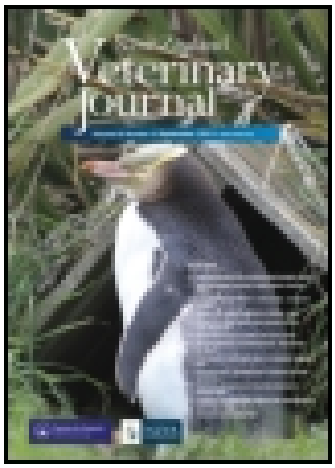

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Pharmacokinetic evaluation of different generic triclabendazole formulations in heifers

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Scientific Article

Pharmacokinetic evaluation of different generic triclabendazole formulations in heifers

P Ortiz*, N Castope*, M Cabrera*, C Farias†, G Suarez‡, C Lanusse† and L Alvarez†§

Abstract

AIMS: To assess the comparative drug systemic exposure of a reference (RF) and four test (Test I, Test II, Test III and Test IV) formulations of triclabendazole (TCBZ) in heifers.

METHODS: Thirty Holstein heifers were randomly distributed into five groups (n=6 per group). Animals in the RF group received the reference formulation (Fasinex), and those in the other groups received different commercially available TCBZ formulations (Test I, Test II, Test III and Test IV). All treatments were orally administered at 12 mg/kg bodyweight. The concentrations of TCBZ metabolites in plasma between 0 and 168 hours after treatment were quantified by high-performance liquid chromatography (HPLC).

RESULTS: Triclabendazole sulphoxide (TCBZ.SO) and TCBZ-sulphone ($TCBZ.SO_2$) were the only analytes recovered in plasma. Only the Test I formulation did not differ from the RF for all pharmacokinetic parameters measured for either metabolite ($p>0.8$). The TCBZ.SO area under the concentration *vs.* time curve for Test II formulation (268.9 µg.h/mL) was lower, and for Test III (619.9 µg.h/mL) and Test IV (683.4 µg.h/mL) was higher, than the RF (418.1 µg.h/mL) ($p<0.005$).

CONCLUSION: Based on the currently available bioequivalence criteria, the only test formulation under evaluation that could be considered equivalent to the RF was the Test I formulation, which demonstrated an equivalent systemic exposure for the active TCBZ.SO metabolite. This comparison of TCBZ pioneer and test formulations in cattle raises awareness of the need for further quality control for drug approval in the veterinary pharmaceutical field in many regions of the world.

KEY WORDS: *Flukicidal control, triclabendazole, generic formulations, bioequivalence*

Introduction

Triclabendazole (TCBZ, 6-chloro-5(2-3 dichlorophenoxy)-2-methyl thio-benzimidazole), a halogenated benzimidazole thiol derivative, shows high efficacy against both the immature and mature stages of *Fasciola hepatica* in sheep and cattle, which is a differential feature compared to other available trematodicidal drugs (Boray *et al.* 1983). As a consequence of its excellent activity against the liver fluke it has been extensively used and this has inevitably promoted the selection of TCBZ-resistant populations, which is now a worrying problem in several areas of the world (Fairweather 2005, 2009). TCBZ resistance has been reported in both Europe (Coles *et al.* 2000; Moll *et al.* 2000; Alvarez-Sánchez *et al.* 2006) and South America (Olaechea *et al.* 2011a, b; Ortiz *et al.* 2013).

The benzimidazole anthelmintics require extensive hepatic oxidative metabolism to achieve sufficient polarity for excretion. TCBZ is rapidly oxidised to form triclabendazole sulphoxide (TCBZ.SO) and triclabendazole sulphone ($TCBZ.SO_2$) (Hennessy *et al.* 1987; Sanyal 1995). The parent compound is short lived, and both TCBZ.SO and $TCBZ.SO_2$ are the main unconjugated analytes recovered in the bloodstream and bile of treated sheep (Hennessy *et al.* 1987). It has been demonstrated that both metabolites may be capable of inducing severe disruption to *F. hepatica in vitro* (Halferty *et al.* 2009). Furthermore, we have shown that oral ingestion is a main route of drug entry into adult flukes *in vivo* exposed to TCBZ metabolites (Moreno *et al.* 2014). Consequently, flukicidal activity may be mainly related to a complementary activity of both systemically available TCBZ metabolites, although only a limited *in vivo* activity (41% efficacy on 28-day-old liver flukes) has been shown for the sulphone metabolite (Büscher *et al.* 1999).

