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Pharmacokinetic evaluation of four ivermectin generic formulations in calves

A. Lifschitz [a,b], J. Sallovitz [a], F. Imperiale [a], A. Pis [a], J. Jauregui Lorda [c], C. Lanusse [a,b,*]

[a] *Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro, Campus Universitario, 7000 Tandil, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
[c] *Pfizer SRL, Buenos Aires, Argentina*

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Abstract

The plasma concentration profiles of four randomly chosen ivermectin (IVM) generic formulations (IVM G1–G4) were compared after their subcutaneous (SC) administration to healthy calves. The disposition of other avermectin-type endectocide compounds, doramectin (DRM) and abamectin (ABM), was also assessed in the same pharmacokinetic trial. Forty-two parasite-free Aberdeen Angus male calves were randomly allocated into six treatment groups. Animals in each group ($n = 7$) received SC treatment (200 μg/kg) with one of the commercially available endectocide formulation used in the trial. Blood samples were taken into heparinised vacutainer tubes from the jugular vein prior to and up to 35 days post-treatment. The recovered plasma was analysed by HPLC with fluorescence detection. Large kinetic differences were observed among the DRM, ABM and IVM formulations under evaluation. The DRM plasma concentration profiles were higher than those measured for ABM and all the IVM generic formulations. The higher and sustained plasma concentrations of DRM accounted for greater area under concentration–time curve (AUC) and longer mean residence time (MRT) values compared to those obtained for both ABM and the IVM generic preparations. The pattern of IVM absorption from the site of subcutaneous administration showed differences among the generic formulations under evaluation. The IVM G2 preparation showed higher peak plasma concentration and AUC values ($P < 0.05$) compared to those obtained after the administration of the IVM G1 formulation. Longer ($P < 0.05$) MRT values were obtained after the administration of the IVM G3 compared to other IVM generic preparations. The kinetic behaviour of ABM did not show significant differences with that described for most of the IVM

* Corresponding author. Tel.: +54-2293-447108; fax: +54-2293-422357.
E-mail address: clanusse@vet.unicen.edu.ar (C. Lanusse).

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formulations. This study demonstrates that major differences on drug kinetic behaviour may be observed when using different endectocide injectable formulations in cattle.

Keywords: Pharmacokinetics; Ivermectin generic formulations; Cattle

1. Introduction

The macrocyclic lactones are broad-spectrum antiparasitic drugs, extensively used in veterinary medicine. They are known as "endectocide" compounds based on their unique activity against endo-and ectoparasites (Shoop et al., 1995). The macrocyclic lactons include two chemical families: avermectins (abamectin, ivermectin, doramectin, eprinomectin and selamectin) and milbemycins (nemadectin, moxidectin, D-milbemycin, etc.), which are commercially available to use in livestock and pet 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 the absorption process and the resultant drug concentration profiles in the bloodstream (Lo et al., 1985; Wicks et al., 1993) and at the sites of target parasite location (Lifschitz et al., 2000). The level and duration of endectocide drug concentrations in contact with different stages of target endo- and/or ectoparasites are relevant for the efficacy and persistence of their antiparasitic activity (Lanusse and Prichard, 1993). The oily based vehicle of the available commercial formulation of doramectin contributes to the higher concentration profiles and extended residence in plasma (Lanusse et al., 1997; Toutain et al., 1997) and target tissues (Lifschitz et al., 2000) compared to IVM following their subcutaneous administration to cattle. Also it has been shown that the so-called long-acting IVM formulations are essentially oil-based preparations that account for a slow absorption process from the subcutaneous space and a long persistence of concentrations in the bloodstream and tissues of parasite location (Lifschitz et al., 1999).

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 formulation (Ivomec®, Merial). Most of the available IVM generic preparations (some of them are now very well established in the pharmaceutical market), contain basically the same vehicle composition used in the innovator IVM formulation, but there is no available information on the comparative kinetic behaviour of generic preparations in a standardised pharmacokinetic trial. The work reported here evaluated the comparative plasma concentration profiles of four randomly chosen IVM generic formulations (commercially available as 1% injectable solutions) after their subcutaneous (SC) administration to healthy calves. The plasma kinetics of other avermectin-type endectocides worldwide used, doramectin (DRM) and abamectin (ABM), was also characterised in the same trial.

