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Ivermectin (3.15%) long-acting formulations in cattle: Absorption pattern and pharmacokinetic considerations

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Abstract

Ivermectin (IVM) is a broad-spectrum antiparasitic drug extensively used in veterinary medicine. The composition of the pharmaceutical preparation affects IVM absorption and its systemic availability. After the introduction of the first approved IVM formulation (propylene glycol/glycerol formal 60:40) used at 200 μg/kg, different pharmaceutical modifications have been assayed to extend IVM persistent endectocide activity. Recently, IVM 3.15% long-acting (IVM-LA) preparations to be administered at 630 μg/kg to cattle were introduced into the veterinary pharmaceutical market. The work reported here was designed to evaluate the comparative IVM absorption pattern and plasma concentration profiles obtained after subcutaneous administration of the classic pioneer IVM formulation (1%) and two different commercially available IVM-LA preparations (3.15%) to cattle. Twenty-eight Holstein heifers were divided in four experimental groups ($n = 7$) and treated subcutaneously as follows—Group A: IVM 1% given at 200 μg/kg, Group B: IVM 1% administered at 630 μg/kg, Group C: IVM-LA (A) injected at 630 μg/kg and Group D: IVM-LA (B) given at 630 μg/kg. Blood samples were taken between 0.5 and 90 days post-treatment and IVM plasma concentrations were determined by HPLC with fluorescence detection. There were no differences in the persistence of IVM plasma concentrations after the administration of IVM 1% formulation at the two used dose levels (200 and 630 μg/kg). Higher peak plasma concentration (C_{max}) and shorter mean residence time (MRT) were obtained for IVM 1% given at 630 μg/kg (Group B) compared to the treatments with both IVM-LA preparations. The IVM-LA (A) formulation showed a more extended absorption process than IVM-LA (B) preparation, which accounted for a longer persistence of detectable IVM plasma concentrations. The parasitological implications of the observed differences in peak plasma concentrations (C_{max} values) and in the IVM concentration levels measured from day 20, and afterwards until day 90 post-treatment, between the different preparations assayed need to be elucidated. The characterization of the absorption patterns and kinetic behaviour obtained after injection of these novel long-acting formulations used at three times the therapeutic dose recommended for the classic IVM preparation in cattle is a further contribution to the field.

Keywords: Ivermectin; Long-acting preparations; Pharmacokinetics; Cattle

1. Introduction

The time duration of the antiparasitic effect has been considered a relevant attribute in parasite control programs in livestock. The persistence of the antiparasitic activity is the period elapsed between the

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administration of a single therapeutic dose and the time when the establishment of a subsequent parasite infection becomes possible (Barth and Rehbein, 1997). The concept of antiparasitic persistence acquired a great clinical significance after the discovery and commercial development of the avermectin and milbemycin endectocide compounds for use in ruminant species.

Ivermectin (IVM) is a semi-synthetic avermectin broad-spectrum compound active against endo and ectoparasites of clinical relevance in veterinary and human medicine. IVM is commercially available to use in livestock animals as injectable, oral and/or pour-on formulations (McKellar and Benchaoui, 1996). It has been shown that differences in drug formulation may affect the pharmacokinetic disposition of endectocide drugs in different animal species. The vehicle in which these compounds are formulated may influence their absorption process and the resultant drug concentration profiles achieved in the bloodstream (Lo et al., 1985; Wicks et al., 1993) and at the tissues of target parasite location (Lifschitz et al., 2000). After the introduction of the first approved IVM formulation (propylene glycol/glycerol formal 60:40) used at 200 μg/kg, different pharmaceutical modifications have been assayed to extend the IVM persistent endectocide activity in cattle. The first so-called long-acting IVM 1% formulations are essentially oil-based preparations that account for a slow absorption process from the subcutaneous space and an extended persistence of concentrations in the bloodstream and tissues of parasite location (Lifschitz et al., 1999) compared to the original preparation.

More recently, in an attempt to further extend the antiparasitic persistent period with a single treatment, highly concentrated (3.15%) long-acting (LA) IVM preparations to be administered to cattle at 630 μg/kg, were introduced into the veterinary pharmaceutical market. These novel long-acting formulations administered at three times the recommended IVM dose rate have been tested in terms of antiparasitic efficacy (Bridi et al., 2001). However, further knowledge addressed to establish the relationship between pharmacokinetic behaviour and persistent activity against different target endo and ectoparasites is needed. The work reported here was designed to evaluate the comparative IVM absorption pattern and plasma concentration profiles obtained after subcutaneous administration of the classic pioneer IVM formulation (1%) and two different commercially available IVM-LA preparations (3.15%) to cattle.

