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Short communication

Pharmacokinetics of abamectin in sheep, goat and deer

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ABSTRACT

Previous studies and anecdotal reports have generally suggested that goats and deer have higher metabolic rates of anthelmintics as compared to sheep. If this is correct then these animals are effectively under dosed if treated with the sheep dose rate. Underdosing is an accepted risk factor for developing anthelmintic resistance as it allows resistant, and even some partly resistant parasites to survive. Despite this risk, there has been no study to date, which has quantified the pharmacokinetic profile of abamectin in sheep, goats and deer rates under similar conditions. The objective of this research was to compare the pharmacokinetic parameters of interest esp. Cmax, Tmax and AUC in sheep, goats and deer given 0.2 mg/kg abamectin per os and then manipulate the dose rate for any variation to match that of sheep. The blood samples obtained from these animals were analysed by HPLC-Fluorescence detector using a validated method of analysis. Goats at the same dose rate had significantly lower Cmax (11.5 ± 1.6 ng/mL) as compared to sheep (20.1 ± 7.3 ng/mL) and deer (26.4 ± 7.0 ng/mL) with the latter two not being different to each other. Goats dosed with a double oral dose of abamectin (0.4 mg/kg) achieved a Cmax (20.2 ± 5.69 ng/mL) which was similar sheep and deer orally dosed with 0.2 mg/kg abamectin. The results of this study suggest that goats are being potentially underdosed at the sheep dose rates and efficacy studies of abamectin should be conducted in goats at both the dosing regimens. In contrast, these results do not support the hypothesis that deer are metabolising abamectin more rapidly than sheep and hence this dose rate would seem to be appropriate for deer but should be confirmed by further study.

1. Introduction

Abamectin (ABM) is a natural fermentation product of *Streptomyces avermitilis* and has been used as an anthelmintic since at least 1985. Abamectin is a mixture of two homologs and contains at least 80% of avermectin B_{1a} and not more than 20% of avermectin B_{1b} (Shoop and Soll, 2002). It is also the precursor for the synthesis of the widely used anthelmintic ivermectin (IVM). In ruminants ABM is generally used at the same dose rate as IVM and at an equal dose rate has a higher potency against some gastrointestinal nematodes such as *Teladorsagia* and *Haemonchus* (Shoop et al., 1995; Leathwick et al., 2000; Lloberas et al., 2013). Although it is the parent compound for IVM there is much less known about the pharmacokinetics of ABM as IVM was favoured as the pioneer anthelmintic product for the macrocyclic lactone (ML) family of anthelmintics as it had a wider spectrum of activity and was generally safer for the animals compared to ABM (Shoop and Soll, 2002; Alka et al., 2004).

Most research in ruminants has focused on the use of ML anthelmintics in sheep and cattle with very little directed to their use for goats and deer. Indeed, there has only been a limited number of useful pharmacokinetic and efficacy studies for any anthelmintics in goats and deer over time. From the few studies published for IVM in goats the general trend indicates plasma levels tending to be lower than those in cattle and sheep (Canga et al., 2009; Hennessy and Alvinerie, 2002). The aim of this present study was to compare the pharmacokinetics of ABM in goats and deer against those in sheep and then manipulate the dose for these other hosts to more closely resemble the pharmacokinetics of ABM in sheep.

2. Material & methods

2.1. Experimental animals

2.1.1. Sheep

Six sheep approximately 6 months of age and of the Romney breed were housed and fed lucerne chaff for the duration of the experiment. All animals were treated with monepantel (Zolvix, Elanco NZ Ltd) at the time of housing which was 2 weeks before the start of the experiment.

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2.1.2. Goats

Six adult non-lactating goats of the Saanen breed were housed and fed lucerne chaff for the duration of the experiment. As for the sheep they were treated with monepantel but at 10 mg/kg 2 weeks before the start of the experiment

2.1.3. Deer

Seven red deer (*Cervus elaphus*) which were about 10 months of age were housed and treated with oxfendazole at 10 mg/kg two weeks prior to the start of the experiment. All animals were fed with commercial baleage (FibreFresh NZ Ltd) for the duration of the experiment.

2.2. Study design

All animals were dosed with ABM at 0.2 mg/kg (abamectin 1 g/L; Genesis Hi-Mineral, Ancare NZ Ltd) on Day 1. All animals were bled on Day -7 to confirm there was no detectable ABM in their blood and faecal sampled to confirm they were free of nematode infections. The sheep were bled prior to dosing then 3, 6, 9, 12, 15, 18, 24, 72, 120 and 168 h after the administration of drug. Goats and deer were bled at a slightly lower frequency. All animals were bled from the jugular vein into heparinized 10 mL vacutainer tubes (BD Vacutainer®). The blood was immediately centrifuged and the recovered plasma stored in glass vials at −20 °C until analysed. The goats were kept indoors and their plasma periodically checked for any residual ABM being detected. When all six goats were shown to be abamectin-free they were again treated but with 0.4 mg/kg ABM and serially bled as before. All the experiments were approved by Massey University Animal Ethics Committee.

