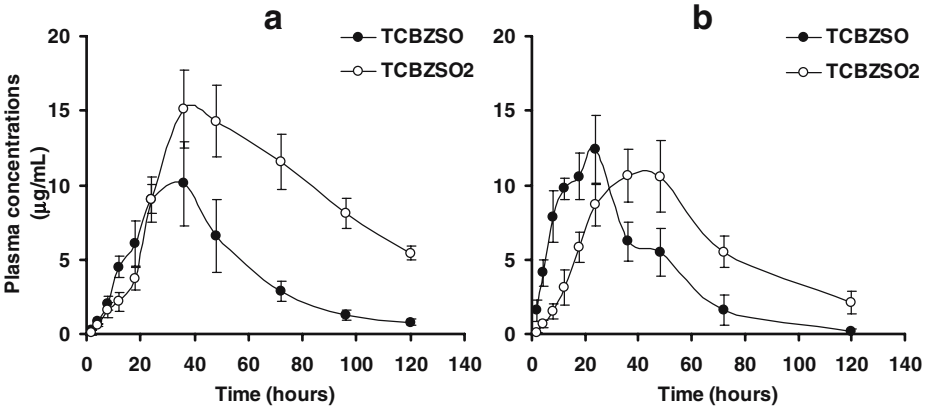

Fig. 2 Plots of plasma concentration-time profiles (mean ± SD) of triclabendazole sulfoxide (TCBZ-SO) and triclabendazole sulfone (TCBZ-SO_2) obtained after oral administration of triclabendazole (Biofasiolex, Biogénesis S.A., Argentina) at a dose rate of 10 mg/kg in calves (a) and sheep (b)

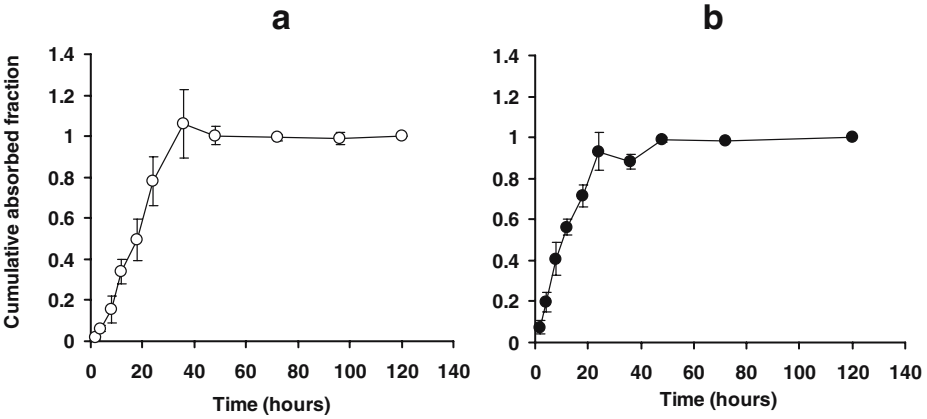

Fig. 3 Plot of cumulative absorbed fraction of triclabendazole sulfoxide (TCBZ-SO) as a function of time expressed as mean±SD (n=6) constructed by the Wagner-Nelson method from plasma concentration-time profiles of TCBZ-SO obtained after oral administration of triclabendazole (Biofasiolex, Biogénesis S.A., Argentina) at a dose rate of 10 mg/kg in calves (a) and sheep (b)

where k_0 is the zero-order constant of apparent absorption, V_d is the volume of distribution, and t is time. Other symbols are as defined previously. A graphical representation of the pharmacokinetic model is presented in Fig. 1.

The pharmacokinetic parameters were calculated directly from the plasma concentration-time profiles by weighted non-linear least-squares regression analysis using the software WinNonlin Professional, Pharsight Corporation, Version 4.1. Weightings used for the xth measured drug concentration were 1, $1/x^{1/2}$, $1/x$ and $1/x^2$. Plots of the weighted residuals were inspected and the best weighting scheme was selected based on the scatter and random distribution of the residuals around the abscissa axis.

