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Effect of Parasitism on the Pharmacokinetic Disposition of Ivermectin in Lambs

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With 3 figures and 1 table

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Summary

The aim of this study was to investigate the effect of parasitism on plasma availability and pharmacokinetic behaviour of ivermectin (IVM) in lambs. Fourteen greyface Suffolk lambs (26.8 ± 2.2 kg body weight) were selected for this study. Seven pairs of lambs were allocated into two groups in order to obtain an approximately even distribution. Group I (non-parasitized) was pre-treated by three repeated administrations of 5 mg/kg of fenbendazole (Panacur®), in order to maintain a parasite-free condition. The lambs in group II (parasitized) did not receive any anthelmintic treatment and the natural infection was sustained by an oral inoculation of infective stages of nematode parasites. After the 85-day pre-treatment period both groups of animals were treated with IVM (200 μg/kg, Ivomec®) by subcutaneous injection in the shoulder area. Both groups of animals were maintained under similar conditions of feeding and management. Blood samples were collected by jugular puncture at different times between 0.5 h and 25 days post-treatment. After plasma extraction and derivatization, samples were analysed by high-performance liquid chromatography with fluorescence detection. A computerized kinetic analysis was performed and data were compared using the unpaired Student's *t*-test. The parent molecule was detected in plasma between 30 min and either 12 (parasitized) or 20 (no parasitized) days post-IVM treatment. The area under the curve values of the parasitized group (75.2 ± 15.5 ng × d/ml) were significantly lower that those observed in the parasite-free group (134.3 ± 15.7 ng × d/ml). The mean residence time (MRT) of the parasitized group (2.93 ± 0.16 days) was significantly lower than the MRT of healthy group (3.93 ± 0.29 days). The results of this study have shown that a change in body condition followed by a parasitic infection is associated with significant changes in plasma disposition of IVM when it is administered subcutaneously to parasitized lambs. Therefore, variations in the condition induced by parasitism should be considered when these anthelmintics are used for treating parasitized animals.

Introduction

Ivermectin (IVM) is a highly effective anthelmintic for use against adult and larval forms of many ovine gastrointestinal and lung nematodes (Zajac et al., 1992). It is also effective against some ovine ectoparasitic diseases (Campbell and Benz, 1984; Reinemeyer and Courtney, 2001).

The plasma disposition kinetics of IVM have been studied in sheep (Prichard et al., 1985; Marriner et al., 1987; Bogan and McKellar, 1988; Barber et al., 2003); it is characterized by a long persistence in the body and a large volume of distribution, a linear pharmacokinetic pattern and has significant effects of formulation and/or route of administration on bioavailability (McKellar and Benchaoui, 1996). Other factors such as animal species, level of feed intake, nutritional status/body condition, have been shown to substantially affect the systemic availability of macrocyclic lactones in sheep and cattle (Lanusse, 2003).

The clinical efficacy of macrocyclic lactones is closely related to pharmacokinetic behaviour; the time of parasite exposure to active drug concentrations is relevant to obtain optimal and persistent antiparasitic activity (Lanusse, 2003). The extended permanence of the drug in the body is largely because of high lipophilicity and extensive distribution in fat. The subsequent rate of release from this lipid reserve into bloodstream is considered to be a major determinant of the pharmacokinetic behaviour of these compounds (Craven et al., 2001).

Anthelmintics are thoroughly tested before licensing so that the pharmacokinetics, the plasma concentrations after therapeutic doses and tissue residues are known. The pharmacokinetic data obtained are assumed to indicate the behaviour of the same dose under field conditions (Taylor et al., 1992). Normally, these pharmacokinetic studies on anthelmintics have been carried out in non-parasitized animals. However, it seems likely that parasitic burdens could influence the plasma kinetics and tissue and gastrointestinal disposition of anthelmintic drugs (McKellar et al., 1991). Some conditions of parasitic disease may alter the host's physiology and modify the plasma availability of macrocyclic lactones. For example, a more rapid absorption of IVM together with a reduced area under the curve (AUC) has been observed in sheep with poor physical condition as a result of mange infestation compared with healthy unaffected controls (Echeverría et al., 2002). A recent study by Lespine et al. (2004) has demonstrated that gastrointestinal parasitism produces significant changes in moxidectin disposition with an increase in drug clearance and a reduction in the half-life of elimination.

