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Pharmacokinetics of monepantel and its sulfone metabolite, monepantel sulfone, after intravenous and oral administration in sheep

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The pharmacokinetic properties of the developmental Amino-Acetonitrile Derivative (AAD), monepantel and its sulfone metabolite, monepantel sulfone were investigated in sheep following intravenous (i.v.) and oral administrations. The sulfone metabolite was rapidly formed and predominated over monepantel 4 h after dosing, irrespective of the route of administration. The steady-state volume of distribution, total body clearance and mean residence time of monepantel were 7.4 L/kg, 1.49 L/(kg·h) and 4.9 h, respectively and 31.2 L/kg, 0.28 L/(kg·h) and 111 h, respectively for monepantel sulfone. The overall bioavailability of monepantel was 31%, but it was demonstrated that approximately the same amount of monepantel sulfone was produced whether monepantel was given intravenously or orally ($AUC_{(0-\infty)}$ oral/$AUC_{(0-\infty)}$ i.v. of 94% for monepantel sulfone), making oral administration a very efficient route of administration for monepantel in terms of the amount of sulfone metabolite generated. Because monepantel sulfone is the main chemical entity present in sheep blood after monepantel administration and because it is also an active metabolite, its pharmacokinetic properties are of primary importance for the interpretation of future residue and efficacy studies. Overall, these pharmacokinetic data aid in the evaluation of monepantel as an oral anthelmintic in sheep.

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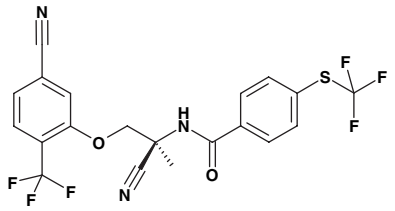
INTRODUCTION

Extensive use and improper dosage of anthelmintics in conjunction with other factors have resulted in drug resistance to one or more of the current broad-spectrum anthelmintic families (benzimidazoles, imidazothiazoles and macrocyclic lactones) thereby causing a serious threat to the effective control of helminth infections in livestock (Prichard, 1994; Coles, 1999; Sangster & Gill, 1999; Köhler, 2001). As farmers continue to rely on anthelmintics as their primary means of nematode management (Brunsdon *et al.*, 1983; Lawrence *et al.*, 2007), there is a serious requirement for a new class of anthelmintics with a new mode of action to be discovered and developed.

Despite intensive efforts in medicinal chemistry over the past 25 years (Woods *et al.*, 2007), only two new classes of broad-spectrum anthelmintics have emerged, the cyclodepsipeptides (Scherkenbeck *et al.*, 2002) and the paraherquamides (Lee *et al.*, 2002). However, neither class has resulted in a marketed product for farmed livestock as yet. Discovery of a newer class of anthelmintics, the Amino-Acetonitrile Derivatives (AADs) has recently been reported (Ducray *et al.*, 2008; Kaminsky *et al.*, 2008a). The AADs demonstrate a wide spectrum of activity against parasitic nematode species in ruminants (Ducray *et al.*, 2008; Kaminsky *et al.*, 2008a). Most significantly, they demonstrate activity against nematode strains resistant to the currently available broad-spectrum anthelmintics. This is due to a novel mode of action involving a unique nematode-specific clade of acetylcholine receptor subunits (Kaminsky *et al.*, 2008a).

Monepantel, a new drug candidate from this class, is currently being evaluated as an oral anthelmintic in sheep (Kaminsky *et al.*, 2008b). Monepantel exists in two enantiomeric forms. Only one of the enantiomers, the S-enantiomer, is biologically active (Ducray *et al.*, 2008). The pharmacokinetics of the enantiomers differ considerably for this class of compounds (Jung *et al.*, unpublished data) and only the active enantiomer has been selected for development.

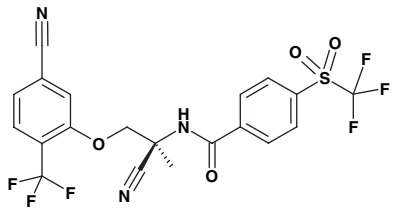
Preliminary unpublished studies show that monepantel is rapidly metabolized after oral dosing to monepantel sulfone (Figs 1 & 2). The sulfone is the major metabolite found in sheep blood, with trace levels of the intermediate sulfoxide also seen at early time points. However, as the conversion of the parent drug to the sulfone is much faster than the elimination of the sulfone,



IUPAC name: N-[(1S)-1-cyano-2-(5-cyano-2-trifluoromethyl-phenoxy)-1-methyl-ethyl]-4-trifluoromethylsulfanyl-benzamide

MW = 473.39, cLogP = 4.7

Fig. 1. Chemical structure of monepantel.



IUPAC name: N-[(1S)-1-cyano-2-(5-cyano-2-trifluoromethyl-phenoxy)-1-methyl-ethyl]-4-trifluoromethylsulfonyl-benzamide

MW = 505.39, cLogP = 4.1

Fig. 2. Chemical structure of monepantel sulfone.

the pharmacokinetics are dominated by the sulfone (Jung *et al.*, unpublished data).

Dose determination studies with monepantel administered to sheep as an oral solution (Hosking *et al.*, 2008; Kaminsky *et al.*, 2009) have demonstrated >95% efficacy against most nematodes at 2.5 mg/kg. Therefore, oral doses of 1, 3 and 10 mg/kg were used to characterize the pharmacokinetic behaviour of monepantel and monepantel sulfone.

The ultimate objective of this study was to establish the pharmacokinetic parameters of monepantel and its sulfone metabolite, following intravenous (i.v.) and oral administration in sheep, as such data are important for judicious use of monepantel as an oral anthelmintic in sheep.

MATERIALS AND METHODS

In vitro *assays*

Monepantel and monepantel sulfone were tested, as previously described (Ducray *et al.*, 2008), by serial dilution in dimethyl sulfoxide (DMSO) to determine the effective concentration (EC_{100}), which corresponds to the minimal dose at which 100% efficacy was still achieved.

The strains of nematode used in these assays were recently isolated from the field and characterized for their susceptibility to currently used anthelmintics such as benzimidazole and levamisole.