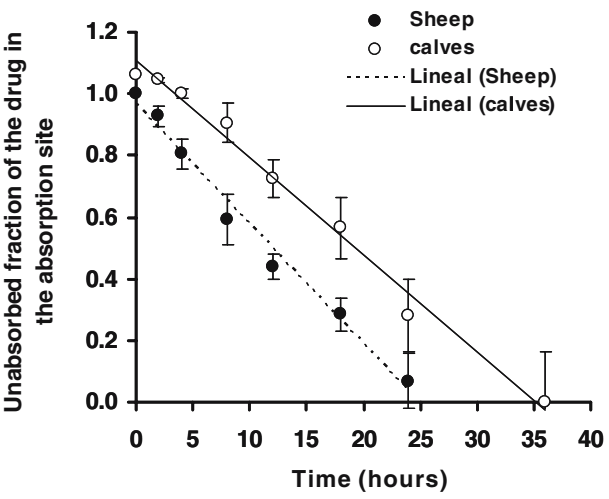

Fig. 4 Plot of fraction of triclabendazole sulfoxide (TCBZ-SO) expressed as mean±SD (n=6) remaining at the absorption site as a function of time constructed by the Wagner-Nelson method from plasma concentration-time profiles of TCBZ-SO obtained after oral administration of triclabendazole (Biofasiolex, Biogénesis S.A., Argentina) at a dose rate of 10 mg/kg in calves and sheep. The lines represent the mean values estimated from a first-order equation

Table 1 Pharmacokinetic parameters (mean±SD) of triclabendazole sulfoxide (TCBZ-SO) in calves and sheep estimated by non-compartmental analysis of its plasma concentration profiles obtained after administration of triclabendazole (Biofasiolex, Biogénesis S.A., Argentina) at a dose rate of 10 mg/kg by the oral route

Kinetic parameter[a]	Calves (n=6)	Sheep (n=6)
$AUC_{0-\infty}$ (μg h/ml)	525.7±100.8	512.7±101.5[NS]
$AUMC_{0-\infty}$ (μg h^2/ml)	26 120±4 908	17 663±5 740
$MRT_{(t)\ 0-\infty}$ (h)	49.9±3.88	33.8±4.68
MAT (h)	15.0±3.29	11.0±1.55
t_{max} (h)	30.0±6.57	22.0±3.10
C_{max} (μg/ml)	10.7±2.32	12.7±2.16[NS]
λ_z (h^{-1})	0.030±0.0049	0.046±0.008
$t_{1/2}(\lambda_z)$ (h)	23.8±3.85	15.5±2.93

[a] $AUC_{0-\infty}$, area under the plasma drug concentration-time curve (from time zero to infinity); $AUMC_{0-\infty}$, area under the first moment curve (from zero to infinity); MRT and MAT, mean residence time and mean absorption time respectively; t_{max}, observed time after drug administration at which peak plasma concentrations occurs; C_{max}, observed maximum peak concentration of the drug in plasma; λ_z, elimination rate constant; $t_{1/2}(\lambda_z)$, half-life.

[NS] Non-significant difference between species.

Pharmacokinetic analysis of TCBZ-SO_2

The pharmacokinetic study was carried out by non-compartmental analysis of the plasma profiles of TCBZ-SO_2. The ratio of the AUC values of TCBZ-SO_2 and TCBZ-SO (SO_2/SO) was calculated.

Statistical analysis

The pharmacokinetic parameters are reported as mean ± standard deviation (SD) and were statistically compared using the Mann-Whitney U-test. Mean values were considered significantly different at $p<0.05$.

Table 2 Pharmacokinetic parameters (mean±SD) of triclabendazole sulfone (TCBZ-SO_2) in calves and sheep estimated by non-compartmental analysis of its plasma concentration profiles obtained after administration of triclabendazole (Biofasiolex, Biogénesis S.A., Argentina) at a dose rate of 10 mg/kg by the oral route

Kinetic parameter[a]	Calves (n=6)	Sheep (n=6)
$AUC_{0-\infty}$ (μg h/ml)	1 516.7±69.7	812.2±120.7
$AUMC_{0-\infty}$ (μg h^2/ml)	15 0674±14 831	55 661±15 924
$MRT_{0-\infty}$ (h)	99.5±11.0	67.4±10.7
t_{max} (h)	42.0±6.6	38.0±9.0[NS]
C_{max} (μg/ml)	15.6±2.3	11.8±2.1
λ_z (h^{-1})	0.0134±0.0027	0.0217±0.0051
$t_{1/2}$ (λ_z) (h)	52.9±8.5	33.5±8.1
SO_2/SO	2.96±0.48	1.63±0.40

[a] SO_2/SO, ratio between the AUC values of TCBZ-SO_2 and TCBZ-SO. Other symbols are as defined in Table 1.