Ivermectin and moxidectin are two closely related 16-membered macrocylic lactones that share a common mode of action and broad spectrum of anti-parasitic activity. However, differences in physicochemical properties among them may account for differences in kinetic behaviour and in the potency and persistence of their anti-parasitic activity (Lanusse et al., 1997). As the anti-parasitic activity of macrocyclic lactones depends on drug concentrations and time of parasite exposure

to them, an evaluation of the comparative pharmacokinetic profiles between parasitized and non-parasitized animals may help to estimate and optimize drug efficacy. The aim of the current study was to investigate the effect of parasitism on the pharmacokinetic behaviour of IVM in lambs.

Materials and Methods

Fourteen greyface Suffolk lambs (eight castrated males and six females) aged between 3 and 4 months with 26.8 ± 2.2 kg body weight were selected for this study. During the experimental period the lambs were maintained outdoors during the day and stabled during the night. They were fed daily with a mixture of ryegrass and clover hay and supplementary concentrate. Water and hay were provided *ad libitum*. They also had access to natural grass from the pasture of the paddock. All lambs were weighed before the treatments by means of a digital scale.

Faecal examinations were performed on all lambs to determine faecal egg counts (FEC) to determine the level of natural infection. Quantitative pre- and post-treatment FEC were performed using a modified McMaster technique (Zajac, 1994) during a period of 90 days previous and 70 days after IVM treatment. All faecal samples were obtained from the rectum with an interval of 7 days in between. A minimum of 200 eggs per gram (epg) of faeces was established to consider the incorporation of lambs in the experimental groups.

Experimental groups

Animals were allocated into two experimental groups of seven pairs of lambs selected according to a randomized block design, considering the variables body weight and sex, in order to obtain an approximately even distribution of animals. In group I, animals were treated three times by the oral administration of 5 mg/kg fenbendazole (Panacur®; Intervet, Santiago, Chile), in order to maintain a healthy parasite-free condition for a period of 85 days. In group II (parasitized), the natural infection was sustained by oral inoculation with cultures of the infective stages of nematodes (mainly *Ostertagia*, *Trichostrongylus* and *Cooperia* genus). An infective dose containing approximately 5000 larvae was given orally once a week for 3 weeks. This group did not receive any anthelmintic treatment, in order to maintain their parasitized condition for the same period.

After the 85-day pre-treatment period, both groups of animals were treated with IVM (200 μg/kg body weight) by subcutaneous injection in the shoulder area using a commercially available formulation (Ivomec®; Merial, Harlem, the Netherland). Both groups of animals were maintained together under similar conditions of feeding and management.

Sampling

Heparinized blood samples were collected from the jugular vein prior to the treatments and at 0.5, 1.0, 2.0, 4.0, 8.0, 12.0 and 24.0 h and 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 20.0 and 25.0 days post-treatment. Plasma was separated by centrifugation and stored at −18°C until analysis.

After the treatments, the animals were observed continuously for a 4-h period and at least twice daily within 2 days of treatment for any signs of adverse reaction.

Analytical procedures

Ivermectin was assayed by high-performance liquid chromatography (HPLC) with fluorescence detection after solid phase extraction, according to the procedures previously described by De Montigny et al. (1990) and Alvinerie et al. (1993, 1995).

Drug extraction and derivatization

Drug-free plasma samples (1 ml) were fortified with IVM to reach final concentrations of 0.1, 0.5, 1.0, 5.0, 10.0, 25.0 and 50.0 ng/ml. Fortified and experimental plasma samples were homogenized and solid phase extraction was performed after 15 min of incubation at room temperature. Briefly, 1 ml of acetonitrile and 0.25 ml of water was added to 1 ml of plasma. After mixing for 20 min, the samples were centrifuged at 2000 ***g*** for 5 min, and the supernatant transferred to a Supelco C18 cartridge (Supelco Inc., Bellefonte, PA, USA). After washing with water, IVM was eluted with 1.0 ml of methanol (MeOH). The eluate was evaporated to dryness under a gentle stream of nitrogen, and the residue was dissolved in 100 μl of *N*-methylimidazole solution in acetonitrile (1:2 v/v). To initiate the derivatization, 150 μl of trifluoroacetic anhydride solution in acetonitrile (1:2 v/v) was added. After completion of the reaction (< 30 s), an aliquot (100 μl) of this solution was injected directly into the chromatograph. To avoid photoisomerization of the derivatives under ambient light conditions (Sklavounos et al., 1994) solutions were stored in amber glassware during HPLC analysis (Nowakowski et al., 1995).

