Pharmacokinetics of abamectin in combination with monepantel is not impacted by cytochrome P450 induction

Mary Smal[1], Martin Jung[2], Sevim Adams[1] and Günther Strehlau[2]

[1]Elanco Australasia Pty Ltd, 245 Western Rd, Kemps Creek NSW 2178, Australia
[2]Elanco Animal Health c/o Novartis Animal Health, 4002 Basel, Switzerland

Introduction

ZOLVIX® MONEPANTEL

Monepantel is a new anthelmintic drug, marketed as Zolvix®, intended for the treatment of gastrointestinal roundworm infestations in sheep. Monepantel is rapidly transformed, by oxidation, via the sulfoxide, to the active metabolite monepantel sulfone, which is eliminated rather slowly through the feces [1]. Metabolism *in vitro* and *in vivo* was studied with mass spectrometric methods [2,3]. These researchers continued with investigations of monepantel effects on the cytochrome P450 induction, as these are the most relevant enzymes for Phase I metabolism [4]. They found that **monepantel significantly increased all cytochrome P450-related activities**, and increased expression of CYP3A24 mRNA in sheep, and proposed that that **co-administration of other anthelmintics with monepantel may have serious pharmacological and/or toxicological consequences**.
Co-administration of two different anthelmintics is an option to delay the development of resistance in nematodes, and indeed we have combined monepantel with abamectin, registered as Zolvix Plus for sheep. This product contains 25 mg/mL monepantel and 2 mg/mL abamectin in solution, formulated identically as Zolvix. This combination product has an extended spectrum of efficacy against gastrointestinal worms in sheep.
We have studied the pharmacokinetic profile of this combination product in sheep with direct comparison to the mono-products, each containing the single active drug in identical formulations.

Materials and Methods

Animals and study design
The study was conducted as a 3-way cross-over design, with 18 Merino sheep, about 11 months old and 33-46 kg at the start, and equal numbers of each sex. Sheep were maintained in pens with roughage mix and pellets and ad lib access to water. The study was approved by the local animal ethics committee and conducted in compliance with GLP. There was an 8 weeks interval between treatments. The cross-over design is shown below:

Group	Number	Phase 1	Phase 2	Phase 3
A	6	Monepantel (MON)	MON+ABA	Abamectin (ABA)
B	6	MON+ABA	ABA	MON
C	6	ABA	MON	MON+ABA

Treatment
Each animal was weighed one day before each treatment and this bodyweight was used to calculate the volume of formulation (0.15 mL/kg BW, equivalent to 3.75 mg/kg monepantel (MON) and/or 0.3 mg/kg abamectin (ABA)). The required volume was administered via disposable syringe slowly into the mouth of the animal.
Blood collection
Whole blood (about 5 mL) was collected from the jugular vein, using EDTA as anticoagulant. Tubes were stored frozen at about -20°C until analysis.
Analysis and analytical methods
Whole blood samples were analysed for MON, monepantel sulfone (MONSUL) and/or ABA B1a. Control and fortified blood samples (QC) were included in every analytical batch to assess method performance. Both analytical methods were validated prior to use. In summary, they involved protein precipitation followed by SPE and subsequent LC-MS/MS analysis. The range of the methods was 2-200 ng/mL (MON, MONSUL) and 0.15 – 100 ng/ML (ABA). Method details are given in the Conference Proceedings of this conference (Euroresidue VIII).
Statistics
Non-compartmental PK analysis using validated SAS macros. For MON, MONSUL, the sum MON+MONSUL and ABA B1a, calculation of Cmax, Tmax, AUC (0-t), where t is the last time point with levels >LOQ. Values below LOQ were replaced by missing values. 90% confidence intervals were calculated on a log scale. Treatment group comparisons for Cmax and AUC(0-t) (relative bio-availability ratios) were calculated using 90% confidence intervals and tested for significance at 0.05. Bioequivalence was assessed, and two products were considered bioequivalent if the 90% confidence interval for the corresponding bio-availability ratio is entirely enclosed by the standard bioequivalence range of [0.80-1.25].