2. Materials and methods

2.1. Experimental animals, treatments and sampling

Forty-two parasite-free, healthy 8-month-old Aberdeen Angus male calves 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 weighted (160 ± 20 kg) and randomly allocated into six treatment groups of seven calves each. Animals in each group received one of the following SC treatments (200 μg/kg) with a commercially available endectocide formulation (10 mg/ml injectable solutions) for use in cattle: DRM (Dectomax®, Pfizer, Inc.), abamectin (ABM) (Duotin®, Merial) and four different IVM generic formulations (G1–G4). The labelling of the IVM G1–G4 preparations (1% injectable solutions) used in the current trial does not contain indication of any difference with the vehicle composition of the first approved pioneer IVM product. Blood samples were taken into heparinised vacutainer tubes prior to and at 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30 and 35 days after treatments. Blood samples were centrifuged at 3000 rpm for 20 min and the recovered plasma was kept in labelled vials at $-20\,^{\circ}$C until HPLC analysis.

2.2. Analytical procedures

2.2.1. Chemical extraction and derivatisation

The extractions of IVM, DRM and ABM from spiked and experimental plasma samples were carried out following the technique first described by Alvinerie et al. (1993) and slightly modified by Lifschitz et al. (1999). Basically, 1 ml-aliquot of plasma sample was combined with 10 ng of the internal standard compound (ABM for the analysis of the IVM and DRM experimental samples and IVM for the ABM experimental assay), and then mixed with 1 ml of acetonitrile–water (4:1). After mixing for 20 min, the solvent–sample mixture was centrifuged at $2000 \times g$ during 15 min. The supernatant was manually transferred into a tube that was then placed on the appropriate rack of a 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 deionised water. The cartridge was flushed with 1 ml of water and 1 ml of water/methanol (4:1). The analytes 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). Derivatisation was initiated adding 150 μl of trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) 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, ABM and DRM 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 a acetic acid 0.2% in water/methanol/acetonitrile (3.8/40/56.2 for IVM, 4.2/40/55.8 for ABM and 5/40/55 for DRM, respectively) mobile phase at a flow rate of 1.5 ml/min at 30 °C. The analytes were detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). IVM, ABM and DRM concentrations were determined by the internal standard method using the Class LC 10 Software version 1.2 (Shimadzu Corporation, Kyoto, Japan) on an IBM compatible AT computer. The peak area ratios were considered to calculate the IVM, ABM and DRM 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 each endectocide molecule was performed before starting analysis of the experimental samples obtained during the pharmacokinetic trial. Calibration curves in the range between 0.1–5 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, ABM and DRM concentrations/detector responses relationship. Percentages of IVM, ABM and DRM recoveries from plasma were obtained in the range between 0.1 and 40 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, ABM and DRM concentrations (2 and 20 ng/ml) on different working days. The limits of drug detection and quantification were established. The mean baseline noise at the IVM, ABM and DRM peak retention times 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.

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} were determined applying the method of residuals to the first portion of the plasma concentration–time curve. The areas under the concentration–time curves (AUC) were calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). Statistical moment theory was applied to calculate the mean residence time (MRT) for IVM, ABM and DRM as follows:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

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

4. Results

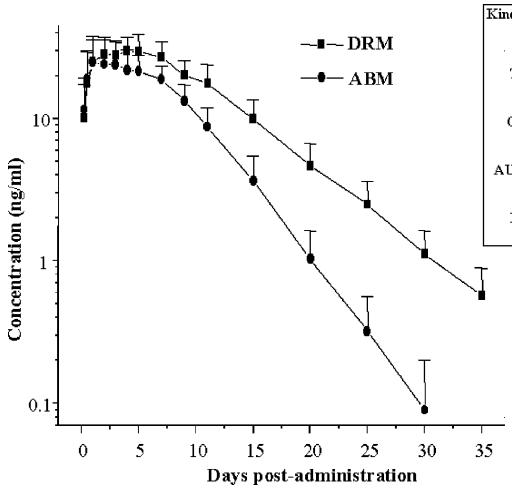
The analytical procedures including chemical extraction, derivatisation and HPLC analysis for IVM, DRM and ABM were adequately validated. The linear regression lines for the different molecules under study in the range between 0.10–5 and 5–100 ng/ml showed correlation coefficients greater than 0.998. Mean drug recoveries from plasma were 80.4% (IVM), 78.1% (ABM) and 75.0% (DRM). The inter-assay precision of the extraction and chromatographic procedures for IVM, DRM and ABM showed coefficients of variation between 2.38 and 3.00%. The validation parameters for each endectocide compound are summarised in Table 1.