Chromatographic conditions

The mobile phase consisted of acetic acid (0.2% in water), MeOH and acetonitrile (4:32:64, v/v/v) passing at a flow rate of 1.5 ml/min through a Supelcosil C18 column (3 μm, 4.6 mm i.d. × 150 mm; Supelco Inc.) with fluorescence detection at an excitation wavelength of 383 nm and an emission wavelength of 447 nm (RF.551 Fluorescence detector; Shimadzu, Kyoto, Japan).

Method of calibration

Calibration graphs for IVM in the range of 0.1–50 ng/ml were prepared using drug-free plasma of non-treated lambs. Pooled plasma samples were taken through the procedure, and calibration graphs were plotted using the peak area as a function of analyte concentration. Linear regression analysis was used to determine the slopes and correlation coefficients of the different calibration curves ($n = 5$). The extraction efficiency of the drug under study was measured by comparison of the peak area from the spiked plasma samples with the peak area resulting from direct injections of the standard in MeOH carried through the derivatization procedure. The inter-assay precision of the extraction and chromatography procedures were evaluated by processing replicate aliquots of plasma samples (quintuplicate determinations) containing known amounts of the drug on different days.

The analytical method used to extract, derive and quantify the plasma concentration of IVM by chromatographic analysis using the fluorescence detector was validated as follows: the regression lines between peak areas and drug concentrations showed correlation coefficients ranging between 0.9998 and

0.9987. The mean extraction recoveries from plasma was 83.8 ± 6.5% at the spiked concentrations between 0.1 and 50 ng/ml. The inter-assay precision showed variation coefficients of 7.4 ± 4.2%. The limit of quantification of the method was defined as the lowest concentration that would have a coefficient of variation of < 20%; this was found to be 0.1 ng/ml.

Data analysis

The plasma concentration versus time curves obtained after treatment in each individual animal was fitted with the PK Solutions computer program (Farrier, 1997) (goodness-of-fit was evaluated by comparing observed concentration and calculated concentration and residue repartition). A tri-exponential equation resulted in the best-fit concentration–time curves and was used to describe the plasma disposition kinetics of IVM

$$C_t = A_1^{-\alpha t} + A_2^{-\beta t} - A_3^{-kabt},$$

where A_1, A_2 and A_3 are the intercepts, C is the plasma concentration at time t, kab is the first-order rate constant of IVM absorption and α and β are the first-order rate constants for distribution and elimination respectively. The terminal (elimination) half-life ($t_{1/2\beta}$), intermediate (distribution) half-life ($t_{1/2\alpha}$) and absorption half-life ($t_{1/2ab}$) were calculated as $\ln 2/\beta$, $\ln 2/\alpha$ and $\ln 2/ka$ respectively. The peak concentrations (C_{max}) and time to peak concentrations (T_{max}) were read from the plotted concentration–time curve of each drug in each individual animal. The area under the plasma concentration–time curves (AUC) from time zero to the last time t with a measurable concentration (C_p) was calculated using linear trapezoidal approximation. The mean residence time (MRT) was calculated by the linear trapezoidal rule without extrapolation to infinity, using the formula:

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}},$$

where AUMC is the area under the first moment curve and AUC is the area under the plasma concentration time curves as previously defined.

The pharmacokinetic parameters are reported as mean ± SD and were statistically compared by the unpaired Student's t-test with Welch's correction. A linear regression analysis was performed to find the relationship between the level of parasitism measured through the FEC and the pharmacokinetic parameters such as $t_{1/2\beta}$, MRT and AUC. All statistical analyses were performed through the software Graph Pad Instat (version 3.0; Graph Pad, San Diego, CA, USA). Mean values were considered significantly different at $P < 0.05$.