2.3. Sample analysis

2.3.1. Chemical extraction and derivatization

To extract ABM from plasma samples, 2.5 mL of methanol (Merck, New Zealand) was added to a 500 µL plasma sample and then the mixture was vortexed for 5 min. The samples were then chilled in a −80 °C freezer for 10 min and subsequently centrifuged at 5000*g* for 15 min (at 4 °C). The supernatant was pipetted into clean tubes and was evaporated at 45 °C using a rotatory evaporator. After evaporation, the dried residues were cooled for 5 min at room temperature and reconstituted with 300 µL of 1-methylimidazole + acetronitrile solution (1:1, v/v; Millipore Cooperation, USA and Merck, New Zealand). This mixture was vortexed for 5 s and put on a water bath at 50 °C for 5 min. With the tubes still in the water bath 200 µL of acetonitrile + trifluoracetic anhydride (2:1, v/v; Sigma Aldrich, Germany) was added, mixed by pipetting up and down, then the mixture was left to react in the water bath. After one minute, 200 µL of the mixture was pipetted into amber coloured glass tubes. The vials were placed in the high performance liquid chromatography (HPLC) auto sampler and 50 µL was injected into the chromatographic system.

2.3.2. Chromatographic conditions

The plasma concentration of ABM was analysed by HPLC with fluorescence detection. The mobile phase consisted a combination of methanol:acetonitrile:MilliQ water (95:3:2, v/v/v; MilliQ PF plus system, Millipore Cooperation, USA) and with 1 mL/min flow rate. The fluorescent detection was carried out at the excitation wavelength of 365 nm and emission wavelength of 470 nm (Sari et al., 2006). The chromatography was performed on a HPLC system equipped with two pumps (LC-20AD, Shimadzu), an auto sampler (SIL-20ACht, Shimadzu), column oven (CTO-20 A, Shimadzu) and fluorescence detector (RF-10Axl, Shimadzu). A C18 reverse-phase column (Synergi hydro-RP 80A, 4 µm, 150 × 4.6 mm) was used for separation at 40 °C.

2.3.3. HPLC method validation

Linearity was determined by spiking 500 µL sheep plasma with ABM (Sigma, Aldrich) to create 0.8, 0.96, 1.92, 9.6, 19.2, 96.0 and 192.0 ng/mL standard concentrations of ABM. Chromatographic peak areas and standard concentrations were plotted against each other to establish a calibration curve using the least-square method with linear regression analysis using the software Prism 7 (GraphPad Software Inc., USA). The standard stock solution was prepared every week in 100% methanol. The working standards were prepared daily in Milli Q water.

Intraday and interday precision was calculated for all seven concentrations 0.8, 0.96, 1.92, 9.6, 19.2, 96.0 and 192.0 ng/mL. To determine the precision of the method the relative standard deviation (RSD) was calculated in percentages. The mean recovery was calculated in percentage to classify extraction efficiency. The lower limit of quantification (LLQ) in plasma was measured by running a series of low concentrations of ABM spiked with blank plasma of each species. The LLQ was set at the lowest concentration showing a signal to noise ratio of 10.

2.4. Pharmacokinetic analysis

Pharmacokinetic parameters were determined by a compartmental approach from the individual plasma concentration data using PKSolver 'add-on' for Excel 2010 (Zhang et al., 2010). The method of residuals and Akaike's information criteria were used to determine the goodness of fit in the above model. The rate constants of the absorption (K_a) and terminal phase (K_{10}) were calculated by linear regression of the logarithmic plasma concentration. Half-lives of the absorption ($t_{½\alpha}$) and terminal ($t_{½\beta}$) phases were calculated as $\ln2/K_a$ and $\ln2/K_{10}$, respectively. The area under the curve (AUC) and the area under the first moment (AUMC) were determined using the linear trapezoidal method. Mean residence time (MRT) was calculated as AUMC/AUC.

2.5. Statistical analysis

The data are reported in mean ± standard deviation. The pharmacokinetic parameters from sheep, goats (both doses) and deer were compared using One-way Analysis of Variance (ANOVA) using Prism 6 for Macintosh (GraphPad Software, Inc, CA, USA). Significance was set at $p < 0.05$.

3. Results

The HPLC method used for analysis of ABM in sheep, goat and deer plasma was linear from 0.8 to 192 ng/mL. The correlation coefficient was 0.9967. The inter- and intra-day variation ranged from 4.23 to 9.30 and 0.07 to 6.7%, respectively. The overall recovery of ABM ranged from 75 to 83%, for sheep, goat and deer plasma.

The semilog plots of the concentration time curve in sheep, goats and deer are shown in Fig. 1. The pharmacokinetic parameters calculated by single compartmental analysis are shown in Table 1. The Cmax was significantly higher in sheep (20.1 ± 7.3 ng/mL) and deer (26.4 ± 7.0 ng/mL) as compared to goats (11.5 ± 1.6 ng/mL) dosed

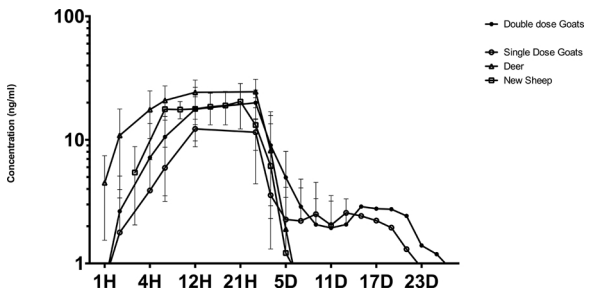

Fig. 1. Semilog plot for Abamectin after oral administration in deer (open triangles), sheep (open squares), goats (open circles) at 0.2 mg/kg dose and goats (solid circles) at 0.4 mg/kg dose. Each data point represents mean and standard deviation of 6 animals in each group. error bars represent standard deviation.