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Experimental Treatment of Fallow Deer (*Dama dama*) with Abamectin

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Abstract: Macrolide Endectocides (ME) are antiparasitic agents that are daily applied in pharmacotherapy of food-producing animals. Worldwide numbers of pharmacokinetics studies performed on various matrixes of domestic animals with diverse detection methods for ME determination were developed. However, only few studies on deer matrixes were published although ME are licensed and in use for treatment of red deer, fallow deer and reindeer in some countries of European Union, America and Australia. The purpose of this study was to follow some pharmacokinetic parameters of abamectin in fallow deer plasma 48 h after subcutaneous application of therapeutic dose. Biochemical and haematological parameters were also followed for 120 h after application of antiparasitic agent.

Key words: Antihelmintics, abamectin, pharmacokinetics, drugs, blood, fallow deer

INTRODUCTION

In last few decades, Macrolide Endectocides (ME) were extensively used in veterinary pharmacotherapy with food-producing animals. Ivermectin (IVM), Doramectin (DOR), Moxidectin (MOX) and Abamectin (ABM) were experimentally introduced in some countries with animals bred in enclosures and free-living animals as well (Pound *et al*., 2004; Lamka *et al*., 1997; Young *et al*., 2000). Subcutaneous injection, oral drench, feed sprayed with diluted injectable solution or topical applications are common routes of administration of commercially available ME. Most studies that concerned the use of IVM were focused on antihelmintic efficacy of the drug against lungworm, gastrointestinal nematodes and ectoparasites with various deer species such as red deer (Connan, 1996), fallow deer (Malczewski *et al*., 1998), roe deer (Lamka *et al*., 1997), white-tailed deer (Pound *et al*., 2004) and reindeer (Haugerud *et al*., 1993). However, according to Haugerud *et al*. (1993) IVM was first licensed and used ME for the treatment of warbles, throat bots and nematodes in reindeer in Finland, Sweden and Norway. Lately ABM, that was before also frequently used to control insects and mites of a range of agronomic, fruit, vegetable and ornamental crops, has been approved in Australia for the use as an antihelmintic drug with deer in Pour-on formulation.

Numerous analytical methods for the determination of ME residues in body fluids, tissues and excreta in domestic animals were published. Analytical methods for the determination of ME in plasma using high performance liquid chromatography with fluorescence detection have been previously reported with sheep (Cerkvenik, 2001) cattle (Wei and Li, 2001) swine (Scott and Mckellar, 1992) and horses (Gokbulut *et al*., 2001). However, the efficacy, toxicity and metabolism of ME have to be investigated independently with deer. To our knowledge, only few studies on disposition kinetic of IVM and DOR with deer were reported (Mackintosh *et al*., 1985; Andrews *et al*., 1993; Oksanen *et al*., 1995). Although deer have not been introduced as farmed animals for a long time, venison has rapidly become a part of the human consumption. Therefore, the detection of drug residues is also important with deer. However handling with fallow deer requires special attention. Captured animals reactions of fear, panic and attempts of escape represent serious limitations in performing pharmacokinetic studies. In this study, the results of an examination of the concentration-time course of ABM and its influence on blood parameters in fallow deer plasma after subcutaneous application of 200 μg of ABM per kilogram of body weight (bw) were investigated.

MATERIALS AND METHODS

Four fallow deer female (2-6 years old) with no history of ABM use were chemically immobilised using mixture of xylazine (125 mg mL^{-1}; Rompun®, Bayer, Germany) and

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ketamine (100 mg mL^{-1}; Ketamin 10%®, Veyx Pharma, Germany). Hinds for the trial were randomly selected from a heard of 30 animals, weighed, treated and placed into the wooden crates with strap system. Animals identified by ear tags numbers of 1 and 2 were ranged into group T (treated animals) and those numbered with 3 and 4 were ranged into group C (controls). Clinical examinations were performed before the experiment. Hinds were observed before, at and after each sampling. All animals were in good physical condition, none of them showed any physical reaction to ABM at the time of the trial. During the trial, all animals were fed according to their regular feeding regimen (hay, maize, beet, turnip, carrot).