Results

Faecal egg counts of lambs are shown in Figure 1. At the beginning of the assay the sheep of the parasitized group (group II) had a mean FEC of 383 epg of faeces (range 200–500 epg). While in group I, the mean FEC was 262 (range 100–500 epg). After 85 days of assay, the FEC of the parasitized group (group II) increased to 797 epg (range 500–1000 epg). On the contrary, in the non-parasitized group the FEC was significantly reduced to zero after the first two treatments with fenbendazole, and a mean value of 115 epg

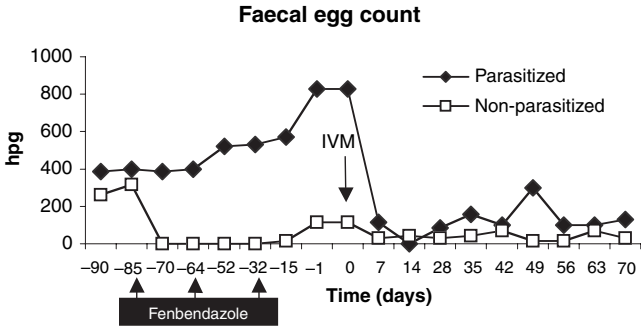

Fig. 1. Arithmetic mean of faecal egg counts in two groups of lambs before and after subcutaneous administration of ivermectin (IVM).

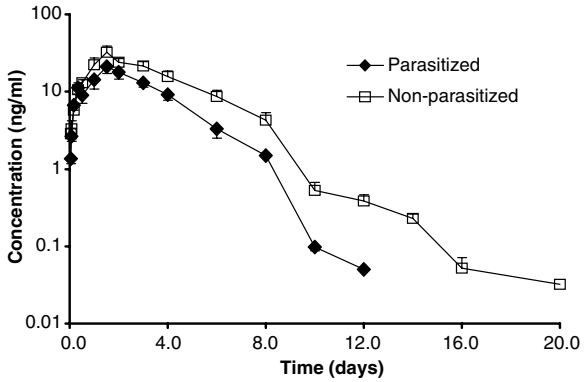

Fig. 2. Mean (±SEM) plasma ivermectin concentrations (ng/ml) following subcutaneous administration in healthy and parasitized lambs.

(range 0–400 epg) was observed after the third treatment (Fig. 1). The administration of IVM in both group of lambs significantly reduced the FEC and maintained it at levels close to zero for the first 14 days following drug administration (Fig. 1).

At the beginning of the experimental period the mean body weight of lambs was 26.9 ± 2.2 kg in group I and 26.1 ± 2.6 kg for group II. A significant increase in body weight was observed in lambs of group I (36.5 ± 1.9 kg) which were maintained free of parasites through the pre-treatment with fenbendazole in comparison with the body weight observed in group II (28.7 ± 2.1 kg) of parasitized animals. Although the differences seem to persist for a period of 70 days after IVM administration, where values of 48.4 ± 3.2 kg for group I and 43.1 ± 3.6 kg for group II (parasitized lambs) were obtained, these results were not statistically different ($P > 0.05$).

The mean plasma concentrations of IVM obtained in the two groups of lambs are shown in Fig. 2. Concentrations > 0.1 ng/ml (limit of quantification) were detected in plasma between 0.5 h and either 20 (group I) or 12 (group II) days post-IVM treatment.

The pharmacokinetic parameters that describe the disposition of IVM after subcutaneous administration of 0.2 mg/kg in both groups of lambs are shown in Table 1. Although a slightly higher and faster level of absorption was observed in the parasite-free lambs of group I, these differences were not significant. However, the AUC values of parasitized group

Table 1. Plasma pharmacokinetic parameters (mean ± SEM) of ivermectin (IVM), at 200 μg/kg body weight after subcutaneous administration in healthy and parasitized lambs