AUC	Area under the concentration *vs.* time curve
C_{max}	Peak concentration
HPLC	High-performance liquid chromatography
LOQ	Limit of quantification
MRT	Mean residence time
RF	Reference formulation
TCBZ	Triclabendazole
TCBZ.SO	Triclabendazole sulphoxide
$TCBZ.SO_2$	Triclabendazole sulphone
T_{max}	Time to the peak concentration
$T_{1/2}$	Elimination half life

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Several generic formulations of TCBZ have been introduced into the pharmaceutical market in different regions of the world after the expiration of the original patent of the first approved (pioneer) TCBZ formulation (Fasinex, Novartis Animal Health, Basel, Switzerland). In Peru, about 15 TCBZ formulations are available for use in cattle and sheep. Most of them contain basically the same vehicle composition used in the pioneer TCBZ formulation, but there is no available information on the comparative kinetic behaviour of generic preparations in standardised pharmacokinetic trials. Differences in excipient composition or quality, quality of the active ingredient, etc. may determine differences in gastrointestinal absorption, plasma drug exposure and, eventually, in the clinical efficacy of the preparations. In fact, differences in plasma availability of the active albendazole sulphoxide metabolite were observed among different albendazole formulations orally administered to sheep (Suarez *et al*. 2011). Additionally, different ivermectin systemic exposures were observed in cattle after subcutaneous administration of different commercial formulations (Lo *et al*. 1985; Lifschitz *et al*. 2009). The current comparison of TCBZ pioneer and generic formulations in cattle contributes to knowledge about the quality and pharmacokinetic behaviour of different TCBZ formulations, which appears to be important in the context of resistance development. The obtained results may be useful not only for Peru, but also to other countries where different anthelmintic formulations containing the same active ingredient are available in the pharmaceutical veterinary market. The current experimental goal was to assess the comparative drug systemic exposure of a reference (RF) and four test TCBZ formulations, after their oral administration to heifers at the same dose.

Materials and methods

Chemicals

Pure (97–99%) reference standards of TCBZ and its TCBZ.SO and TCBZ.SO_2 metabolites were provided by Novartis Animal Health (Basel, Switzerland). Oxibendazole, used as an internal standard, was from Sigma-Aldrich Co (St Louis, Mo, USA). The following commercially available formulations were used in the current experiment (in alphabetical order): Bilevon (12% suspension, Bayer, Lima, Perú); Fasinex (10% suspension, Novartis, Lima, Perú); Trisan (12% suspension, Montana S.A., Lima, Perú), Zolinex Dorado, (12.5% suspension, Laboratorio Biomont S.A., Lima, Perú) and Zoliplus, (12% suspension, Innova Andina, Lima, Perú). Fasinex was considered the RF as it was the original pioneer product and the first authorised product with a full dossier. The four test formulations were randomly designated as Test I, Test II, Test III and Test IV. The different solvents (high-performance liquid chromatography (HPLC) grade) and buffer salt used for sample extraction or chromatographic methods were purchased from Mallinckrodt Baker (Xalostoc, Edo. Mexico, Mexico).

Animals and experimental design

The animal phase of the current trial was performed on a farm located in Cajamarca, Peru. Thirty healthy Holstein heifers, with an average bodyweight of 350 (SD 25.0) kg were used in this experiment. The animals were kept on pasture over the duration of the study. Water was provided *ad libitum*. All animals were fed *ad libitum* up to 30 minutes prior to treatment. Animal procedures and management protocols were carried out in accordance with the Animal Welfare Policy of the Faculty of Veterinary Medicine, Universidad Nacional de Cajamarca, Cajamarca, Peru (www.unc.edu.pe), and the ethical standards described in Anonymous (2000).