Blood samples; The dose of 200 μg ABM per kilogram of the body weight was administered to each hind. Deer 1 and 2 in the group T were single-dosed via s/c injection of ABM (Abamitel L.A.®, Krka, Slovenia). Placebo (aqua pro injectione) was used with deer 3 and 4 in the group C. Blood samples were collected from the jugular vein prior to the drug administration and 1, 2, 4, 8, 12, 24, 36 and 48 h after drug application. All blood samples were centrifuged at 3000 rpm for 10 min and plasma was distributed into tubes. Serum was stored frozen at-20°C before analysis.

Blood samples for plasma biochemistry were collected before treatment (0 h) and 8, 36 and 120 h after drug application into EDTA K_3 tubes. Lithium heparin tubes were used to collect plasma for electrolyte analysis. For performing biochemical tests, blood in plain tubes was left to clot at room temperature.

Analytical procedure: Plasma samples were analysed for ABM concentration using HPLC with fluorescence detection as described elsewhere (Nordlander and Johnsson, 1990; De Montigny *et al*., 1990). ABM was extracted from samples with acetonitrile and extracts were cleaned-up by solid phase extraction on extraction columns Bakerbond speTM Octyl C8 (J.T.Baker, Mallinckrodt Baker, Phillipsburg, NY, USA). Eluates were evaporated with nitrogen to dryness, derivatized with trifluoroacetic anhydride and N-methylimidazole, diluted with acetonitrile and injected into HPLC system (Waters Alliance 2690, Waters, Milford, MA, USA) equipped with a computer with a Millennium program for the system control and data processing and a column Supelcosil™ LC-8DB 5 μm, 4,6 mm×15 cm (Supelco, Bellefonte, USA), heated to 27°C. The mobile phase was a mixture of acetonitrile, methanol and deionised water (475 + 475 + 50) and the flow rate was 1,1 mL min^{-1}. The injection volume was 50 μL, excitation wavelength 364 nm and emission wavelength 470 nm. The validation of the procedure for the determination of ABM residues in deer plasma was performed prior to the experiment with spiked blank fallow deer plasma.

Haematological parameters were obtained using a haematological analyzer Vet ABC (ABX Diagnostics, Montpellier, France), while biochemical values were determined with a biochemical analyser COBAS MIRA (Roche Diagnostics, Rotkreuz, Switzerland) with the enzyme assay performed at 37°C and the value at 30°C was calculated using a suitable factor. Blood smears were prepared by Pappenheim method using May-Grünwald Giemsa staining.

RESULTS AND DISCUSSION

The plasma concentrations of ABM within 48 h after application are presented in hind 1 and hind 2. With the hind 1, the concentration of ABM in plasma started to rise slowly in the first 2 h after application. In the next 2 h the measured value increased to 23,6 ng mL^{-1} and 4 h later the concentration reached the peak value of 43,9 ng mL^{-1}. Because of unexpectedly low quantity of plasma after centrifugation, the concentration was not determined 12 h after application. Twenty four hour after application the concentration decreased to approximately half of a peak value (24.6 ng mL^{-1}) and remained roughly at that level for further 24 h. With the animal 2 the maximal concentration of 95 ng mL^{-1} was reached 24 h after drug application. In the next 24 h the value decreased rapidly to 6 ng mL^{-1}. At the hind 2, the half-life of elimination was 20.8 h. No significant differences were found in serum biochemistry values and haematological values between hinds of T and C group at 0, 8, 36 and 120 h after drug application.