The parent endectocide molecules were detected in plasma between 6 h and either 30 (ABM and the IVM G4 formulation) or 35 (DRM and the IVM generic preparations G1, G2 and G3) days post-treatment. The DRM plasma concentration profiles were higher than those measured for ABM and all the IVM generic formulations. The mean plasma concentrations and pharmacokinetic parameters obtained for DRM and ABM after their SC administrations to cattle are shown in Fig. 1. The sustained higher plasma concentrations of DRM accounted for greater AUC, and longer MRT values compared to those obtained for ABM and the IVM generic formulations. The IVM plasma concentration profiles obtained after the administration of the IVM G1–G4 formulations are compared in Fig. 2. The pattern of IVM absorption from the site of SC administration showed differences among the generic formulations under evaluation. Consequently, large kinetic differences were observed among the IVM generic formulations investigated in this trial. The IVM G2 preparation showed higher C_{max} and AUC values ($P < 0.05$) compared to those obtained

Table 1
Validation of the analytical methodology used to measure ivermectin (IVM), doramectin (DRM) and abamectin (ABM) concentrations in bovine plasma

	IVM	DRM	ABM
Limit of quantification (ng/ml)	0.05	0.05	0.04
Recovery (%)	80.4 (8.3)	75.0 (6.5)	78.1 (5.3)
Linearity (*r*)	0.999	0.999	0.998
Coefficient of variation (%)	2.38	2.63	3.00

Values presented in this table were obtained as defined in the analytical methodology section. Values in brackets represent the coefficient of variation for the recovery assays. *r*: correlation coefficients.



Kinetic parameters	DRM	ABM
Tmax (days)	3.86 ± 1.77 (*)	2.14 ± 1.22
Cmax (ng/ml)	32.0 ± 9.34	25.4 ± 12.5
$AUC_{0\text{-}35\,d}$ (ng d/ml)	391 ± 110 (*)	244 ± 83.3
MRT (days)	9.19 ± 1.29 (*)	6.59 ± 0.65

Fig. 1. Comparative mean (±S.D.) ($n = 7$) plasma concentrations of doramectin (DRM) and abamectin (ABM) obtained after their subcutaneous administration to calves (200 μg/kg). Some pharmacokinetic variables obtained after both drug treatments are shown in the inserted table. T_{max}: time to peak plasma concentration. C_{max}: peak plasma concentration. $AUC_{0-35\,days}$: area under the concentration vs. time curve between drug administration and 35 days post-treatment. MRT: mean residence time. (*) Mean kinetic parameters for DRM are significantly different to those obtained for ABM at $P < 0.05$.