Animals were randomly allocated to five groups of six animals each. Animals in the RF group received Fasinex and those in Test I, Test II, Test III and Test IV groups received the different test formulations. In all cases, TCBZ was administered orally at 12 mg/kg bodyweight. The doses were calculated individually according to bodyweight. Blood samples (5 mL) were taken by jugular venipuncture into heparinised vacutainer tubes (Becton Dickinson, Franklin Lakes, USA) before administration (Time 0) and at 1, 3, 6, 9, 12, 18, 24, 30, 36, 48, 72, 120, 144 and 168 hours post-treatment. Plasma was separated by centrifugation at 3000g for 15 minutes, placed into plastic tubes and frozen at −20°C until analysed by HPLC.

Analytical procedures

The analytical procedures used to quantify TCBZ metabolites in plasma samples obtained from treated animals were performed in the Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina.

Drug quantification by HPLC analysis

Triclabendazole and its metabolites were extracted from plasma as previously described (Virkel *et al*. 2006). Experimental and fortified plasma samples were analysed by HPLC to determine the concentration of TCBZ and its metabolites following the methodology previously described (Virkel *et al*. 2006). The elution from the stationary phase (Selectosil C_{18} column, 5 µm, 250 × 4.6 mm, Phenomenex, CA, USA) was carried out at a flow rate of 1.2 mL/min, using a mixture of acetonitrile/ammonium acetate (0.025 M, pH 6.6) as mobile phase. Fifty µL of each previously extracted sample were injected into a Shimadzu 10A HPLC System (Kyoto, Japan), using a gradient pump, UV detector set at 300 nm, an autosampler and a controller (Shimadzu Class LC10, Kyoto, Japan). Analytes were identified by the retention times of pure reference standards. Chromatographic retention times were: 4.18 (oxibendazole), 6.66 (TCBZ.SO_2), 8.18 (TCBZ.SO) and 14.48 (TCBZ) minutes. Blank plasma samples were fortified with each analyte in a range between 0.1 and 30 µg/mL, plus the internal standard (1 µg/mL). The analytical calibration curves for the parent drug and its metabolites in plasma samples were obtained using the linear least squares regression procedure, using the run test and ANOVA to determine if the data differed from a straight line. It showed correlation coefficients ≥0.9992. Mean absolute recoveries and CV within the concentration range between 0.5 and 15 µg/mL (n=6) were 91.5% (CV 6.8%; TCBZ), 90.3% (CV 8.3%; TCBZ.SO) and 92.2% (CV 7.1%; TCBZ.SO_2). Precision (intra- and inter-assay) was determined by analysing replicates of fortified plasma samples (n=6) with each compound at three different concentrations (0.1, 5 and 10 µg/mL). The CV ranged from 3.89 to 6.04%. The limit of quantification (LOQ) was defined as the lowest measured concentration with a CV < 20% and accuracy of ±20% and an absolute recovery ≥70%. The LOQ defined for the three molecules assayed was 0.1 µg/mL. Values below LOQ were not included in the pharmacokinetic analysis.

Pharmacokinetic analysis

Concentration versus time curves for TCBZ metabolites in plasma for individual animals were fitted using the PKSolutions

computer programme (Summit Research Service, Ashland, USA). Pharmacokinetic analysis of the experimental data was performed by non-compartmental analysis. The elimination half life ($T_{1/2}$) was calculated as ln 2/β, where β represent the terminal slope (h^{-1}). The observed 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) from time 0 up to the limit of quantification ($AUC_{0\text{-}LOQ}$) was calculated by the trapezoidal rule (Gibaldi and Perrier 1982) and further extrapolated to infinity ($AUC_{0\text{-}\infty}$) by dividing the last experimental concentration by the terminal slope (β). Additionally, C_{max}/AUC was estimated in order to compare the rate of drug absorption between the reference and the different test formulations. Statistical moment theory was applied to calculate the mean residence time (MRT) for TCBZ metabolites in plasma, as follows:

$$MRT = AUMC/AUC$$

where AUC is as defined previously and AUMC is the area under the curve of the product of time and the plasma drug concentration versus time from zero to infinity (Gibaldi and Perrier 1982).