Parameter	Non-parasitized	Parasitized	*P*-value
Body weight (kg)	36.5 ± 1.9	28.7 ± 2.1	0.044*
Dose (μg/kg)	200	200	
$t_{1/2ka}$ (h)	20.2 ± 3.97	13.7 ± 0.77	0.149
C_{max} (ng/ml)	34.5 ± 6.13	21.7 ± 3.96	0.179
T_{max} (days)	1.75 ± 0.1	1.71 ± 0.1	0.981
$t_{1/2\beta}$ (days)†	2.52 ± 0.69	1.6 ± 0.17	0.069
AUC_{Clast} (ng × d/ml)	134.3 ± 15.7	75.2 ± 15.5	0.05*
AUMC (ng × d^2/ml)	496.8 ± 70	228 ± 58	0.025*
MRT_{Clast} (days)	3.81 ± 0.29	2.93 ± 0.16	0.025*

$t_{1/2ab}$, absorption half-life; C_{max}, peak plasma concentration; T_{max}, time to peak plasma concentration; AUC_{Clast}, area under the concentration–time curve from t_0 to the last measurable concentration (*C*last); $t_{1/2\beta}$, terminal half-life; MRT_{Clast}, mean residence time from t_0 to the last measurable concentration.
*$P < 0.05$.
†Values represent the arithmetic mean for $t_{1/2ab}$, $t_{1/2\alpha}$ and $t_{1/2\beta}$ (days) respectively.

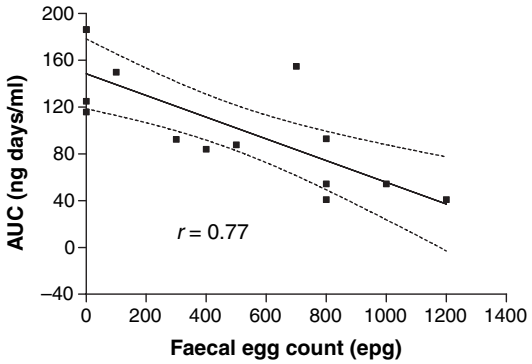

Fig. 3. Linear regression analysis for the correlation between nematode faecal egg counts and the area under the ivermectin concentration versus time curve.

(75.2 ± 15.5 ng × d/ml) were significantly lower than those observed in the parasite-free group (134.3 ± 15.7 ng × d/ml). There was a high (0.77) and significant ($P < 0.05$) correlation was observed between the FEC and the calculated values of AUC_{0-t} (Fig. 3). The MRT of the parasitized group (2.93 ± 0.16 days) was significantly lower than the MRT of healthy group (3.81 ± 0.3 days). Low and non-significant correlations were determined for the parameters $t_{1/2\beta}$ or MRT.

Discussion

The pathogenesis of gastrointestinal nematode infection is associated with inappetence and increased loss of protein into the gastrointestinal tract (Holmes, 1987; Arneberg et al., 1996). Resultant changes in the host metabolism account for the poor productivity of infected animals observed through growth retardation and lower average daily weight gain. In addition to changes in body weight, alterations in body composition also occur: the percentage of water is higher and the deposition of fat, protein and skeletal calcium and phosphorus is lower than in nematode-free animals (Holmes, 1987).

According to the results observed in the current study, the effect of parasitism on infected lambs, during almost a 3-month period, can modify the weight and body condition of these animals. Studies performed by Sykes (1978), have demonstrated that in parasitized sheep a major factor in reducing weight gain is a severe reduction in the efficiency of utilization of digested energy which is demonstrated mainly through the reduced fat deposition. On the other hand, the higher live weight gain, in the lambs pre-treated with fenbendazole, obviously is based on the fact that as the parasites are eliminated, the feed is appropriately digested, and the nutrients are absorbed and metabolized for the optimum use by the body.

Reduced plasma availability, and a lower permanence of the plasma concentrations of IVM, were observed in the parasitized lambs in comparison with healthy animals. These results agree with those described for moxidectin by Lespine et al. (2004) in sheep, where a reduced availability, measured as AUC was observed in sheep with poor physical condition as a result of gastrointestinal parasitism compared with healthy unaffected controls. However, they are not in agreement with those described by McKellar et al. (1991), where no significant differences in IVM availability was observed between parasitized lambs exposed to a moderate challenge with *Nematodirus battus* in comparison with parasite-free lambs. Possibly differences in the type of parasite and lamb's physical condition between both studies could explain these differences.