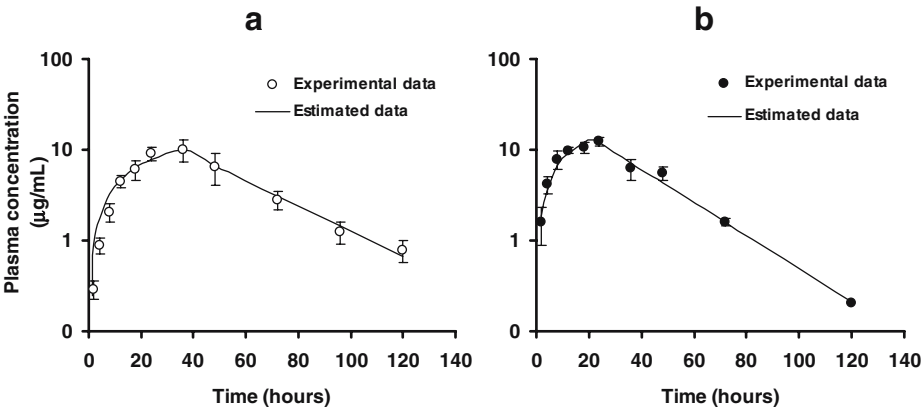

Fig. 5 Mean±SD plasma log concentration-time profiles of triclabendazole sulfoxide (TCBZ-SO) obtained after oral administration of triclabendazole (Biofasiolex, Biogénesis S.A., Argentina) at a dose rate of 10 mg/kg in calves (a) and sheep (b)

Results

Using the chromatographic conditions described, TCBZ-SO, TCBZ-SO_2 and MBZ were well resolved with retention times of 3.75±0.07, 2.28±0.06 and 2.65±0.06 min, respectively, and no interference was seen from the endogenous compounds.

Plots of plasma concentration versus time profiles of TCBZ-SO and TCBZ-SO_2 obtained in cattle and sheep, expressed as mean±SD values, are presented in Fig. 2.

Plots of cumulative fractional absorbed TCBZ-SO as a function of time obtained in cattle and sheep, expressed as mean±SD values, are presented in Fig. 3.

Values of the mean±SD fractions of drug remaining in the absorption site as a function of time were plotted for calves and sheep; their corresponding values estimated by linear regression are presented in Fig. 4.

Some pharmacokinetic parameters of TCBZ-SO and TCBZ-SO_2 in calves and sheep calculated by the non-compartmental method, expressed as mean±SD, are presented in

Table 3 Pharmacokinetic parameters (mean±SD) of triclabendazole sulfoxide (TCBZ-SO) in calves and sheep estimated by compartmental analysis of its plasma concentration profiles obtained after administration of triclabendazole (Biofasiolex, Biogenésis S.A., Argentina) at a dose rate of 10 mg/kg by oral route

Kinetic parameter[a]	Calves (n=6)	Sheep (n=6)
k_0/F (µg kg h)	350±36.9	514±31.5
$V_{d\ (area)}/F$ (ml/kg)	750±93.4	534±53.3
Cl_B/F (ml kg h)	23.5±3.01	24.9±3.55^{NS}
$AUC_{0\text{-}\infty}$ (µg h/ml)	431±56.9	407±57.4^{NS}
t_{abs} (h)	34.9±2.76	22.5±1.62
C_{max} (µg/ml)	9.66±2.15	12.7±1.70^{NS}
λ_z (h^{-1})	0.031±0.0056	0.047±0.0081
$t_{1/2}(\lambda_z)$ (h)	22.5±4.58	15.1±2.78

[a] k_0/F, apparent zero-order constant of absorption; $V_{d(area)}/F$, volume of distribution associated with the elimination phase; Cl_B/F, apparent total clearance; t_{abs}, estimated time at which the zero-order absorption process ends. Other symbols as defined in Table 1.

Tables 1 and 2, respectively. The values of $AUC_{0-\infty}$ and C_{max} for TCBZ-SO did not differ between species. while other kinetic parameters except for λ_z had higher values in calves than in sheep. In the case of TCBZ-SO_2, t_{max} was the only parameter that did not differ between species, while other kinetic parameters except for λ_z had higher values in calves than in sheep.

The optimal weighting scheme for fitting the plasma concentration-time profiles of TCBZ-SO in a compartmental analysis was $w=1$, this was selected because of a more homogeneous scatter and random distribution of residuals about the abscissa axis in a plot of weighted residuals as ordinate versus time as abscissa.