Haemonchus contortus was demonstrated to be highly resistant to the benzimidazole compounds albendazole (Resistance factor, R/S = 3000) and thiabendazole (R/S = 1000).

Trichostrongylus colubriformis displayed rather low resistance towards both benzimidazole compounds (R/S = 30 and 100, respectively) but an R/S of 1000 towards levamisole when compared to the laboratory-used strains.

Validation of the blood method

The validated linear range for both parent and the sulfone metabolite was determined by using six concentrations: 2.5, 5, 10, 100, 500 and 1000 ng/mL.

The limit of detection (LOD) was determined by injecting six control samples onto the HPLC system with a range of standards. The detector response at the respective retention times was measured and an estimation of the corresponding concentration determined by extrapolation of the individual calibration curve for each analyte. The mean responses (at the retention times of monepantel and monepantel sulfone) and standard deviations were calculated. The LOD was calculated as the mean response plus three times the standard deviation (SD) of the mean for each analyte.

The limit of quantification (LOQ) was defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. For practical reasons, the tested LOQs were the same for each analyte and set at 3 ng/mL. The LOQs of 3 ng/mL for each analyte were validated by analysing a minimum of six replicate samples at this concentration.

The coefficients of variation (CVs) were used to assess precision. Repeatability was assessed by carrying out analysis by the same analyst using the same apparatus and identical reagents over a period of several weeks. Reproducibility was assessed for each analyte by analysis of a set of six fortified samples at LOQ, with the analysis carried out by another analyst.

Stability (postpreparative, freeze/thaw, short-term and long-term) was also evaluated. Postpreparative stability of fortified and control samples (re-constituted for final HPLC quantification) for one set of analysis was checked after storage at room temperature for 16–24 h. This was performed by re-injection onto the HPLC system.

To evaluate freeze/thaw stability, blood samples fortified with the analytes at two fortification levels (10 and 200 ng/mL, in triplicate) were subjected to freeze/thawing (three cycles) and analysed at the end of the third cycle for evaluation.

Short-term stability was assessed by thawing triplicate aliquots at two fortification levels (10 and 200 ng/mL) and keeping them at room temperature for about 4 h. A similar set of fortified samples were kept frozen and thawed immediately prior to analysis.

Incurred specimens at two different levels were pooled and used to assess stability during storage in the freezer (at about −20 °C). Stored frozen incurred specimens were analysed (in

triplicate at two levels) at 1, 2 and 4 months. Specimens for T = 0 were extracted on the same day of pooling.

Animals

The study was conducted in 36 cross-bred sheep, 6–8 months of age and of mixed-sex, weighing from 30 to 50 kg at commencement. Clinically healthy animals were allocated to five treatment groups based on sex and body weight. Each group was planned to have an equal number of females and castrated males. Groups 1 and 2 consisted of six animals, while groups 3–5 had eight animals each.

All the animals were maintained in indoor pens and fed a 'chaff' mix of lucerne hay/oaten hay/straw/oats with an added vitamin premix, salt and canola oil. Water was available *ad libitum*. Animals were fasted overnight (approximately 18 h) before drug administration and fed following the 0.5 h postadministration blood collection.

This study was approved by the Novartis Animal Ethics Committee and conducted under an Australian Pesticides and Veterinary Medicines Authority (APVMA) small-scale trial permit.

Drug administration

Monepantel and its sulfone metabolite (supplied by CarboGen AG with a purity of 99.2 ± 1.3% and 99.2 ± 0.2%, respectively) were prepared on the morning of administration by dissolving 0.5% (w/v) of each drug in separate placebo formulations. The placebo formulation (supplied by Novartis Animal Health Inc.) consisted (w/v) of 40% glycofurol, 40% DMSO, 10% absolute ethanol and 10% Solutol HS 15. The solutions were homogeneous before administration. Treatment doses for each animal were calculated on individual body weights.

Groups 1 and 2 were administered an i.v. injection, slowly over *ca.* 1 min via a jugular catheter, of 0.5% w/v monepantel (1 mg/kg) and 0.5% w/v monepantel sulfone (1 mg/kg), respectively. Groups 3, 4 and 5 were respectively administered an oral treatment of 1, 3 or 10 mg/kg of a 2.5% w/v solution of monepantel. Administrations were made to the back of the buccal cavity, over the tongue with a suitably sized plastic disposable syringe.

Sampling

Blood samples were collected by jugular venipuncture from each animal into 5 mL blood collection tubes containing the anticoagulant, ethylenediaminetetraacetic acid. Blood samples were taken before drug administration and at 2, 5, 10, 30 min and 1, 2, 4, 8, 12, 24, 36, 48, 72, 96 h and 7, 10, 14, 21 and 28 days after i.v. administration. Similarly, blood samples were collected before drug administration and at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96 h and 7, 10, 14, 21, 28 and 35 days after oral administration.

Faecal samples were also collected using collection bags attached over the anus of the animal from groups 1, 2 and 4 over a 8-h period before drug administration. In addition faecal samples over 8-h periods were collected from group 1 (at 24–32 h and 48–56 h), group 2 (at 48–56 h and 168–176 h) and group 4 (at 24–32 h and 48–56 h) postdrug administration. Prior to analysis, the faecal samples were thawed to room temperature and then well minced in a horizontal food cutter.

All samples were stored frozen (at about −20 °C) before and after analysis.

Analytical procedures

Blood concentrations of monepantel and monepantel sulfone were determined simultaneously by high-performance liquid chromatography (HPLC) using a validated analytical method. Briefly, 0.5 mL blood was vigorously mixed with 0.5 mL water and 1.3 mL acetonitrile in a 10 mL polypropylene test tube. After vortex mixing, the tube was centrifuged for 15 min at 2600 ***g***. The supernatant was mixed with 5.0 mL water and loaded on to a polymeric sorbent solid phase extraction cartridge (Strata-X 33 μm Polymeric Sorbent 60 mg, Phenomenex Torrance, CA, USA) conditioned with 1.0 mL acetonitrile and 1.0 mL water. The cartridge was washed with 2.0 mL of acetonitrile:water (30:70, v/v). The analytes were eluted with 1.0 mL of acetonitrile and evaporated to dryness under a stream of nitrogen. Following reconstitution in 0.5 mL of mobile phase (acetonitrile:methanol:water 50:10:40, v/v/v), 50 μL was injected onto the HPLC.