To assess the bioequivalence between the RF and each of the test formulations, TCBZ.SO C_{max} and $AUC_{0\text{-}LOQ}$ were considered the primary outcome variables. The formulations were considered bioequivalent if the ln ratios for the 90% CI of the LSM of the pharmacokinetic parameters were within the predetermined bioequivalence range of 0.8–1.25 (Anonymous 2001, 2006).

Statistical analysis

Parametric (unpaired *t* test) or non parametric (Mann-Whitney) tests were used for the statistical comparison of the pharmacokinetic data between the RF and the different test formulations. In all cases a value of $p<0.05$ was considered statistically significant.

Results

In all experimental groups, TCBZ.SO and $TCBZ.SO_2$ were the only analytes recovered in plasma after the oral administration of TCBZ. Both metabolites were measured in plasma up to 168 hours post-treatment. However, the main TCBZ metabolite found in plasma was $TCBZ.SO_2$, with $AUC_{0\text{-}LOQ}$ values representing 86 (RF group), 84 (Test I), 91 (Test II), 82 (Test III) and 83% (Test IV) of the total drug recovered from plasma in the different groups. The comparative mean concentration profiles of TCBZ.SO in plasma obtained after the oral administration of TCBZ as the RF and the different test formulations are shown in Figure 1.

Table 1 summarises the plasma pharmacokinetic parameters obtained for TCBZ.SO after the oral administration of the different formulations. Only Test I formulation did not differ from the RF for all pharmacokinetic parameters measured ($p>0.8$). For the other test formulations (Test II, III and IV) there were significant differences compared with the RF. Lower $AUC_{0\text{-}LOQ}$ was observed for Test II, whereas this parameter was higher for groups treated with Test III and IV formulations ($p<0.005$), compared to the RF. The plasma pharmacokinetic parameters obtained for