2. Materials and methods

2.1. Experimental animals, treatments and sampling

Twenty-eight parasite-free, healthy Holstein heifers were selected from the same cattle ranch (area of Tandil, Province of Buenos Aires, Argentina) and identified with ear tags. Animals were in optimal nutritional condition and had free access to food and water during the entire experimental period. The experimental animals were weighed (140 ± 20 kg) using a digital scale and randomly allocated into four treatment groups of seven animals each. The individual injectable doses were calculated based on the weight of the experimental animals and the IVM concentration of the preparations used in the current trial. Animals in each group received one of the following subcutaneous treatments in the shoulder area—Group A: IVM 1% (Ivomec®, Merial) given at the recommended 200 μg/kg dose rate; Group B: IVM 1% (Ivomec®, Merial) administered at three times the recommended dose (630 μg/kg); Group C: animals were treated with one of the 3.15% IVM-LA preparation (formulation A) (Ivomec Gold®, Merial) injected at the recommended dose of 630 μg/kg and Group D: IVM-LA (formulation B) (Vermectin LA Premium®, Laboratorio Over, Argentina) given at the same dose rate of 630 μg/kg. Blood samples were taken into heparinized vacutainer tubes prior to and at 0.5, 1, 2, 3, 4, 7, 10, 15, 20, 30, 40, 50, 70 and 90 days after treatments. Blood samples were centrifuged at 3000 rpm for 20 min and the recovered plasma was kept in labeled vials at −20 °C until HPLC analysis.

2.2. Analytical procedures

2.2.1. Chemical extraction and derivatization

The extraction of IVM (22,23 dehydro-avermectin B1a), from spiked and experimental plasma samples was carried out following the technique first described by Alvinerie et al. (1993) slightly modified by Lifschitz et al. (1999). Basically, 1-ml aliquot of plasma sample was combined with 10 ng of the internal standard compound (abamectin) and then mixed with 1 ml of acetonitrile–water (4:1). After mixing for 20 min, the solvent–sample mixture was centrifuged at 2000 *g* for 15 min. The supernatant was manually transferred into a tube that was then placed on the appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France). The supernatant was injected to a Supelclean LC_{18} cartridge (Supelco, Bellfonte, PA,

USA), previously conditioned by passing 2 ml methanol and 2 ml deionized water. The cartridge was flushed with 1 ml of water and 1 ml of water/methanol (4:1). The compounds were eluted with 1.5 ml of methanol and concentrated to dryness under a stream of nitrogen. The re-suspension was done with 100 μl of a solution of *N*-methylimidazole (Sigma Chemical, St. Louis, MO, USA) in acetonitrile (1:1) (De Montigny et al., 1990). Derivatization was initiated adding 150 μl of trifluoroacetic anhydride (Sigma Chemical) solution in acetonitrile (1:2). After completion of the reaction (<30 s), an aliquot (100 μl) of this solution was injected directly into the chromatograph.

2.2.2. Chromatographic conditions

IVM concentrations were determined by high performance liquid chromatography (HPLC) using a Shimadzu 10 A HPLC system with autosampler (Shimadzu Corporation, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase C_{18} column (Phenomenex, 5 μm, 4.6 mm × 250 mm) and an acetic acid 0.2% in water/methanol/acetonitrile (3.8/40/56.2) mobile phase at a flow rate of 1.5 ml/min at 30 °C. IVM was detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). IVM concentrations were determined by the internal standard method using the Class LC 10 Software version 1.2 (Shimadzu Corporation) on an IBM compatible AT computer. The peak area ratios were considered to calculate the IVM concentrations in spiked (validation) and experimental plasma samples. There was no interference of endogenous compounds in the chromatographic determinations. The solvents (Baker, Phillipsburg, NJ, USA) used during the extraction and drug analysis were HPLC grade.