Faecal concentrations of monepantel and its sulfone metabolite were also determined simultaneously, by liquid chromatography coupled to a tandem mass spectrometer (LC/MS/MS). Briefly, acetonitrile (9.0 mL) was added to 1.0 g faeces. Monepantel and monepantel sulfone were extracted by shaking on a mechanical shaker for 10 min at approximately 350 rpm and sonicating for 10 min. Following centrifugation for 10 min at 1900 ***g***, a portion of supernatant (about 2.0 mL) was passed through a polytetrafluoroethylene filter (0.45 μm). To 0.5 mL of filtered extract was added 0.1 mL methanol and 0.4 mL water and 10 μL was injected onto the LC/MS/MS.

Chromatographic conditions

Blood analysis

Monepantel and monepantel sulfone concentrations in blood were determined with a Shimadzu HPLC system (Shimadzu, Kyoto, Japan). The HPLC analysis was undertaken with the use of two-column switching, that is using a C18 column (Luna 3 μm; 3 mm × 150 mm; Phenomenex) with a C18 guard column (3 mm × 4 mm; Phenomenex) as the first column and a phenyl column (Novapak Milford, MA, USA phenyl 4 μm; 3.9 mm × 150 mm; Waters) as the second column. The columns were kept in a column oven at 40 °C (CTO-10A VP; Shimadzu) and detection was by UV detector (SPD-10A; Shimadzu) at 230 nm. The mobile phase consisted of acetonitrile:methanol:water 50:10:40 (v/v/v) at isocratic flow rates of 0.5 and 0.4 mL/min on column 1 and 2, respectively (LC-10AD VP; Shimadzu).

Faecal analysis

Monepantel and monepantel sulfone concentrations in faeces were determined with the use of a Shimadzu HPLC system (Shimadzu) coupled to a 4000 QTrap MS/MS (Applied Biosystems/MDS Sciex, Toronto, ON, Canada) in negative Turbo Ion Spray mode, using multiple reaction monitoring (MRM). The separation was performed on a C18 column (Atlantis T3 3 μm; 2.1 mm × 150 mm; Waters) kept in a column oven at 30 °C (CTO-20A; Shimadzu). Eluents were water:acetonitrile 95:5 v/v (A) and acetonitrile:methanol 50:50 v/v (B). Isocratic conditions were used, with flow rates of 0.1 and 0.2 mL/min of mobile phases A and B, respectively (LC-20AD; Shimadzu). The analytes were detected by monitoring the following MRM transitions: 472 → 186 for monepantel and 504 → 186 for monepantel sulfone, using electrospray ionization in negative mode.

Method of calibration and within study validation

Calibration curves in the range of 2.5–1000 ng/mL were prepared using nonmatrix matched standards (peak area) for the blood analysis. Quality Control (QC) samples were freshly prepared by fortifying control blood samples with known amounts of analyte(s). Calibration curves, QC levels and incurred levels were determined by linear regression, with origin excluded and $1/x^2$ weighting. The LOQs were 3 ng/mL for both parent and metabolite.

For the faecal analysis, calibration curves in the range of 50–10 000 μg/kg were prepared using matrix matched standards (peak area). QC samples were freshly prepared by fortifying control faecal samples with known amounts of analyte(s). The method used for the analysis of faecal specimens was not validated. Calibration curves, QC levels and incurred levels were determined by linear regression, with origin excluded and 1/x weighting.

Pharmacokinetic analysis

The following pharmacokinetic parameters were calculated for individual animals using the statistical software SAS®, Version 9.1.3: the area under the concentration curve (*AUC*), by the linear trapezoidal rule; the mean residence time (*MRT*); the clearance per kg of body weight (*Cl*), defined as dose per kg of body weight/*AUC*, for the i.v. groups; the volume of distribution at steady-state per kg of body weight (V_{ss}), which is $Cl \times MRT$, for the i.v. groups; the terminal half-life ($t_{1/2}$), by log-linear regression over a suitable time interval for the sulfone metabolite only; the apparent volume of distribution per kg of body weight (V_{area}), which is $Cl \times t_{1/2}/\ln(2)$, for i.v. group 2 only. For the linear trapezoidal rule, values below the LOQ (3 ng/mL) were treated as zero.

Faecal clearance (Cl_{fec}) was calculated as the total amount of the drug found in the faecal sample, divided by the duration of the collection period (approximately 8 h) and also divided by the estimated mean concentration in the blood during this collection period and by the body weight. The total amount of drug excreted in the faeces divided by body weight, A_{fec}, was calculated as $Cl_{fec} \times AUC_{(0-\infty)}$ (normalized to a dose of 1 mg/kg), with $AUC_{(0-7days)}$ being substituted for $AUC_{(0-\infty)}$ in the case of the parent drug. This is the amount of drug (expressed in μg/kg) excreted in the faeces (per kg body weight), if 1 mg/kg of the parent drug (in group 1 or 4) or of the sulfone metabolite (in group 2) was administered, the resulting unit being μg/mg.

Statistical analysis

Summary statistics, such as geometric means, SDs and CVs were calculated. The arithmetic mean was also calculated, but it is not reported because the results were similar to the geometric mean and because the normality of the residuals for most parameters was better after log transformation (for half-life harmonic means and SDs were calculated). Only the median, minimum and maximum values were calculated for T_{max}.

All blood pharmacokinetic parameters except T_{max} were submitted to an analysis of variance (ANOVA), to compare groups. Dose-normalized *AUC*s after log transformation were compared for groups 1 and 3, the two groups receiving 1 mg/kg of monepantel, to assess the bioavailability of the oral application and for groups 3–5, the three oral groups, to assess dose proportionality.