The plasma disposition of IVM is characterized by a long persistence in the body due to a low plasma clearance and to an extensive distribution into fat. Ivermectin is a highly lipophilic drug that has a preferential distribution to adipose tissue and represents an important reservoir that can influence the persistence in the body. Ivermectin distribution to the body fat is likely to be of significant importance in defining the pharmacokinetic behaviour of this drug. A more rapid peak in plasma IVM concentrations and a less persistent drug profile in pigs with reduced fat has been described by Craven et al. (2002). The MRT of subcutaneously administered macrocyclic lactones is influenced by the body condition of the animal.

The plasma elimination half-life of IVM, after subcutaneous administration, appears to be longer in the non-parasitized lambs. Although the differences were not significant, they were achieved by a reduction in the mean values of clearance (data not shown). Therefore, the low persistence of IVM concentrations observed in parasitized lambs may be the result of a higher elimination rate. More recently, it has been demonstrated that IVM has a high level of binding to plasma lipoproteins which probably is involved in the delivery to adipose tissue, and subsequently to their long residence time in the body (Lespine et al., 2003). Changes in protein synthesis and in lipid turn-over may occur in parasitized lambs which accounts for a faster rate of IVM elimination.

In the current study, a high and significant correlation between FEC and the AUC values was determined for the lambs, where the animals with the highest values of FEC showed the lowest values of AUC, indicating that a greater level of parasitism reduces the systemic availability of IVM.

Host physio-pathological changes occurring during parasitic diseases may also influence the fate of drug. An important step in understanding the pathophysiology of gastrointestinal infections was the observation that they are usually associated with hypoalbuminaemia and in some cases with anaemia

(Holmes, 1987). The increased permeability of the digestive epithelium to plasma proteins in the gastrointestinal lumen reduces the half-life of plasma albumin and immunoglobulins up to 50% (Barriga, 1996). In addition to the loss of plasma proteins into gastrointestinal tract, it is likely that there are important protein losses in the form of exfoliated epithelial cells and mucus (Holmes, 1987). Binding studies in humans and dogs show that IVM bound extensively to plasma proteins (Okonkwo et al., 1993) including albumin and lipoproteins (Rohrer and Evans, 1990). Consequently, the plasma protein leakage into the gut might be an important feature in reducing the plasma availability of IVM in the parasitized lambs of the current study.

Parasitism is also associated with an increase of intestinal secretions and gut motility. Because large amounts of unmodified IVM are excreted in faeces via bile (Bogan and McKellar, 1988; Chiu et al., 1990; Lifschitz et al., 2000) and intestinal secretions (Laffont et al., 2002), these changes may also contribute to the increased IVM elimination observed in infected lambs.

It is generally accepted that the anti-parasitic effect of IVM depends on drug concentration and the time of parasite exposure to the compound (Lanusse, 2003). The differences observed between the groups of lambs should be considered during the strategic timing of anthelmintic administration because it is based on the persistence of therapeutic anthelmintic concentrations in the treated hosts. It has been established that the actual dose of an extravascularly administered drug is better represented by the systemic AUC than by the administered dose (Riviere, 1999). The differences in systemic availability observed between parasitized and healthy lambs of the current study might indicate that in the highly parasitized animals the overall exposure of parasites to the drug could be reduced.

From the pharmacokinetic point of view the main factors that contribute to the reduced IVM plasma availability in the parasitized lambs are: (i) the reduction in the volume of distribution of the drug because of the reduced body fat content that limits the drug reserves; and (ii) the reduced half-life of elimination of the drug due to the physiopathological changes produced by the parasites on the gastrointestinal epithelium, which may induce an important loss of the drug through plasma protein leakage and increased gut motility.

In conclusion, this study has shown that parasitic disease can modify the body condition of animals inducing significant changes in the plasma availability and disposition of IVM when the compound is administered subcutaneously to parasitized lambs. Therefore, the reduction in the permanence of IVM in plasma observed in parasitized animals could have consequences in the duration of the anthelmintic effect of the drug, particularly in those animals in which the effect of the parasitic disease is more intense, producing a marked loss of body weight.

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