Results

In-life part
All animals completed the study in good health and the average weight gain was about 33% over the entire study period.
Analytical part
Samples were analyzed within the validated storage stability period. Performance of the validated analytical methods was good: For ABA B1a, mean accuracy and CV of QC samples in the range 0.25 to 100 ng/ml was 96-100% and 5.9-9.2% respectively.
For MON and MONSUL, in the range 3 to 200 ng/ml accuracy was 89-97% and 90-96% respectively. CVs were <9.4%. The method performed well for both analytes. The 8 week wash-out period was acceptable as pre-treatment blood levels were <LOQ for all analytes and all treatment phases.
Pharmacokinetic (PK) analysis
Results of PK parameters and blood profiles are displayed below. The 90% confidence intervals for the relative bioavailability ratios for all analytes were enclosed by the range [0.8 – 1.25] with significances >0.05, indicating bioequivalence.

Parameter	Formulation	MON	MONSUL	ABA B1a
Cmax	MON+ABA	31.5	113.9	17.8
(ng/mL)	MON	30.9	103.8	-
	ABA	-	-	18.5
Tmax	MON+ABA	0.3	0.7	0.8
(days)	MON	0.3	0.9	-
	ABA	-	-	0.8
AUC(0-t)	MON+ABA	35.8	500	62.4
(days.ng/mL)	MON	36.5	505	-
	ABA	-	-	66.3

Table 1 . Geometric mean pharmacokinetic (PK) parameters of
- Monepantel (MON)
- monepantel sulfone (MONSUL)
- abamectin (ABA) B1a
after administration of MON + ABA (combi product) and the respective mono product.

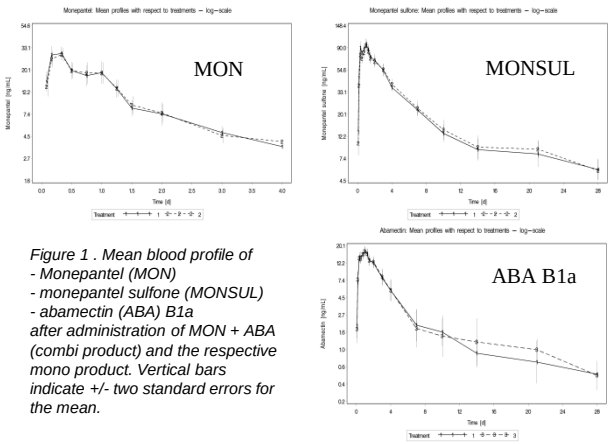

Figure 1 . Mean blood profile of
- Monepantel (MON)
- monepantel sulfone (MONSUL)
- abamectin (ABA) B1a
after administration of MON + ABA (combi product) and the respective mono product. Vertical bars indicate +/- two standard errors for the mean.

Discussion

This study clearly demonstrates the absence of drug-drug interactions between monepantel and abamectin in sheep. It further demonstrates that monepantel and abamectin as a combination in a Zolvix-type formulation are bioequivalent to each of the drugs in the corresponding mono-formulation. Bioequivalence was demonstrated with respect to both AUC (0-t) and Cmax for MON, for MONSUL, for the sum (MON+MONSUL) as well as for ABA B1a. Moreover, there were no adverse or toxic effects on the target animals in this study, nor have any such effects been observed in efficacy studies [5], which also demonstrated high efficacy against major gastrointestinal parasites present in sheep, including macrocyclic-lactone-resistant strains. Indeed, this combination is now a registered oral product for sheep in Australia and New Zealand

Conclusions

The presence of monepantel or monepantel sulfone does not affect the levels of abamectin found in the blood (no enhancement or depression) suggesting that any potential effect of monepantel on the cytochrome P450 liver enzymes that metabolise abamectin, is either non-existent or not perceptible and not at all clinically relevant.

References:

[1] Karadzovska D, Seewald W, Browning A, Smal M, Bouvier J, Giraudel JM. J Vet Pharmacol Therap 2009; 32:359-367
[2] Stuchlikova L, Jirasko R, Vokral I, Lamka J, Spulak M, Holcapek M, Szotakova B, Bartikova H, Pour M, Skalova L. Anal Bioanal Chem 2013; 405:1705-1712.
[3] Stuchlikova L, Jirasko J, Vokral I, Valat M, Lamka J, Szotakova B, Holcapek M, Skalova. Drug Test Anal 2014; 6:1055-1062.
[4] Stuchlikova L, Matouskova P, Bartikova H, Vokral I, Lamka J, Stolcova T, Petnikova H, Szotakova B, Kubicek V, Skalova L. Chemico-Biol Interactions 2015; 227:63-68.
[5] Rolfe P, Pathiaux V, Griffiths T, Nottingham R. Proceedings of the 24th International Conference of the World Association for the Advancement of Veterinary Parasitology; 2013 August 25-29; Perth, Australia, p. 400.