Mass balance calculations

Mass balance calculations used dose normalized values and geometric means. The amount of parent drug (in relation to the administered dose of parent drug) transformed into the sulfone metabolite, after i.v. administration of the parent drug (group 1), is given by:

$$A_{(P\rightarrow M)} = Cl_M(\text{group2}) \times AUC_{M(0-\infty)}(\text{group1}) \times \frac{MW_P}{MW_M}$$

where, P is the parent drug and M is the metabolite. In this formula, Cl_M from group 2 is used, as it is not known for group 1 and it can be assumed that they are similar in the two groups. MW denotes the molecular weight.

The amount of sulfone metabolite (in relation to the administered dose of parent drug) excreted in faeces, after intravenous administration of the parent drug (group 1), is given by:

$$Cl_{fec-M}(\text{group1}) \times AUC_{M(0-\infty)}(\text{group1}) \times \frac{MW_P}{MW_M}$$

For the mass balance of the parent drug (group 1), the fractions of parent drug, which are excreted in faeces, transformed to the sulfone metabolite, or metabolized otherwise, are respectively given by:

$$A_{fec-P} = Cl_{fec-P} \times AUC_{P(0-7days)};$$

$$A_{(P\rightarrow M)}$$

$$1 - A_{fec-P} - A_{(P\rightarrow M)}$$

For the mass balance of the sulfone metabolite (group 2), the amounts of sulfone metabolite, which are excreted in faeces or metabolized further are respectively given by:

$$A_{fec-M} = Cl_{fec-M} \times AUC_{M(0-\infty)};$$

$$1 - A_{fec-M}$$

Finally, the ratio of exposure to M over exposure to P, after i.v. and oral administration of the parent drug (group 1), is given by:

$$\frac{AUC_{M(0-\infty)}}{AUC_{P(0-7days)}} \times \frac{MW_P}{MW_M}$$

RESULTS

In vitro *assays*

Monepantel displayed relevant biological activity against both nematode larvae with an EC_{100} = 0.001 ppm [0.01 μg/mL]. Monepantel sulfone displayed similar activity with an EC_{100} = 0.0032 ppm [0.0032 μg/mL]. It is concluded that both compounds have the same intrinsic activity against the two parasitic nematodes (one dilution factor not being considered as a significant difference in this *in vitro* assay), suggesting that the main metabolite contributes significantly to the *in vivo* biological activity.

Validation of the blood method

The linearity for both parent and metabolite was demonstrated over the range of 2.5–1000 ng/mL. The response was indeed found to be linear over this range for the 14 calibration curves generated during the validation. The relative deviations from the curve of all standard points and the total variance of all these deviations were calculated. The standard deviation for monepantel and monepantel sulfone of the entire curve was 5.3 ± 1.9% and 3.6 ± 0.6%, respectively, which is less than 10% (in accordance to in-house Standard Operating Procedures).

The LODs were found to be 0.50 and 0.65 ng/mL for monepantel and monepantel sulfone respectively. At the LOQ, accuracy (93% for both analytes) and precision (CVs ≤ 9.1 and 18% for parent and metabolite, respectively) were acceptable.

In addition to the LOQ, a minimum of six replicates of five other concentrations were evaluated: 5, 10, 200, 500 and 1000 ng/mL. The method was demonstrated to be accurate for both parent and metabolite with an accuracy ranging from 89% to 100%.

Precision, under repeatability conditions, was evaluated using CVs, which were ≤ 10%. Under reproducibility conditions, accuracy and precision for each analyte of six fortified samples at LOQ was acceptable with accuracy ranging from 88% to 119% and CVs ≤ 16%.

No degradation of either analyte was observed in postpreparative samples, in blood samples subjected to freeze/thaw or to storage at room temperature for 4 h and in samples stored 4 months in the freezer.

Method of calibration and within-study validation

Quality Control samples analysed with the study blood samples demonstrated the method to be accurate (accuracy ranged from 89% to 102%) and precise (CVs ≤ 10%), for both parent and metabolite.

For the faecal analysis, the response was found to be linear over the concentration range of 50–5000 μg/kg. Samples outside this range were diluted to be within the range of the calibration curve. The LOQs were 50 μg/kg for both parent and metabolite. QC samples analysed with the study faecal samples demonstrated the method to be accurate (accuracy ranged from 79% to 103%) and precise (CVs ≤ 15%), for both parent and metabolite.

In both blood and faeces, the presence of one analyte did not interfere with the quantification of the other.

Animals

All animals treated intravenously showed signs of urinary discoloration within 30 min of drug administration. However, the animals did not show any signs of distress and the urinary discolouration disappeared 2–4 h later. DMSO was the vehicle for both the parent compound and metabolite and it is well known that DMSO damages red cells, releasing hemoglobin, which is eliminated via the urine (Samoszuk *et al.*, 1983; Santos *et al.*, 2003). One male animal in group 1 was replaced by a spare female animal as it was under-dosed during the i.v. administration, therefore group 1 had four female animals and two males. No abnormal events, relating to drug administration, occurred following oral administration.

Pharmacokinetic results

The pharmacokinetic parameters of monepantel and monepantel sulfone are summarized in Tables 1 & 2 and drug concentration vs. time profiles are shown in Figs 3–6.

After i.v. administration of 1 mg/kg of monepantel, blood concentrations declined rapidly, from an average initial value of 710 ng/mL, reaching the LOQ before or at 48 h. It was not possible to determine the terminal half-life of monepantel. Total blood clearance (1.49 L/kg·h) was relatively high and corresponded to an overall extraction ratio of 27%. Mean $AUC_{(0-7days)}$ was 671 ng·h/mL and *MRT* was 4.9 h. High blood concentrations of the sulfone metabolite were observed very early, with T_{max} at 2 h and a mean C_{max} of 61.4 ng/mL. Monepantel sulfone persisted in the blood much longer than monepantel, with a mean $AUC_{(0-\infty)}$ of *ca.* 3590 ng·h/mL and an estimated terminal half-life of 143 h.