2.2.3. Validation procedures

A complete validation of the analytical procedures used for extraction and quantification of IVM was performed before starting analysis of the experimental samples obtained during the pharmacokinetic trial. Calibration curves in the range between 0.1–5 ng/ml and 5–100 ng/ml were prepared for each compound. Calibration curves were established using least squares linear regression analysis and correlation coefficients (r) and coefficient of variations (CV) calculated. Linearity was established to determine the IVM concentrations/detector responses relationship. Percentages of IVM recovery from plasma were obtained in the range between 0.1 and 50 ng/ml. The inter-assay precision of the extraction and chromatography procedures was estimated by processing replicate aliquots ($n = 4$) of pooled cattle plasma samples containing known IVM concentrations (1 and 40 ng/ml) on different working days. The limits of drug detection and quantification were established. The mean baseline noise at the IVM plus three standard deviations was defined as the detection limit. The mean baseline noise plus 10 standard deviations was defined as the theoretical quantification limit. Concentration values below the quantification limit were not considered for the kinetic analysis of experimental data.

2.2.4. Determination of IVM concentration in the IVM-LA preparations

The IVM concentrations in the IVM-LA formulation A and B were determined by HPLC analysis with fluorescent detection after serial dilutions of commercial preparations in organic solvents.

3. Pharmacokinetic and statistical analyses

Pharmacokinetic parameters were determined using a model-independent method. The peak concentration (C_{max}) and the time to peak concentration (T_{max}) were read from the plotted concentration–time curve for each individual animal. The absorption half-life ($T_{1/2\ ab}$) was calculated as $(\ln 2)/K_{ab}$, where K_{ab} represents the first-order absorption rate constant (h^{-1}). The K_{ab} was determined applying the method of residuals to the first portion of the plasma concentration–time curve. The area under the concentration–time curves from time zero to the last measurable concentration (AUC_{0-last}) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). Statistical moment theory was applied to calculate the mean residence time (MRT) for IVM as follows:

$$MRT = \frac{AUMC_{0-last}}{AUC_{0-last}}$$

where AUC is as defined previously, and AUMC is the area under the curve of the product of time and drug concentration versus time from zero to infinity (Gibaldi and Perrier, 1982). Mean pharmacokinetic parameters are reported as mean ± S.E.M. Mean parameters obtained after the administrations of the different formulations were statistically compared by analysis of variance (ANOVA). A non-parametric test (Kruskal–Wallis test) was used where significant differences among standard deviations were observed. A value of $P < 0.05$ was considered significant.

4. Results

The methodology used to quantify IVM in plasma was validated following well-established analytical standards. The linear regression lines showed correlation coefficients ≥0.998. The mean recoveries of IVM from plasma were in a range between 74 and 81%. The inter-assay precision of the analytical procedures obtained after HPLC analysis of IVM spiked standards (1 and 40 ng/ml) on different working days, showed a CV < 6%. The limit of quantification was established at 0.05 ng/ml. There were no statistical differences in the IVM concentration found in the commercial IVM-LA preparations assayed in the current trial. The equivalent IVM concentration measured in the 3.15% preparations accounts for the similar values of the plasma AUC obtained after the treatment with both formulations given at 630 μg/kg.

IVM was measured in plasma up to 40 (Group A), 50 days (Group B) and 90 days post-treatment (Groups C and D). The C_{max} and AUC values obtained after the administration of the IVM 1% preparation given at 630 μg/kg were significantly higher compared to those obtained after the treatment with the same formulation at 200 μg/kg. However, the mean MRT value was similar for both groups. The IVM plasma concentration profiles and the main pharmacokinetic parameters obtained after the treatment with the 1% classic preparation given at the different dose rates are shown in Fig. 1.