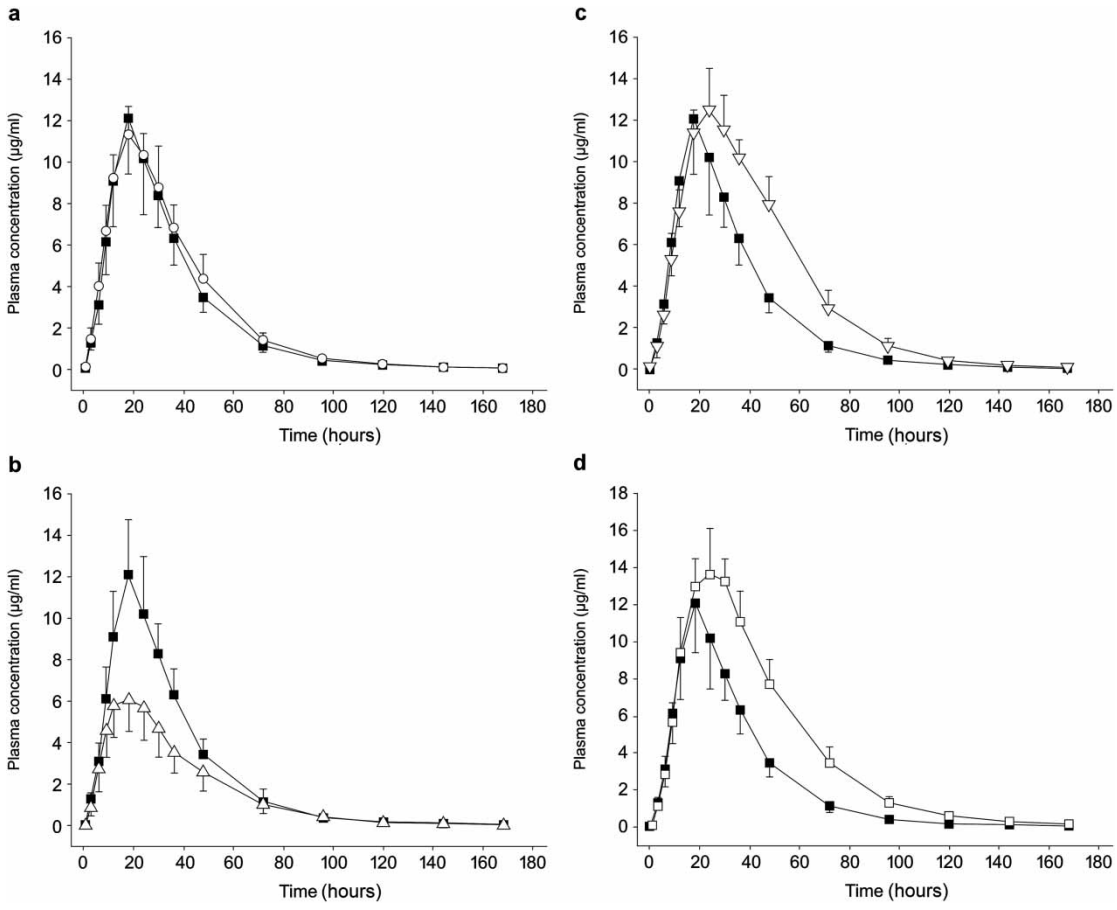

Figure 1. Mean (±SD) concentrations of triclabendazole sulphoxide in plasma measured after the administration of different formulations of triclabendazole to heifers, comparing the reference formulation (■) with a) Test I (○), b) Test II (△), c) Test III (▽) and d) Test IV (□).

Table 1. Mean (± SD) plasma pharmacokinetic parameters for triclabendazole sulphoxide, obtained after the oral administration of triclabendazole (12 mg/kg) to heifers (n=6 per group) as either a reference formulation (RF, Fasinex, Novartis), or different commercially available Test formulations. Significance of difference between Test formulations and RF is given in brackets.

Pharmacokinetic parameters	RF	Test I	Test II	Test III	Test IV
C_{max} (µg/mL)	12.1 ± 2.67	11.5 ± 1.08	6.32 ± 1.59	12.6 ± 1.88	14.3 ± 2.11
		(0.954)	(<0.001)	(0.966)	(0.196)
T_{max} (hours)	19.0 ± 2.45	18.0 ± 3.80	15.9 ± 6.46	24.0 ± 3.79	24.0 ± 3.79
		(0.982)	(0.528)	(0.159)	(0.159)
$AUC_{0\text{-}LOQ}$ (µg.hours/mL)	418.1 ± 60.2	446.6 ± 66.3	268.9 ± 78.1	619.9 ± 73.0	683.4 ± 85.5
		(0.901)	(0.005)	(<0.001)	(<0.001)
$AUC_{0\text{-}\infty}$ (µg.hours/mL)	419.0 ± 60.2	447.4 ± 66.5	270.5 ± 78.6	622.8 ± 74.5	687.8 ± 86.7
		(0.906)	(0.006)	(<0.001)	(<0.001)
MRT (hours)	33.2 ± 2.90	33.9 ± 2.57	37.3 ± 2.60	41.6 ± 3.67	42.6 ± 3.36
		(0.986)	(0.093)	(<0.001)	(<0.001)
$T_{1/2}$ (hours)	17.2 ± 1.40	16.5 ± 0.78	20.4 ± 0.70	19.2 ± 1.97	20.6 ± 1.57
		(0.803)	(0.001)	(0.055)	(<0.001)
$C_{max}/AUC_{0\text{-}t}$	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
		(0.969)	(0.589)	(0.077)	(0.013)

$AUC_{0\text{-}LOQ}$ = area under the concentration *vs.* time curve from 0 up to the limit of quantification; $AUC_{0\text{-}\infty}$ = area under the concentration *vs.* time curve extrapolated to infinity; C_{max} = peak concentration; MRT = mean residence time; T_{max} = time to peak concentration; $T_{1/2}$ = elimination half-life.