After i.v. administration of 1 mg/kg of monepantel sulfone, the mean $AUC_{(0-\infty)}$ was *ca.* 3560 ng·h/mL, remarkably similar to that observed after i.v. administration of the parent. Total blood clearance of the metabolite was much lower than that of the

Table 1. Geometric mean ± SD of pharmacokinetic parameters of monepantel and monepantel sulfone obtained after i.v. administration of 1 mg/kg of each active ingredient

Parameter	Group 1 (Monepantel)		Group 2 (Monepantel sulfone)
	Monepantel	Monepantel sulfone	Monepantel sulfone
Actual dose (mg/kg)	1.11 ± 0.02	NA	1.18 ± 0.02
C_{max} (ng/mL)	710 ± 232	61.4 ± 11.0	632 ± 133
AUC (ng·h/mL)	671 ± 93	3592 ± 712	3564 ± 1103
Cl (L/h·kg)	1.49 ± 0.21	ND	0.28 ± 0.09
V_{area} (L/kg)	ND	ND	47.6 ± 15.2
$t_{1/2}$ (h)	ND	143 ± 28	105 ± 61
V_{ss} (L/kg)	7.4 ± 2.4	ND	31.2 ± 8.4
MRT (h)	4.9 ± 2.1	168 ± 43	111 ± 50

Harmonic mean and SD calculated for half-life; otherwise SD represents the geometric standard deviation. C_{max} and *AUC* values were dose-normalized; $AUC_{(0–7days)}$ is given for monepantel and $AUC_{(0–\infty)}$ for monepantel sulfone.
NA, not applicable; ND, not determined.

Table 2. Geometric mean ± SD of pharmacokinetic parameters of monepantel and monepantel sulfone after oral administration of monepantel at nominal doses of 1, 3 and 10 mg/kg

Actual dose (mg/kg)	Monepantel			Monepantel sulfone		
	T_{max}* (h)	C_{max}† (ng/mL)	$AUC_{(0–7days)}$† (ng·h/mL)	T_{max}* (h)	C_{max}† (ng/mL)	$AUC_{(0–\infty)}$† (ng·h/mL)
1.35 ± 0.10	8 (2–8)	6.8 ± 1.8	211 ± 91	24 (24–24)	29.9 ± 4.8	3376 ± 1126
3.57 ± 0.09	16 (4–24)	17.9 ± 6.6	671 ± 214	24 (24–24)	94.3 ± 15.6	11125 ± 3279
11.45 ± 0.07	4 (4–8)	98.8 ± 75.5	1920 ± 446	24 (4–24)	276 ± 101	19110 ± 2009

*Median (Minimum–Maximum) is given for T_{max}.
†C_{max} and *AUC*s were normalized to the nominal dose.
SD represents the geometric standard deviation.

parent (0.28 L/kg·h) and corresponded to an overall extraction ratio of 5%. The V_{ss} was much higher for monepantel sulfone (31.2 L/kg) than for monepantel (7.4 L/kg). As a consequence, the terminal half-life of the sulfone metabolite was high and estimated to be 105 h.

Blood drug concentrations after oral administration of monepantel to sheep at doses of 1, 3 and 10 mg/kg were more prolonged than after i.v. administration. Following oral administration of 1, 3 and 10 mg/kg monepantel, mean $AUC_{(0–7days)}$ was *ca.* 211, 671 and 1920 ng·h/mL, respectively and *MRT* was *ca.* 23, 30 and 22 h, respectively for the parent compound. Blood concentrations of monepantel declined over time, reaching the LOQ before or at 96 h. Monepantel sulfone was detected 1 h after administration and by 4 h, its concentration was greater than that of the parent compound, with maximal concentrations being observed at 24 h. The mean $AUC_{(0–\infty)}$ of monepantel sulfone was 3376, 11 125 and 19 110 ng·h/mL for groups treated at 1, 3 and 10 mg/kg of monepantel respectively and the *MRT* was *ca.* 133, 165 and 100 h respectively. The profiles of monepantel and monepantel sulfone obtained following oral administration of 1, 3 and 10 mg/kg are shown in Figs 5 & 6.

The bioavailability of monepantel after oral administration of 1 mg/kg of the parent was *ca.* 31%. Dose normalized $AUCs_{(0–\infty)}$ for the sulfone metabolite after intravenous and oral administration of monepantel were also compared: the exposure to the sulfone metabolite after oral administration of monepantel represented 94% of the exposure after intravenous administration at the same molar dose.

Dose proportionality for the parent appears to hold for oral administrations of 1–10 mg/kg of monepantel. However, for the sulfone, the proportionality of $AUC_{(0–\infty)}$ with respect to the monepantel dose was only demonstrated for doses ranging from 1 to 3 mg/kg. It is hypothesized that the metabolism of the sulfone approaches saturation at the highest dose but further experimental work is required to confirm this hypothesis. At the highest dose of 10 mg/kg, *AUC* (dose-normalized) and *MRT* are indeed significantly lower than for the lower dose groups. However, C_{max} for monepantel sulfone (dose-normalized) was not significantly different across the three dose groups. Additionally, for the 10 mg/kg oral groups, the observed T_{max} for both parent and metabolite was shorter than for the two lower oral dose groups.

The faecal clearance values were relatively consistent over the two samples within each animal and also between animals. The overall faecal clearance, summarized in Table 3, was *ca.* 0.05 L/(kg·h) for the parent drug and *ca.* 0.08 L/(kg·h) for the sulfone metabolite, after i.v. administration of the respective drugs.

Through the mass balance calculations (Tables 4 & 5), it was shown that a rather small fraction of the parent drug (37 μg/mg or 3.7%) is excreted via faeces; the remaining drug is mainly converted to the sulfone metabolite. The estimated sum of these two fractions is 981 μg/mg or 98.1%, suggesting a tiny fraction of