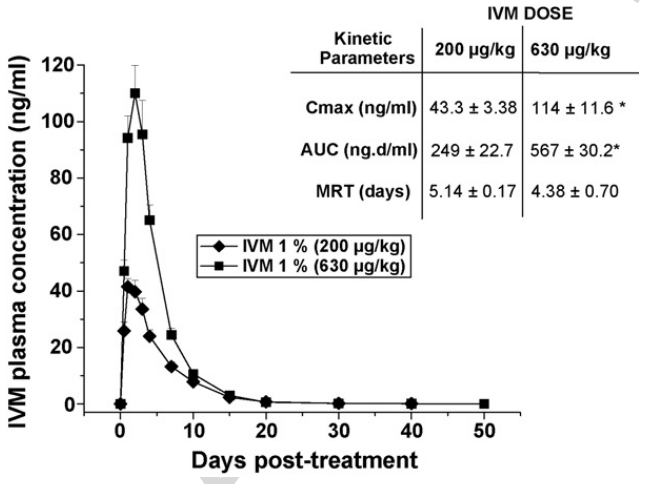

Fig. 1. Comparative mean ivermectin (IVM) plasma concentrations ($n = 7$) obtained after the subcutaneous administration of the classic IVM (1%) formulation given at 200 and 630 μg/kg to cattle. The insert shows the comparison of the main pharmacokinetic parameters. *Values are statistically different at $P < 0.05$. (C_{max}) peak plasma concentration; (AUC) area under the concentration–time curve from time zero to the last time with a measurable concentration; (MRT) mean residence time.

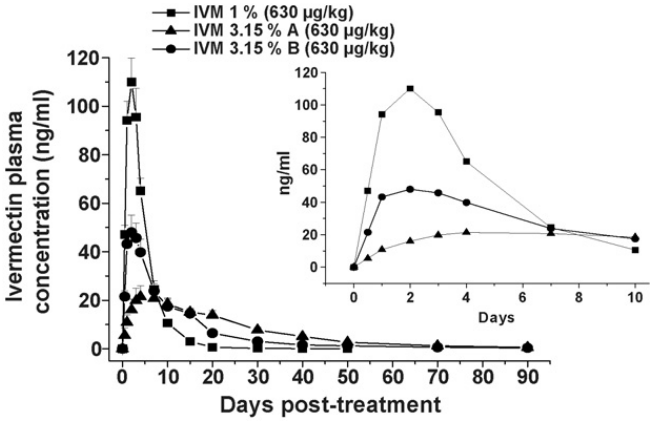

Fig. 2. Mean ivermectin (IVM) plasma concentration ($n = 7$) obtained after its subcutaneous administration as the classic IVM (1%) formulation given at 630 μg/kg and as two different long-acting (3.15%) formulations (630 μg/kg) to cattle. The insert shows the comparative absorption patterns expressed as the plasma concentrations measured during the first 10 days after the administration of the different IVM preparations.

The detection of IVM in plasma was extended after the administration of the 3.15% formulations compared to the 1% preparation administered at equivalent dose rates (630 μg/kg). The IVM plasma concentrations obtained after the administration of the 1 and 3.15% preparations are shown in Fig. 2. The main plasma pharmacokinetic parameters obtained for IVM after the treatment with the 1 and 3.15% formulations are summarized in Table 1.

Marked differences on the absorption kinetics were observed between the two 3.15% long-action preparations under evaluation. The peak plasma concentration was higher after the administration of the IVM 3.15% B compared to the IVM 3.15% A preparation. Extended absorption half-life and longer MRT values were observed after treatment with formulation A. The IVM plasma concentrations measured between 30 and 90 days post-treatment with both 3.15% preparations are compared in Fig. 3. The estimation of the percentage of the total drug availability (AUC) achieved at 15 days post-treatment was useful to assess the comparative pattern of IVM absorption among the assayed formulations. This comparison is shown in Fig. 4.

5. Discussion

The main therapeutic features of the avermectin and milbemycin endectocides are their high efficacy against endo and ectoparasites and the long persistence of their antiparasitic activity (McKellar and Benchaoui, 1996). Recognizing the need for extended duration of parasite control with a single injectable treatment in cattle,

Table 1
Comparative mean (±S.E.M.) ($n = 7$) kinetic parameters for ivermectin (IVM) obtained after its subcutaneous administration as the classic (1%) formulation given at 630 μg/kg and as two different long-acting (LA) (3.15%) formulations (630 μg/kg) to cattle