Figure 5 (logarithmic scale) shows the mean±*SD* plasma concentration-time profiles of TCBZS-SO obtained in calves (A) and sheep (B) and the mean estimated values obtained by fitting the profiles of each animal with compartmental analysis.

Pharmacokinetic parameters of TCBZ-SO in calves and sheep estimated by means of compartmental analysis using a one-compartmental open model with zero-order absorption processes and apparent first-order of elimination are presented in Table 3. Values of Cl_B/F, $AUC_{0-\infty}$ and C_{max} did not differ between species, while other kinetic parameters except λ_z were larger in calves.

Discussion

The absence of TCBZ in plasma of calves and sheep and the presence of their active (TCBZ-SO) and inactive (TCBZ-SO_2) metabolites in plasma of both species indicate that TCBZ was extensively metabolized presystemically (Sanyal 1995). This phenomenon is explained by the fact that TCBZ, during absorption from the digestive tract, can be metabolized either in the intestine or in the liver (Hennessy et al. 1987; Virkel et al. 2006). However, loss of drug from the digestive tract by the action of enzymes of the digestive microflora (rumen and intestine) should not be underestimated.

It is assumed that any compound once it has entered into the systemic circulation exhibits a degree of diffusion to tissues; therefore, for any drug; a distribution process is almost always operative. However, in the absence of an intravascular pharmacokinetic study of the drug and for didactic reasons, a one-compartmental open model was assumed as the best way to explain the kinetic behaviour of TCBZ-SO in bovines and ovines. Therefore, individual plots of cumulative fractional absorbed TCBZ-SO as a function of time were constructed by means of the Wagner-Nelson method (Wagner and Nelson 1963). Visual inspection of these curves shows that the fractional values increase proportionally from time zero until a time at which a 'plateau' (30 h in calves and 22 h in sheep) is achieved, this 'plateau' corresponds with the time at which the apparent absorption process ends, or t_{abs}. This agrees with plasma profiles obtained by some authors in sheep, cattle, goats and buffalos (Sanyal 1995; Oukessou and Souhaili 1998, Gokbulut et al. 2006), although none of them mentions the plateau.

These profiles suggest a constant amount of drug entering into the systemic circulation per unit time (Gibaldi and Perrier 1982). This hypothesis is confirmed by the fraction of TCBZ-SO remaining at the absorption site being adequately fitted with a first-order equation. As a result, a zero-order process is proposed as the most suitable to explain the order and the magnitude of the appearance of TCBZ-SO in plasma of calves and sheep in this experiment. Accordingly, a one-compartment open model with apparent first-order rate of elimination and zero-order rate of absorption is the most appropriate to fit and describe the plasma concentration-time profiles of TCBZ-SO in both species.

The value of the zero-order rate constant of apparent absorption (k_0) calculated by the model is an overestimate because the bioavailability (F) is unknown. This is because in the case of extravascular administration of a drug, k_0 is defined as follows:

$$k_0 = (F \times D)/t_{abs}$$

where D is the dose and other symbols are as defined previously. However, in the absence of the F value, k_0 is defined by the relationship:

$$k_0/F = D/t_{abs}$$

In the absence of F, the value of k_0/F gives only relative information about the magnitude of the amount of TCBZ-SO entering into the organism per unit time. Consequently, the differences encountered between species cannot be considered in absolute terms.

The irregular plasma concentration-time profiles of TCBZ-SO during the absorption phase (Fig. 2) suggest that they are the consequence of a sum of absorption pulses in which the amount of drug entering into the general circulation and the elapsed time between them are unpredictable.

The apparent time in which the absorption process takes place (t_{abs}) and the entry of TCBZ-SO into the general circulation as absorption pulses may be explained as follows: (a) the reservoir effect of the rumen, which slows the passage of the digesta towards the posterior digestive tract in which TCBZ and TCBZ-SO are absorbed (Knox and Steel 1997; Oukessou and Souhaili 1998); (b) the emptying of the abomasum giving an intermittent outflow governed by the pyloric pump which releases the contents (and a fraction of the administered dose) to the posterior digestive tract as a series of pulses (Phillipson 1981).