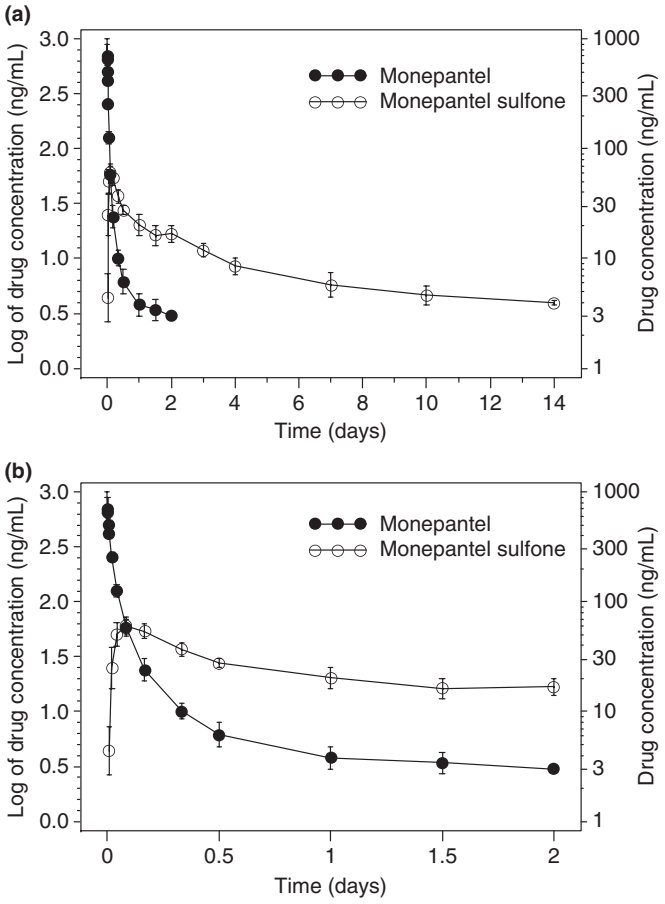


Fig. 3. Mean (±SD) monepantel and monepantel sulfone blood concentration vs. time profiles (a: from 0 to 14 days; b: from 0 to 2 days) for group 1 (i.v. monepantel, dose-normalized to the nominal dose of 1 mg/kg).

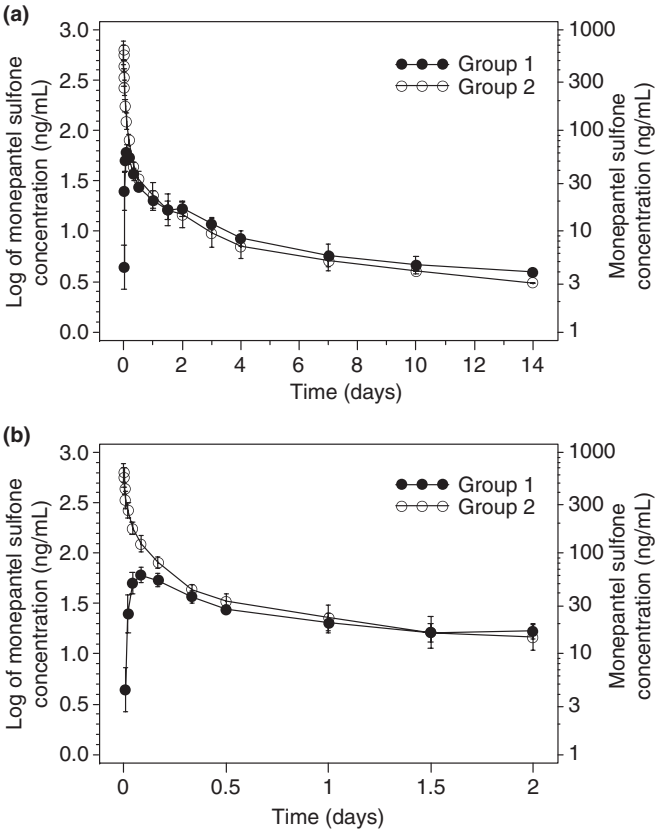


Fig. 4. Mean (±SD) monepantel sulfone blood concentration vs. time profiles (a: from 0 to 14 days; b: from 0 to 2 days) for group 1 (i.v. monepantel, dose-normalized to the nominal dose of 1 mg/kg) and group 2 (i.v. monepantel sulfone, dose-normalized to the nominal dose of 1 mg/kg).

the parent drug is metabolized by other pathways or eliminated as the sulfoxide metabolite, the intermediate metabolite between monepantel and monepantel sulfone. This latter conclusion is based on the assumption that group 1 and group 2 animals have on average the same clearance for the sulfone metabolite.

The sulfone metabolite in turn is excreted via faeces to 272 μg/mg or 27.2%. The remaining 728 μg/mg or 72.8% are further metabolized.

The ratio of *AUC* of the sulfone metabolite over *AUC* of the parent drug, corrected for different molecular weights, is about 5 after i.v. administration and approximately 15 after oral administration of monepantel. The exposure to monepantel sulfone is therefore approximately 15 times higher than the exposure to monepantel following oral administration of the parent compound.

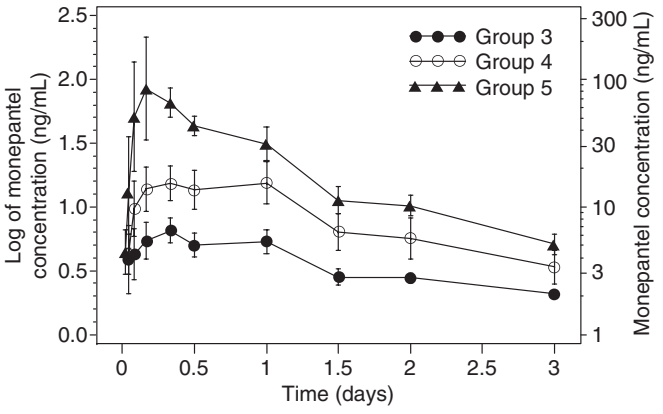


Fig. 5. Mean (±SD) monepantel blood concentration vs. time profiles for groups 3, 4 and 5 (oral monepantel, dose-normalized to the nominal doses of 1, 3 and 10 mg/kg).

DISCUSSION

Following i.v. administration of monepantel at 1 mg/kg, blood levels of the parent drug rapidly declined reaching the LOQ of 3 ng/mL before or at 48 h. Blood concentrations of the sulfone metabolite were observed very early (T_{max} at 2 h) suggesting the conversion of the parent drug to monepantel sulfone was very rapid and the terminal half-life for the sulfone metabolite was the therapeutically relevant half-life of elimination.