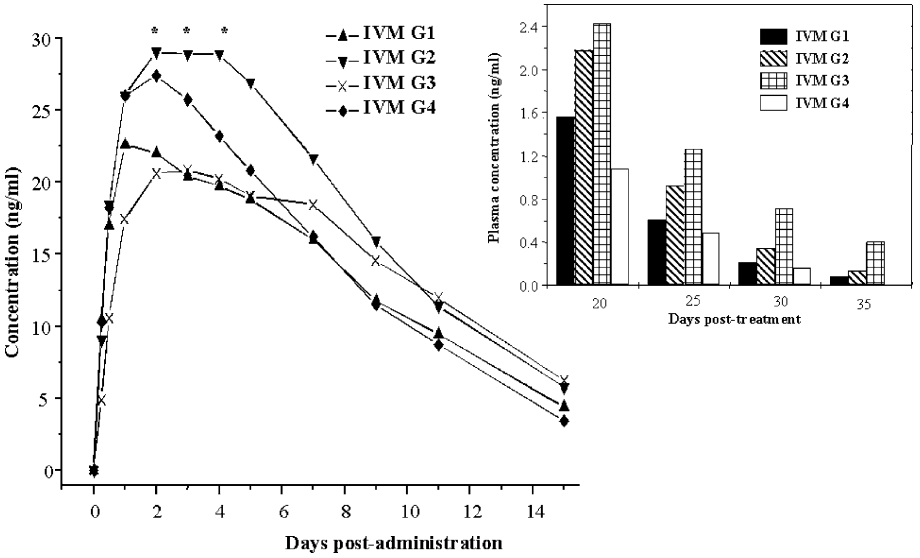


Fig. 2. Comparative mean plasma concentration profiles of ivermectin (IVM) obtained during the first 15 days after the subcutaneous administration of four generic formulations (IVM G1–G4) to calves (200 μg/kg). The comparison of the IVM concentrations measured at 20, 25, 30 and 35 days post-administration of the different generic formulations is shown in the inserted graph. (*) Concentration values for the IVM G2 formulation are statistically different from those obtained for IVM G1 and G3 ($P < 0.05$).

Table 2
Comparative mean (±S.D.) ($n = 7$) kinetic parameters obtained for the four generic ivermectin (IVM) (G1–G4) formulations after their subcutaneous administration (200 μg/kg) to calves

Kinetic parameters	IVM G1	IVM G2	IVM G3	IVM G4
$T_{(1/2)ab}$ (days)	0.67 ± 0.26 a	1.01 ± 0.52 a	1.83 ± 0.56 b	0.93 ± 0.42 a
T_{max} (days)	1.14 ± 0.38 a	2.57± 1.40 bc	4.29 ± 2.06 b	1.86 ± 0.69 ac
C_{max} (ng/ml)	23.6 ± 4.69 a	32.7 ± 4.35 b	22.0 ± 6.86 a	28.4 ± 0.10 ab
$AUC_{0–35\,days}$ (ng day/ml)	231 ± 38.9 a	308 ± 41.8 b	262 ± 67.1 ab	242 ± 40.1 ab
MRT (days)	7.31 ± 1.14 ab	7.29 ± 1.17 ab	9.86 ± 4.49 a	6.60 ± 1.35 b

Within a row, mean kinetic parameters lacking a common letter are significantly different at $P < 0.05$. $T_{(1/2)ab}$: IVM absorption half-life. T_{max}: time to IVM peak plasma concentration. C_{max}: IVM peak plasma concentration. $AUC_{0–35\,days}$: area under the concentration vs. time curve between drug administration and 35 days post-treatment. MRT: mean IVM residence time in the bloodstream.

after the administration of the IVM G1 formulation. Longer MRT values were obtained after the administration of the IVM G3 compared to other IVM generic preparations. The mean pharmacokinetic parameters for the different IVM generic formulations obtained after their SC administration are summarised in Table 2. The cumulative percentages of time over the 35 days post-treatment period, during which the DRM, ABM and IVM plasma concentrations were above 1 ng/ml (a theoretically defined minimal effective concentration), are shown in Fig. 3.

5. Discussion

Large kinetic differences were observed after the administration of the four IVM generic formulations, DRM and ABM to parasite-free calves in the current trial under standardised experimental conditions. In agreement with previous results (Lanusse et al., 1997; Toutain et al., 1997; Lifschitz et al., 2000), significantly higher plasma profiles and extended residence times were observed for DRM compared to both ABM and IVM administered as four generic formulations after SC administration. The higher plasma concentration profiles measured for DRM accounted for the significantly higher plasma availability (measured as AUC) and longer MRT obtained for DRM compared to ABM and the IVM generic formulations assayed. DRM AUC values were between 27 and 69% greater than those obtained for ABM and IVM. The sustained DRM plasma concentrations measured after its SC administration resulted in longer MRT (between 26 and 40%) compared to those obtained after the administration of ABM and the IVM G1, G3 and G4 formulations.