Table 2. Mean (±SD) plasma pharmacokinetic parameters for triclabendazole sulphone, obtained after the oral administration of triclabendazole (12 mg/kg) to heifers (n=6 per group) as either a reference formulation (RF, Fasinex, Novartis), or different commercially available Test formulations. Significance of difference between Test formulations and RF is given in brackets.

Pharmacokinetic parameters	RF	Test I	Test II	Test III	Test IV
C_{max} (µg/mL)	27.7 ± 6.89	23.9 ± 2.23	28.7 ± 2.21	27.9 ± 4.80	32.5 ± 2.79
		(0.975)	(0.372)	(0.965)	(0.122)
T_{max} (hours)	48.0 ± 13.2	52.0 ± 9.80	52.0 ± 9.80	56.0 ± 12.4	62.0 ± 16.0
		(0.825)	(0.825)	(0.619)	(0.227)
$AUC_{0\text{-}LOQ}$ (µg.hours/mL)	2551 ± 543	2385 ± 269	2818 ± 294	2918 ± 563	3338 ± 346
		(0.900)	(0.648)	(0.384)	(0.012)
$AUC_{0\text{-}\infty}$ (µg.hours/mL)	2848 ± 582	2700 ± 336	3277 ± 399	3807 ± 930	4056 ± 548
		(0.978)	(0.545)	(0.033)	(0.006)
MRT (hours)	90.1 ± 8.50	95.2 ± 7.69	98.9 ± 6.58	122.3 ± 20.9	109.3 ± 13.6
		(0.893)	(0.580)	(<0.001)	(0.048)
$T_{1/2}$ (hours)	42.2 ± 5.67	44.4 ± 5.60	49.9 ± 5.86	61.0 ± 14.1	53.2 ± 9.69
		(0.975)	(0.372)	(0.003)	(0.122)

$AUC_{0\text{-}LOQ}$ = area under the concentration *vs.* time curve from 0 up to the limit of quantification; $AUC_{0\text{-}\infty}$ = area under the concentration *vs.* time curve extrapolated to infinity; C_{max} = peak concentration; MRT = mean residence time; T_{max} = time to peak concentration; $T_{1/2}$ = elimination half-life.

Table 3. Statistical evaluation of bioequivalence for different triclabendazole formulations assessed following oral treatment of heifers (n=6 per treatment). Point estimates are ratios of geometric means of the triclabendazole sulphoxide pharmacokinetic parameters. Formulations were either a reference formulation (RF, Fasinex, Novartis), or different commercially available Test formulations.

Ratio	Pharmacokinetic parameter	Point estimate	90% CI	p-value	Bioequivalence
Test I/RF	C_{max}	0.97	0.85–1.10	0.734	Yes
	$AUC_{0\text{-}LOQ}$	1.07	0.95–1.20	0.479	Yes
Test II/RF	C_{max}	0.62	0.47–0.83	0.058	No
	$AUC_{0\text{-}LOQ}$	0.63	0.53–0.74	0.005	No
Test III/RF	C_{max}	1.06	0.92–1.21	0.616	Yes
	$AUC_{0\text{-}LOQ}$	1.49	1.34–1.65	<0.001	No
Test IV/RF	C_{max}	1.19	1.04–1.37	0.281	No
	$AUC_{0\text{-}LOQ}$	1.64	1.81–1.48	<0.001	No

$AUC_{0\text{-}LOQ}$ = area under the concentration *vs.* time curve from 0 up to the limit of quantification; C_{max} = peak concentration.

TCBZ.SO2 are shown in Table 2. Differences were observed between the RF and the Test III and Test IV formulations.

Table 3 summarises the statistical evaluation of bioequivalence between the RF and the different test formulations used, based on the ratios of geometric means of the C_{max} and $AUC_{0\text{-}LOQ}$ for TCBZSO of the RF and the other formulations. Bioequivalence could only be established for Test I, in which the point estimates and the 90% CI for C_{max} and $AUC_{0\text{-}LOQ}$ were within the range of 0.80–1.25. For Test II, the point estimates for C_{max} and $AUC_{0\text{-}LOQ}$ were below 0.80. For Test III and IV, the point estimate for $AUC_{0\text{-}LOQ}$ was above the upper confidence limit of 1.25, as was the C_{max} for Test IV.