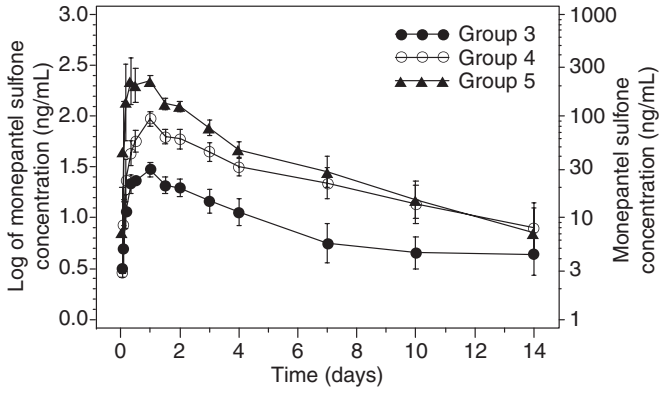


Fig. 6. Mean (±SD) monepantel sulfone blood concentration vs. time profiles for groups 3, 4 and 5 (oral monepantel, dose-normalized to the nominal doses of 1, 3 and 10 mg/kg).

In all oral groups, the C_{max} of the sulfone was generally three times higher than the corresponding parent concentration. When the parent reached C_{max} (*ca.* 4–8 h after drug administration), the sulfone was already about twice as high. Moreover, the ratio of the total *AUC* of monepantel sulfone over the total *AUC* of monepantel, corrected for different molecular weights, was about 5 after i.v. administration (monepantel, 1 mg/kg) and *ca.* 15 after oral administration (monepantel, 3 mg/kg). As the *in vitro* potency of the sulfone and the parent drug against gastro-intestinal nematode larvae have been shown to be similar, these pharmacokinetic results highlight the importance of considering the exposure to monepantel sulfone (and not only to monepantel) when assessing efficacy results. The *in vivo* efficacy should therefore be attributed and interpreted using the sum of the parent drug and the sulfone concentrations, with the sulfone playing the major role.

The overall bioavailability of monepantel was 31%, but it was demonstrated that approximately the same amount of monepantel sulfone was produced whether monepantel was given intravenously or orally ($AUC_{(0-\infty)}$ oral/$AUC_{(0-\infty)}$ i.v. of 94% for monepantel sulfone). This difference in exposure between the parent drug and the sulfone metabolite is most likely the consequence of a high fraction of monepantel being absorbed in the gastro-intestinal tract followed by a strong first-pass metabolism. It can therefore be concluded that the relatively low bioavailability is not detrimental to the efficacy of monepantel and that the oral administration is a very efficient administration route for this compound in terms of the amount of monepantel sulfone generated.

From previous unpublished ADME studies, it is known that renal clearance of both monepantel and monepantel sulfone is zero, so it can be considered that these molecules are either excreted in the faeces or metabolized. By using the pharmacokinetic parameters determined in the current study, it can be predicted that only a rather small fraction of monepantel (approximately 4%) is excreted in the faeces and a great majority of the remaining monepantel is converted to the sulfone (approximately 94%), indicating that the conversion of monepantel to monepantel sulfone is by far the most important metabolic pathway. Approximately, 27% of the sulfone is excreted in the faeces, indicating that the remaining fraction (about 73%) is metabolized further. The sulfone metabolite may therefore be a good candidate to be used as marker residue for future residue studies and withdrawal period determination. It is indeed the major metabolite in the metabolic transformation of monepantel and it is slowly eliminated (clearance is five times lower for the sulfone than for the parent drug) and widely distributed (V_{ss} is three times higher for monepantel sulfone than for monepantel).

By comparison to the leading worldwide anti-parasitic agent for livestock, ivermectin (González Canga *et al.*, 2007), both drugs are highly lipophilic. Ivermectin is known to be widely distributed in the fat, resulting in prolonged blood concentrations (Prichard *et al.*, 1985) and thereby better efficacy. The same may be postulated for monepantel sulfone because of the high LogP (partition coefficient between octanol and water; calculated value = 4.1) and the high V_{ss} (31.2 L/kg). The terminal half-life of this chemical entity in sheep (*ca.* 143 h and 105 h when monepantel and monepantel sulfone were administered i.v., respectively) was also found to be similar to ivermectin (terminal half-life is *ca.* 168 h when administered i.v. at a dose of 200 μg/kg; Prichard *et al.*, 1985).

Metabolically, monepantel bears resemblance to benzimidazoles such as albendazole (ABZ) and fenbendazole (FBZ) as they are metabolized to their respective sulfoxide and sulfone entities. The clearance of the sulfoxide metabolites in sheep is relatively high and the terminal plasma half-lives are about 7 h for albendazole sulfoxide following an oral dose of 5 mg/kg ABZ and about 19 h for oxfendazole (OFZ, the sulfoxide metabolite of FBZ) following an oral dose of 5 mg/kg of FBZ (Lanusse *et al.*, 1995). For benzimidazoles, it was shown that the anthelmintic activity can be attributed to the systemic sum of the parent compound and the sulfoxide metabolite (Hennessy *et al.*, 1989; Lanusse *et al.*, 1995). In contrast, the anthelmintic activity of monepantel is ascribed to the sum of parent compound and sulfone

Table 3. Geometric mean ± SD for faecal clearance of monepantel and monepantel sulfone after i.v. administration of 1 mg/kg of each active ingredient

	Monepantel		Monepantel sulfone	
Administration	*Cl* (L/h·kg)	A_{fec} (μg/mg)	*Cl* (L/h·kg)	A_{fec} (μg/mg)
i.v., Monepantel, 1 mg/kg	0.05 ± 0.02	36.5 ± 9.8	0.06 ± 0.03	232 ± 74
i.v., Monepantel sulfone, 1 mg/kg	Not applicable		0.08 ± 0.02	272 ± 94

SD, geometric standard deviation.