The behaviour of IVM given injectable to cattle as four different generic (1%) formulations showed marked kinetic differences. The pattern of absorption from the SC site of injection was different among the IVM formulations under study. There were statistically significant differences in C_{max}, T_{max} and AUC among the IVM formulations. The AUC values obtained for IVM after the administration of the G2 generic formulation was 33% higher than that obtained after administration of the G1 formulation. The largest differences on peak plasma concentrations (C_{max}) were obtained between the IVM G2 (49% higher) and G3 generic formulations. There were marked differences on the absorption processes

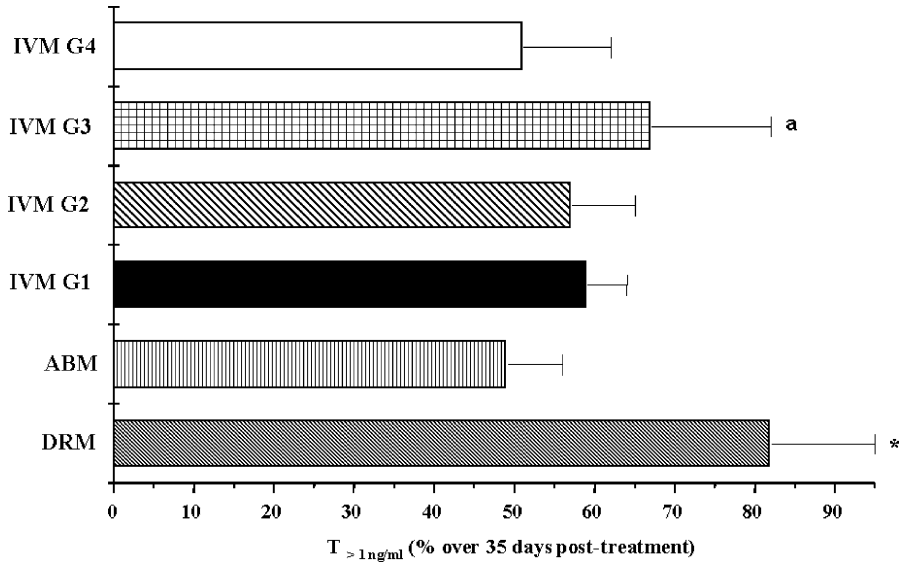


Fig. 3. Cumulative percentage of time over the 35 days post-treatment period during which doramectin (DRM), abamectin (ABM) and ivermectin (IVM) plasma concentrations were above the critical 1 ng/ml value ($T_{>1\,ng/ml}$). This concentration value may be considered as an indirect indicator of the comparative persistence of antiparasitic activity (see Lifschitz et al., 2000) among the endectocide formulations assayed here. (*) The value of $T_{>1\,ng/ml}$ obtained for DRM is significantly higher than those obtained for ABM and all the generic IVM formulations at $P < 0.05$. (a) The mean value for the IVM G3 formulation is significantly different from those obtained for ABM and the IVM G4 formulation at $P < 0.05$.

of the generic IVM formulations. The IVM G3 formulation showed a slower absorption from the SC tissue with a significantly delayed T_{max} and longer absorption half-life compared to the other IVM generic formulations. The $T_{(1/2)ab}$ of IVM after administration of the G3 formulation was 173% longer with a T_{max} value (273%) delayed compared to those obtained for IVM G1. These results reflect a large difference in the absorption process among formulations, which may be due to differences on the composition/quality of their vehicle components. ABM was included in this study to compare its pharmacokinetic behaviour with that of other avermectin-type compounds (DRM and IVM) commercially available in the veterinary pharmaceutical market for injectable use in cattle. The kinetic behaviour of ABM did not show significant differences with that described for most of the IVM formulations under evaluation.