Number of studies on the antiparasitic efficacy of IVM with deer were performed. Only few of them reported some pharmacokinetic characteristics of IVM, DOR and MOX with deer but no information has been given on pharmacokinetic features of ABM and its influence on blood parameters with deer. Pharmacokinetic studies on deer are limited because of relatively late domestification of the species; handling should have the great impact on behaviour responses (Hemmer, 1988). The results of this research describe pharmacokinetic data obtained after subcutaneous administration of ABM to fallow deer. The detected peak ABM concentrations in plasma with two treated animals (43.9 and 95.0 ng mL^{-1}) in this study were comparable to those observed in reindeer and cattle (Oksanen *et al*., 1995; Fink and Porras, 1989) after subcutaneous application of IVM. Conversely, considerably higher concentrations were recorded at red deer (Mackintosh *et al*., 1985; Andrews *et al*., 1993).

Moreover Andrews *et al.* (1993) reported significant elevation of peak concentrations of IVM in plasma after application of 400 μg kg^{-1} bw of IVM with no significant effect on nematode egg output in red deer. The time required to reach maximum concentration (t_{max}) of ABM in plasma in the present study was 8 and 24 h after application at the hind 1 and 2, respectively. With the hind 1, accurate estimation of t_{max} has been difficult because of the amount of sample. t_{max} with the hind 2 was close to t_{max} recorded after treatment with IVM in red deer (Andrews *et al.*, 1993) but shorter than with reindeer, white-tailed deer and cattle, which was approximately 3 days (Oksanen *et al.*, 1995; Fink and Porras, 1989; Pound *et al.*, 2004). Craven *et al.* (2002) also reported differences in time to reach peak plasma concentration and the persistence of IVM and MOX in plasma at pigs due to different body condition.

The results of the present trial indicate that ABM appears in systemic circulation shortly after application. The substance could be quantified within an hour. This is in agreement with previously reported deer's ability to excrete IVM at a faster rate than cattle (Andrews *et al.*, 1993). With the hind 1, the concentration started to rise slowly and constantly after application to the peak value of 43.9 ng mL^{-1}. The values were kept on that level roughly 24 h. Plasma concentrations with the hind 2 were dispersed from the start, after the peak value was reached, concentrations declined constantly. Those dispersions were probably due to the measurement uncertainty. Similar dispersions in individual values of IVM concentrations in plasma within 40 h after treatment were observed at red deer hinds (Andrews *et al.*, 1993) and calves (Mackintosh *et al.*, 1985). However, variations in initial plasma concentrations of IVM after subcutaneous applications were also reported at white tailed deer (Pound *et al.*, 2004). The half-life of ABM in hinds was 47, 2 h and 20.8 h after application. This is much longer than 4 days reported with red deer hinds treated with IVM (Andrews *et al.*, 1993) or 6 days reported with red deer calves treated with IVM (Mackintosh *et al.*, 1985) and 8.3 days reported with cattle (Lo *et al.*, 1985). This is in agreement with faster elimination of IVM with deer as reported by Andrews *et al.* (1993). However considerable variation among individual hinds regarding ABM were observed. Observed differences among animals are probably due to deer metabolism, body condition and composition.

Serum biochemistry and haematological values obtained in this work were in range of previously reported elsewhere (Presidente, 1979; Vengušt *et al.*, 2002; English and Lepherd, 1981; Ranucci *et al.*, 1993; Rehbein *et al.*, 1999; Marco and Lavin, 1999; Chapman *et al.*, 1980).

CONCLUSION

Intensiveness of sampling when performing pharmacokinetics trials may impact on emotional sensitivity that is one of the major causes that affect fallow deer farming (Hemmer, 1988). Therefore in this research, the time of sampling was shortened in order to avoid excessive stress and excitation of the animals. We are aware of low number of animals in study as a consequence of legislation restrictions that concern the use of non domestic animals for research purposes.

As an increasing quantities of deer products are released to the world market the understanding of complex relationships between an animal, drug effect and pharmacokinetics profiles is crucial for optimizing the dosage regime and the efficacy of antihelmintics used at deer. The aim of this study was to gain more information on ABM behaviour in farmed deer. Obtained results may serve as a basis for future experiments of larger scale on the pharmacokinetics of ABM.

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