Kinetic parameters	IVM classic 1% formulation	IVM-LA 3.15% Formulation A	IVM-LA 3.15% Formulation B
$T_{1/2\ ab}$ (days)	$0.50 \pm 0.07^{(a)}$	$2.99 \pm 0.67^{(b)}$	$0.65 \pm 0.03^{(a)}$
T_{max} (days)	$2.29 \pm 0.18^{(a)}$	$9.14 \pm 2.87^{(b)}$	$2.14 \pm 0.14^{(a)}$
C_{max} (ng/ml)	$114 \pm 11.6^{(a)}$	$26.0 \pm 3.55^{(b)}$	$50.6 \pm 6.49^{(c)}$
$T_{1/2\ dist}$ (days)	$2.28 \pm 0.16^{(a)}$	$6.71 \pm 1.34^{(b)}$	$4.14 \pm 0.71^{(b)}$
AUC_{0-t} (ng d/ml)	$567 \pm 30.1^{(a)}$	$600 \pm 40.9^{(a)}$	$558 \pm 46.2^{(a)}$
$T_{1/2\ el}$ (days)	$8.26 \pm 0.50^{(a)}$	$16.5 \pm 0.70^{(b)}$	$11.4 \pm 1.78^{(a)}$
MRT (days)	$7.29 \pm 1.17^{(a)}$	$21.7 \pm 2.13^{(b)}$	$13.9 \pm 1.70^{(c)}$
Cl_B/F (l kg/d)	$1.13 \pm 0.07^{(a)}$	$1.05 \pm 0.06^{(a)}$	$1.15 \pm 0.09^{(a)}$

Within a row, mean kinetic parameters lacking a common superscript letter are significantly different at $P < 0.05$.
($T_{1/2\ ab}$) absorption half-life; (T_{max}) time to peak plasma concentration; (C_{max}) peak plasma concentration; ($T_{1/2\ dist}$) distribution half-life; ($AUC_{(0-last)}$) area under the concentration–time curve from time zero to the last time with a measurable concentration; ($T_{1/2\ el}$) elimination half-life; (MRT) mean residence time; (Cl_B/F) total body clearance, which represents its true value divided by the bioavailability (F).

Merial developed the first 3.15% LA IVM formulation (here referred as the IVM-LA Formulation A). Since then, several other IVM formulations have been introduced in the pharmaceutical market persuading a similar therapeutic goal. The strategic use of these novel IVM 3.15% long-acting formulations is oriented to extend the period of drug protection and to reduce the labour costs of farmers. The improved degree of parasite control results in enhanced animal health and decreased pasture contamination.

Duration of the antiparasitic activity could be associated to the physico-chemical properties of drug molecules, their uptake and release from tissues and the pharmaceutical technology applied to optimize their systemic availability. The dose rate increment of the IVM 1% formulation from 200 to 630 μg/kg assayed in the current experiment did not prolong the persistence of IVM plasma concentrations. The dose-dependent pharmacokinetic parameters, such as C_{max} and AUC were significantly greater after the treatment of the 1% preparation at 630 μg/kg compared to the same formulation administered at 200 μg/kg (Fig. 1). However, as IVM follows a first-order kinetic process, a similar time was needed to eliminate the drug after the administration of the classic propylene glycol/glycerol formal 60:40 IVM 1% formulation injected at 200 and

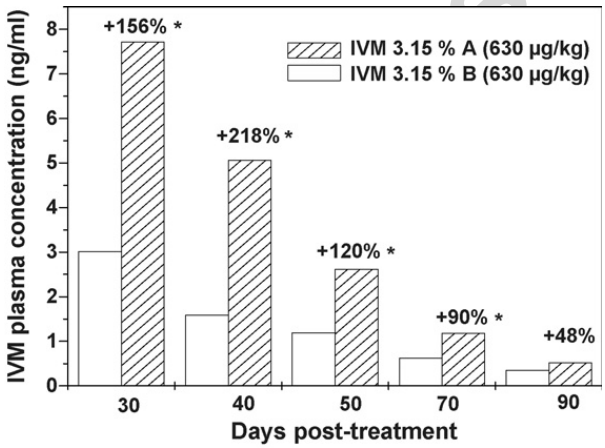

Fig. 3. Comparison of the mean ivermectin (IVM) plasma concentrations ($n = 7$) attained between 30 and 90 days post-treatment after the administration of two different long-acting (3.15%) formulations (630 μg/kg) to cattle. Percentage values indicate the differences on IVM concentrations observed between the two long-acting formulations. *Values are statistically different at $P < 0.05$.