The absorption of BZDs in ruminant species involves many factors including dispersion of the suspended particles in the ruminal fluids, their dissolution, and their slow and sustained passage to the posterior digestive tract for absorption and binding to particulate digesta. On the other hand, TCBZ can enter into the portal circulation unchanged but can be oxidized to TCBZ-SO by the digestive microflora prior to its absorption or by the intestine wall during it. Another factor to be considered is the loss of a fraction of the active drug by formation of inactive metabolites such as TCBZ-SO_2 before or during absorption or by the hepatic first-pass effect. Notwithstanding the good fit of the plasma concentration-time data given by the selected model (Fig. 5), the absorption process in ruminant species appears to be very complex, the model employed here being a simplification of the situation.

The zero-order rate constant of absorption/formation of TCBZ-SO is justified by the slow release of the drugs from the rumen and the intermittent pyloric outflow, which with the presystemic metabolism leads to an absorption profile that closely resembles a zero-order rate of entry of TCBZ-SO into the general circulation. In view of this, we consider that it inexact to refer to a study of absorption of TCBZ-SO but rather the application of a model associated with a zero-order process to study the plasma concentration-time profile of the appearance of TCBZ-SO in plasma.

When the rate of absorption follows a zero-order process, the maximum observed plasma concentration (C_{max}) does not obviously have the same significance as during a first-order process. In the latter case, the C_{max} value increases proportionally to the quantity of drug entering into the general circulation and the time at which it occurs (t_{max}) is inversely proportional to the rate of absorption. On the other hand, when the absorption follows a zero-order process, the plasma concentration-time profile during the phase of absorption is characterized by a constant increase until a time at which the absorption ends,

or t_{abs}. As a consequence, the plasma concentrations tends to achieve a stationary value, the plasma concentration at steady state ($C_{p(ss)}$). Therefore, the observed C_{max} during an absorption process explained by a zero-order rate corresponds to the larger extreme value followed by a series of similar values, which determine the width of the dispersion of the $C_{p(ss)}$ values. This is because even in steady state the measured plasma concentrations show fluctuations due to errors in analytical quantification of the drug as well as variations in the magnitudes of the physiological processes involved in the disposition of the drug.

In the same line of reasoning, the observed t_{max} cannot be related to the speed of the absorption process and should not be considered as synonymous with t_{abs}. The latter should be correlated with the time at which the last plasma concentration previous to the beginning of the elimination phase occurs, which was estimated by compartmental analysis as 34.9± 2.76 h in calves and 22.5±1.62 h in sheep. These values, although slightly different from those observed in the experimental data (Table 1), constitute a better estimate of values not biased by the sampling frequency.

Because the bioavailability F of TCBZ-SO is unknown, it is not possible to establish a linear comparison between the values of estimated F to elucidate differences or similarities between species. It is possible to take into consideration the smaller elimination from the organism, or λ_z, observed in calves (0.030 h^{-1}) in comparison to sheep (0.046 h^{-1}) and the differences found in the pharmacokinetic parameters of TCBZ-SO_2 (Table 2). In this case a larger value of the SO_2/SO ratio found in calves (2.96±0.48) than in sheep (1.63±0.40) indicates a larger capacity for formation of TCBZ-SO_2 which suggests a greater capacity to eliminate the TCBZ-SO from the organism in calves than in sheep.

Conclusion

In a comparative pharmacokinetic study, certain basic assumptions applied constitute the limitations of the theory. In this study, we assume the kinetic behaviour of TCBZ-SO and TCBZ-SO_2 to be linear. In our case, although the entrance/appearance of TCBZ-SO in the organism was best explained as a zero-order process, the resulting values of AUC during the absorption phase show a linear relationship with the quantity of TCBZ-SO absorbed/ formed; thus the pharmacokinetic profile behaves linearly.

It should be recognized that a comparative pharmacokinetic study between species cannot be carried out in an objective way in the absence of intravenous administration data.

Triclabendazole behaves kinetically differently in calves and sheep with regard to the rate at which it is eliminated from the organism. This could be due the protective action of plasma proteins and/or a low capacity of biotransformation/elimination of TCBZ-SO in cattle.

The shapes of the plasma concentration-time profiles are determined by the ruminal reservoir effect, which diminishes the post-ruminal drug availability and delays the release of the active molecules (TCBZ-TCBZS-SO) towards the posterior digestive tract in which they should be absorbed. This phenomenon explains the appearance of TCBZ-SO in the general circulation as a zero-order process. All of the above phenomena contribute significantly to prolonging the presence of TCBZ-SO not only in the systemic circulation but at the site of location of *Fasciola hepatica* and *Fasciola gigantica* also, determining the effectiveness of this drug in these two species.

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