Table 4. Mass balance of monepantel after i.v. administration of 1 mg/kg monepantel

Quantity	Value (%)
Fraction of monepantel excreted in faeces	3.7
Fraction of monepantel metabolized to monepantel sulfone	94.4
Fraction of monepantel metabolized otherwise	1.9
Total	100.0

Table 5. Mass balance of monepantel sulfone after i.v. administration of 1 mg/kg monepantel sulfone

Quantity	Value (%)
Fraction of monepantel sulfone excreted in faeces	27.2
Fraction of monepantel sulfone metabolized further	72.8
Total	100.0

metabolite; the sulfoxide is not considered, as it is a very transitory species and this study demonstrates the extended persistence of monepantel sulfone with a terminal half-life in excess of 100 h (at least five times longer than the benzimidazole active entities). Therefore, more persistent efficacy is possible with the use of monepantel in sheep compared with ABZ, FBZ or OFZ.

This is the first report describing the pharmacokinetic properties in sheep of monepantel, an anthelmintic drug candidate with a novel mode of action and its major metabolite, monepantel sulfone. These pharmacokinetic data will aid in the future evaluation of monepantel as an oral anthelmintic in sheep.

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REFERENCES

Brunsdon, R.V., Kissling, R. & Hosking, B.C. (1983) A survey of anthelmintic usage for sheep – a time for change. *New Zealand Veterinary Journal*, **31,** 24–29.

Coles, G.C. (1999) Anthelmintic resistance and the control of worms (editorial). *Journal of Medical Microbiology*, **48,** 323–325.

Ducray, P., Gauvry, N., Pautrat, F., Goebel, T., Fruechtel, J., Desaules, Y., Schorderet Weber, S., Bouvier, J., Wagner, T., Froelich, O. & Kaminsky, R. (2008) Discovery of amino-acetonitrile derivatives, a new class of synthetic anthelmintic compounds. *Bioorganic and Medicinal Chemistry Letters*, **18,** 2935–2938.

González Canga, A., Sahagún Prieto, A.M., Diez Liébana, M.J., Fernández Martínez, N., Sierra Vega, M. & García Vieitiz, J.J. (2007) The pharmacokinetics and metabolism of ivermectin in domestic animal species. *The Veterinary Journal*, doi:10.1016/j.tvjl.2007.07.011.

Hennessy, D.R., Steel, J.W., Lacey, E., Eagleson, G.K. & Prichard, R.K. (1989) The disposition of albendazole in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **12,** 421–429.

Hosking, B.C., Stein, P.A., Mosimann, D., Seewald, W., Strehlau, G. & Kaminsky, R. (2008) Dose determination studies for monepantel, an Amino-Acetonitrile Derivative, against fourth stage gastro-intestinal nematode larvae infecting sheep. *Veterinary Parasitology*, **157,** 72–80.

Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Schorderet Weber, S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, F., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A. & Mäser, P. (2008a) A new class of anthelmintics effective against drug-resistant nematodes. *Nature*, **452,** 176–180.

Kaminsky, R., Gauvry, N., Schorderet Weber, S., Skripsky, T., Bouvier, J., Wenger, A., Schroeder, F., Desaules, Y., Hotz, R., Goebel, T., Hosking, B.C., Pautrat, F., Wieland-Berghausen, S. & Ducray, P. (2008b) Identification of the amino-acetonitrile derivative monepantel (AAD 1566) as an anthelmintic drug development candidate. *Parasitology Research*, **103,** 931–939.

Kaminsky, R., Mosimann, D., Sager, H., Stein, P. & Hosking, B.C. (2009) Determination of the effective dose rate for monepantel (AAD 1566) against adult gastro-intestinal nematodes in sheep. *International Journal for Parasitology*, **39,** 443–446.

Köhler, P. (2001) The biochemical basis of anthelmintic actions and resistance. *International Journal for Parasitology*, **31,** 336–345.

Lanusse, C.E., Gascon, L.H. & Prichard, R.K. (1995) Comparative plasma disposition kinetics of albendazole, fenbendazole and their metabolites in adult sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **18,** 196–203.

Lawrence, K.E., Leathwick, D.M., Rhodes, A.P., Jackson, R., Heuer, C., Pomroy, W.E., West, D.M., Waghorn, T.S. & Moffat, J.R. (2007) Management of gastrointestinal nematode parasites on sheep farms in New Zealand. *New Zealand Veterinary Journal*, **55,** 228–234.

Lee, B.H., Clothier, M.F., Dutton, F.E., Nelson, S.J., Johnson, S.S., Thompson, D.P., Geary, T.G., Whaley, H.D., Haber, C.L., Marshall, V.P., Kornis, G.I., McNally, P.L., Ciadella, J.I., Martin, D.G., Bowman, J.W., Baker, C.A., Coscarelli, E.M., Alexander-Bowman, S.J., Davis, J.P., Zinser, E.W., Wiley, V., Lipton, M.F. & Mauragis, M.A. (2002) Marcfortine and paraherquamide class of anthelmintics: discovery of PNU-141962. *Current Topics in Medicinal Chemistry*, **2,** 779–793.

Prichard, R.K. (1994) Anthelmintic resistance. *Veterinary Parasitology*, **59,** 259–268.

Prichard, R.K., Steel, J.W., Lacey, E. & Hennessy, D.R. (1985) Pharmacokinetics of ivermectin in sheep following intravenous, intra-abomasal or intra-ruminal administration. *Journal of Veterinary Pharmacology and Therapeutics*, **8,** 88–94.

Samoszuk, M., Reid, M.E. & Toy, P.T. (1983) Intravenous dimethyl sulfoxide therapy causes severe hemolysis mimicking a haemolytic transfusion reaction. *Transfusion*, **23,** 405.

Sangster, N.C. & Gill, J. (1999) Pharmacology of anthelmintic resistance. *Parasitology Today*, **15,** 141–146.

Santos, N.C., Figueira-Coelho, J., Martins-Silva, J. & Saldanha, C. (2003) Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. *Biochemical Pharmacology*, **65,** 1035–1041.

Scherkenbeck, J., Jeschke, P. & Harder, A. (2002) PF1022A and related cyclodepsipeptides – a novel class of anthelmintics. *Current Topics in Medicinal Chemistry*, **2,** 759–777.

Woods, D., Lauret, C. & Geary, T.G. (2007) Anthelmintic discovery and development in the animal health industry. *Expert Opinions on Drug Discovery*, **2**(S1), S25.