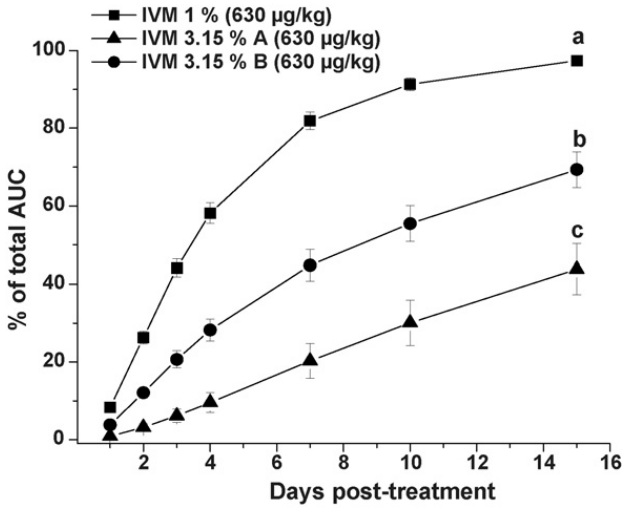

Fig. 4. Assessment of the ivermectin (IVM) absorption process after the subcutaneous administration of different formulations to cattle. The values indicate the percentage of total area under the concentration–time curve (AUC) obtained up to day 15 post-administration of the pioneer classic IVM (1%) formulation and the two long-acting (3.15%) preparations (Formulations A and B) to cattle at the same dose rate (630 μg/kg). The lack of a common superscript letter indicates that the mean percentage values obtained at day 15 days post-treatment are significantly different at $P < 0.05$.

630 μg/kg. Therefore, there was no difference in the time of residence of the drug in the bloodstream (MRT) after the IVM treatment at both dose rate levels. Plasma concentrations were equivalent for both treatments after 15 days post-administration of the 1% formulation (Fig. 1).

The rate of absorption from the subcutaneous space appears to be the rate-limiting step in the disposition of IVM after its subcutaneous administration. The vehicle in which the endectocide molecules are formulated plays a relevant role in their pharmacokinetics (Lo et al., 1985; Lanusse et al., 1997; Lifschitz et al., 1999). The original preparation of IVM 1% contains propylene glycol/glycerol formal 60:40. The innovation introduced to the novel long-acting 3.15% formulations favours a slow absorption from the subcutaneous site and prolongs the persistence of IVM concentrations in the bloodstream, which extend its persistent activity against nematodes. In the current trial, a similar total IVM plasma availability expressed as AUC was obtained after the treatments with the 1 and 3.15% IVM formulations administered at the same high dose (630 μg/kg). However, after the administration of the IVM 3.15% Formulation A, the absorption half-life was 5.82-fold longer and the T_{max} was achieved 3.99-fold later compared to the treatment with the IVM 1%. A significantly shorter residence time in the bloodstream (MRT value) was obtained for IVM 1% given at 630 μg/kg (Group B) compared to those observed after the treatments with both IVM long-acting preparations, which confirms the pharmacokinetic advantage achieved through the modification of the vehicle composition introduced to the LA formulations.

Several generic formulations of IVM have been introduced into the pharmaceutical market in different regions of the world after the expiration of the original patent of the first approved (innovator) IVM 1% formulation (Ivomec®, Merial). Something similar has recently occurred with these novel 3.15% LA preparations, where different generic preparations were introduced into the market in different cattle production areas of the world, shortly after the Ivomec Gold® product developed by Merial. The kinetic behaviour of two different IVM 3.15% preparations available in the veterinary market was compared on the current trial. The pattern of absorption from the subcutaneous site of injection was different between the long-acting IVM formulations under study. This was clearly reflected in the percentage of total drug availability (expressed as AUC) obtained at 15 days post-administration of both 3.15% preparations. The percentage of total AUC was significantly lower after the administration of 3.15% preparation A compared to that obtained after the treatment with the 3.15% B formulation (Fig. 4), which indicates that the process of absorption from the subcutaneous site of injection may differ between both LA formulations. The practical/clinical implications of the pharmacokinetic differences observed between formulations under study may require further evaluation. The IVM 3.15% A formulation showed a more extended absorption process than IVM 3.15% B preparation, which accounted for a longer persistence of detectable IVM plasma concentrations (Fig. 3). The differences observed on the pharmacokinetic behaviour between both 3.15% preparations may affect the efficacy and persistence of their antiparasitic activity. The direct relationship between time of persistence of drug concentrations of endectocide molecules and extended efficacy against endo and ectoparasites has been demonstrated in different trials (Wicks et al., 1993; Bridi et al., 2001; Rehbein et al., 2002). Therefore, the differences observed in the current trial in the plasma concentration profiles between the assayed IVM 3.15% formulations may affect the possibility to switch between both formulations without any observed changes on clinical response. The manufacturer of the IVM 3.15% formulation A declares that the viscosity of the preparation may change according to the mixing speed (tixotropic behaviour). A different vehicle composition and the lack of tixotropic behaviour of the IVM 3.15% B may explain the differential pharmacokinetic behaviour between the both long-acting IVM preparations.