The statistically significant differences observed among the kinetic parameters reflecting the rate and extent of absorption for the different IVM formulations (G1–G4), may support the existence of differences in these pharmaceutical preparations. The composition and quality of the vehicles and/or excipients used in the pharmacotechnical elaboration of IVM formulations may be relevant to its pharmacokinetic behaviour. The practical/clinical implications of the pharmacokinetic differences observed among endectocide molecules and formulations may require further evaluation. However, the differences observed on the systemic availability and drug disposition kinetics among generic formulations may affect

the efficacy and persistence of their antiparasitic activity. The direct relationship between time of persistence of drug concentrations and extended efficacy against endo- and ectoparasites has been demonstrated in different trials. Slight differences in formulation account for changes to the plasma kinetics and exposure of target parasites to active drug concentrations. This has been confirmed by the extended residence times of IVM in plasma and target tissues and the prolonged persistence of its anthelmintic activity, following the administration of a novel oil-based (1%) formulation to cattle, compared to the standard innovator preparation (Lifschitz et al., 1999). Longer protective efficacy against *Psoroptes ovis* in cattle has been demonstrated using a long-acting (1%) IVM preparation compared to other commercially available endectocides formulations (Bridi et al., 2001; Rehbein et al., 2002). The observed influence of differential plasma concentration profiles on the activity of IVM and DRM against *Cooperia oncophora* (Goudie et al., 1993; Wicks et al., 1993), are also indicative of the close relationship between pharmacokinetics and endectocide activity.

Switchability refers to the possibility to switch between equivalent formulations without any observed changes on clinical response (Martinez et al., 2002). The differences observed in the current trial in the plasma concentration profiles among the IVM generic formulations may not affect the efficacy/persistence of the antiparasitic activity against the most susceptible strains of target endo- and ectoparasites, but differences in the activity against the dose-limiting parasites are likely to occur. Thus, the switchability may not be assured among the IVM generic formulations investigated in the current trial. The larger AUC obtained for G2 compared to G1 formulation would be consistent with an increased time of exposure to therapeutic concentrations. This is particularly important if we considered that IVM cattle nematodes have already being reported in different parts of the world. In fact, a lack of IVM efficacy against *Cooperia* spp., the main IVM dose-limiting nematode in cattle, has been recently reported (Anziani et al., 2001; Fiel et al., 2001).

The prolonged persistence of DRM, ABM and IVM contributes to the achievement of drug concentrations in target tissues, where the sustained attainment of drug levels toxic to the parasites is critical for the resultant efficacy. There is a high correlation between IVM and DRM concentrations measured in plasma with those achieved at the tissues where parasites are located (Lifschitz et al., 2000). Endectocide molecules paralyse the pharyngeal pumping activity and also have paralysing effects on the somatic musculature (Geary et al., 1993), but the in vivo minimal concentrations required to achieve these effects remain unknown. Based on pharmacological in vitro assays (Geary et al., 1993; Gill and Lacey, 1998), the theoretical assumption that plasma concentrations above 1 ng/ml (Lifschitz et al., 2000) or 2 ng/ml (Gayrard et al., 1999) would be indicative of the minimal drug level required for optimal antiparasitic activity against some endoparasites may be adopted. Thus, the cumulative percentage of time over the 35 days post-treatment period where the drug concentrations exceed the minimal effective concentration ($T_{>1\,\mathrm{ng/ml}}$) (Mouton et al., 2002) was estimated in the current trial. This parameter was calculated as an indirect indicator of the persistence of antiparasitic activity of the assayed endectocide injectable formulations against nematode parasites. While ABM and IVM plasma concentrations were above 1 ng/ml between 49 and 67% of the 35 days period, DRM profiles remained above that level during 82% of this post-administration time period. Among the generic formulations, the $T_{>1\,\mathrm{ng/ml}}$ of IVM G3 was significantly longer compared to the value obtained for the IVM G4 preparation (Fig. 3).

In conclusion, it is clear from the outcome of this study that major differences in drug kinetic behaviour, may be observed when using different endectocide injectable formulations in cattle. These differences may be reflected in the efficacy and persistence of their antipasitic activity. The study reported here was not designed to identify the "best" or the "worst" IVM generic formulation available in the veterinary market. The rationale behind this trial was to build up "conscience" on the relevance that the switchability among generic formulations of endectocides might have on parasite control. Considering the precautions that should be taken to avoid/delay the development of IVM resistance in cattle nematode parasites, a standardised quality control of generic endectocide formulations may greatly contribute to optimised drug use.

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