Discussion

Triclabendazole has become the most popular flukicidal compound over the world (Fairweather 2011). Its excellent efficacy both against immature and mature flukes has contributed to its widespread use in ruminant species. As has been reported for many other commonly used antiparasitic drugs, there are many countries where several generic formulations are commercially available, which has been linked to under dosing and poor clinical efficacy (Van Wyk *et al.* 1997; Eslami *et al.* 2006; Suarez *et al.* 2011, 2013). The situation of the veterinary pharmaceutical market in Peru, where TCBZ is available as different trade name formulations with similar composition, has been taken as an illustrative example in the work reported here. The work was not undertaken to identify the best TCBZ preparation. Conversely, the scientific approach was intended to demonstrate the need for conducting comparative pharmacokinetic and/or bioequivalence studies in the target animal species during the drug approval process. The comparative plasma disposition kinetics and bioequivalence of five oral TCBZ formulations were assessed in cattle kept under local field conditions.

An ideal bioequivalence study should be undertaken using a cross-over design, ensuring that an appropriate number of clinically healthy subjects are included (Anonymous 2006). The cross-over design has advantages in terms of statistical power and number of animals needed and inter-subject variability is eliminated. In contrast, both inter- and intra-subject variation may be limitations of the parallel design, so a larger number of animals are required. In order to establish the bioequivalence of the new product compared to the reference (pioneer) product, appropriate studies must be implemented, including a sufficient number of animals in a cross-over design. However, as mentioned above, the existing veterinary market situation in many countries around the world is complex, with the availability of a large number of similar commercial formulations, which are not necessarily similar in absorption, relative systemic availability or clinical efficacy. For instance, there are as many as 15 TCBZ formulations available for use in cattle in Peru. Consequently, our primary goal was not to establish the bioequivalence of some of the different TCBZ oral formulations available, but to assess their relative bioavailability to detect gross differences in their absorption patterns and systemic exposure.

An additional difficulty related to the determination of bioequivalence of TCBZ formulations was related to the metabolic pattern of the flukicidal compound. In fact, the parent drug is not detected in the bloodstream after its oral administration, as the sulphoxide and sulphone metabolites are the only analytes identified in plasma of TCBZ-treated animals. After absorption, TCBZ is primarily sulphoxidated to TCBZ.SO, which is then sulphonated to $TCBZ.SO_2$ in sheep (Hennessy *et al.* 1987) and cattle (Sanyal 1995). As the rate of metabolite appearance may be primarily determined by both the rate of drug absorption and/or the rate of metabolite formation (Martinez and Berson 1998), the bioequivalence should be determined through the primary metabolite (TCBZ.SO), which paradoxically accounts for only 20% of the total amount of TCBZ metabolites found in plasma. Thus, aware of the weaknesses of the experimental design, the parallel design permitted us to assess the simultaneous comparative systemic exposure of TCBZ after its oral administration as different formulations to heifers.

There is scarce availability of information related to this topic in the international scientific literature. The comparison of the systemic drug exposure after treating sheep with different albendazole generic formulations has shown large differences among formulations (Eslami *et al.* 2006; Suarez *et al.* 2011). Furthermore, factors related to the quality of the active ingredient have been associated with therapeutic failure of generic rafoxanide formulations against *Haemonchus contortus* in sheep (Van Wyk *et al.* 1997). Conversely, Suarez *et al.* (2013) reported that neither the overall kinetic behaviour nor the systemic exposure differed among all the tested oral formulations of ivermectin in sheep.