Long-acting endectocide formulations are currently used for the advantage of persistent anthelmintic efficacy in strategic programs for controlling nematodes and ectoparasites. A 56 days of protection period against psoroptic mange was reported in cattle treated with IVM 3.15% long-acting preparation (Bridi et al., 2001). A new long-acting formulation of moxidectin protect against endo and ectoparasites between 90 and 150 days post-administration (Ranjan et al., 2003; Cleale et al., 2004; Yazwinski et al., 2006). However, the disadvantages of anthelmintic persistence may be the extended withdrawal period, a reduction in the acquisition of natural immunity and the more rapid selection of resistant parasites by extending their exposure to sub-therapeutic levels of anthelmintic (Yazwinski et al., 1994). The concentration of an endectocide molecule required at the target tissues to inhibit either the development of larval stages or the establishment of different internal and external parasites has not been determined (Lifschitz et al., 1999, 2000). In this perspective, it is not possible to establish the

possible advantage of an IVM formulation achieving either higher C_{max} or greater persistence. Significant differences among the peak plasma concentrations (C_{max} values) reached by the IVM 1% classic preparation and the long-acting formulations were observed (Fig. 2). Moreover, the C_{max} obtained after administration of the IVM 3.15% formulation B was almost two-fold higher compared to that measured after the treatment with the IVM 3.15% formulation A (Table 1).

It is clear the significance of the extended persistence of IVM plasma concentrations for optimal efficacy against ectoparasites in cattle. The duration of effective levels of IVM in the bloodstream is relevant in the treatment of tick infestation as these parasites may feed over a several days period (Jackson, 1989). Mites need between 14 and 17 days to be eliminated after treatment with the classic IVM formulation given at 200 μg/kg remaining infective by 9 days post-administration of the endectocide compound (Wright and Guillot, 1984). Despite some studies on nematode expulsion in sheep, there is no available information about the minimum therapeutic levels required to kill different endoparasites in ruminants. Expulsion kinetic studies suggested that IVM effects on motility are relevant to parasites, such as *Haemonchus contortus* while the action on the pharyngeal pumping may be more important for *Ostertagia circumcincta* (Gill and Lacey, 1998). *In vitro* studies demonstrated that the IVM concentration needed to affect the motility of adult *Trichostrongylus colubriformis* was 10-fold higher compared to that required for adult *H. contortus* (Geary et al., 1993). The IVM concentrations measured in plasma after 20 days post-administration of the IVM 3.15% formulation A were between 1.90 and 3.18-fold higher than those obtained after treatment with the IVM 3.15% B preparation. The parasitological implications of the observed differences in peak plasma concentrations (C_{max} values) and in the IVM concentration levels measured from day 20, and afterwards until day 90 post-treatment, between the different preparations assayed need to be elucidated. However, it is clear that the improved drug profiles obtained for formulation A at critical times post-treatment may impact on a differential persistence of activity against dose-limiting parasites.

As an overall concept, the greater the persistence of a drug the greater must be its efficacy against resistant worms if it is not to select more strongly for resistance (Dobson et al., 1996). When the initial frequency of the allele for resistance is high, the most dangerous treatment seems to be that which kill all the susceptible homozygote but none of the other genotypes (Smith et al., 1999). The selective pressure in South America, as in many other regions of the world, is high due to numerous anthelmintic treatments per year, absence of refugia and intense immigration of new animals with possibly resistant helminthes (Mejia et al., 2003). Therefore, the differences on IVM plasma concentration profiles obtained between 20 and 90 days post-administration of both 3.15% preparations (Figs. 2 and 3) may be sufficient to reach a 100% of efficacy against the susceptible strains, but a differential pattern of activity may be observed as resistance develops. Since the discovery and development of new molecules are long and expensive processes and that non-chemical approaches have had a partial effect (Besier, 2006), the improvement of pharmaceutical preparations and delivery systems for existing drugs has been proposed as a reasonable alternative to achieve sustainable parasite control in livestock. The characterization of the absorption patterns and kinetic behaviour obtained after injection of these novel long-acting formulations used at three times the therapeutic dose recommended for the classic IVM preparation in cattle is a further contribution to the field.

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