Consistent with kinetic data previously obtained in cows (Sanyal 1995), TCBZ.SO and $TCBZ.SO_2$ were the main metabolites recovered in plasma after the oral administration of TCBZ, which has been related to a first-pass oxidation occurring mainly in the liver. TCBZ.SO accounted for 14% of the total analytes found in plasma after the oral administration of the RF in the current study. The percentage of plasma metabolites differed from that reported after the intraruminal or oral administration of TCBZ to sheep (Hennessy *et al.* 1987) and goats (Kinabo and Bogan 1988), in which TCBZ.SO represented 37 and 45%, respectively, of the total TCBZ metabolites recovered in the bloodstream. Both TCBZ metabolites were recovered in plasma for a period of 168 hours post-treatment in the present study. The long persistence of high concentrations of TCBZ.SO and $TCBZ.SO_2$ in plasma compared to other benzimidazole anthelmintics used in cattle such as fenbendazole (Prichard *et al.* 1985), netobimin (Lanusse and Prichard 1992) and albendazole (Sánchez *et al.* 1997), may be due to the strong binding of both metabolites to plasma proteins (Hennessy *et al.* 1987; Chambers *et al.* 2010). This pharmacological property offers some advantage to TCBZ compared to other benzimidazole anthelmintics for its activity against blood-feeding adult flukes, as *in vivo* TCBZ metabolite accumulation into *F. hepatica* appears to be related to their systemic drug exposure (Alvarez *et al.* 2000; Moreno *et al.* 2014). Furthermore, TCBZ, TCBZ.SO and $TCBZ.SO_2$ were capable of disrupting the adult fluke *in vitro* (Halferty *et al.* 2009), indicating that TCBZ action may be due to the additive effects of the parent compound and its main systemically available metabolites.

Although limited by the use of a parallel design, the current comparison of TCBZ pioneer and generic formulations in heifers should raise awareness on the need of further quality control for drug approval in the veterinary pharmaceutical field. This is particularly relevant for *F. hepatica* control in livestock, which is facing the serious threat of the worldwide development of resistance. To meet the regulatory criterion for bioequivalence, the

90% CI for the ratio of the geometric means of C_{max}, $AUC_{0–LOQ}$, and $AUC_{0–\infty}$ between products should fall within the interval from 0.8 to 1.25 (Anonymous 2006). Based on these criteria, the only test formulation under evaluation that could be considered equivalent to the RF was Test I, which demonstrated an equivalent systemic exposure for the active TCBZ.SO metabolite. Use of the other formulations resulted in significantly higher or lower $AUC_{0\text{-}LOQ}$ to that observed for the RF.

It is clear that the observed differences in the TCBZ.SO systemic exposure among pharmaceutical preparations were mostly due to the changes in formulation and not related to the flukicidal active principle. Overall, the generic product being considered for a waiver contains the same active and inactive ingredients in the same dosage form and concentration and has the same pH and physicochemical characteristics as an approved pioneer product (Anonymous 2006). However, the concentration of the different TCBZ formulations assayed in the current trial varied from 10 to 12.5%, with the RF having the lowest concentration (10%). Differences in the quality of the manufacturing procedures, e.g. particle size, surface crystal structure of the active ingredient, type and quality of excipients applied to elaborate the final formulation (suspension) may drastically affect the amount of active drug available. These issues will influence absorption at the gastrointestinal level after the particle dissolution process takes place (Lanusse and Prichard 1993).

A good understanding of the fundamentals of disperse systems is essential in the development of a suitable pharmaceutical suspension. The development of a suspension dosage form follows a complicated path. The selection of the proper excipients, such as surfactants and viscosity imparting agents, is a key issue. The particle size distribution in the finished drug product dosage form is a critical parameter that may impact on the bioavailability and pharmacokinetics of the final product. The large number of available commercial formulations in many countries around the world faces the problem of a lack of information on their absorption patterns. This is important, given the possibility of differences in manufacturing processes and quality of components that may exist among formulations. These differences may substantially affect drug dissolution and the consequent gastrointestinal absorption, which in turn could affect drug effectiveness. Further work is needed to assure the bioequivalence of anthelmintic preparations before their introduction into the market. This is critical considering the worldwide spread of resistance-related therapeutic failures in parasite control.

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