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Comparative depletion of ivermectin and moxidectin milk residues in dairy sheep after oral and subcutaneous administration

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Ivermectin (IVM) and moxidectin (MXD) are broad-spectrum endectocides belonging to the avermectin/milbemycin class of antiparasitic drugs not approved for use in dairy sheep. However, these compounds are widely used extra-label to control endo- and ecto-parasites in lactating dairy sheep. Effects of the route of administration on the pattern of IVM and MXD excretion in milk were comparatively characterized in lactating dairy sheep. The relationship between the milk and plasma disposition kinetics after subcutaneous (s.c.) and oral administration at 200 μg/kg body weight was also evaluated. IVM and MXD concentration profiles were measured in milk and plasma using a specific HPLC-based methodology. IVM and MXD were extensively distributed from the bloodstream to the mammary gland and large quantities, particularly for MXD, were excreted in milk. Residual concentrations of IVM were recovered in milk up to 11 d (oral treatment) or 25 d (s.c. treatment) post treatment. However, high MXD concentrations were detected in milk between 1 h and 35 d after its oral and subcutaneous administration. MXD concentrations as high as 3·77 ng/ml (oral) and 30·3 ng/ml (s.c.) were measured in milk at day 35 post administration. A higher MXD excretion in milk, compared with that of IVM, was obtained for both administration routes. An extensive plasma to milk distribution pattern was observed, being the area under the concentration-time curve of MXD obtained in milk up to 14-fold higher than that measured in the bloodstream. The total fraction of the administered dose excreted in milk for MXD was significantly higher than that for IVM, which agrees with the well known higher MXD lipophilicity. The long persistence of milk residual concentrations of MXD and IVM in lactating dairy sheep should be seriously considered before their extra-label use is recommended.

Keywords: Endectocides, milk, plasma, kinetics.

Avermectins and milbemycins are structurally related antiparasitic drugs (macrocyclic lactones), which are obtained as fermentation products of soil-dwelling actinomycetes (*Streptomyces* spp.). Both groups are included under the term ''endectocides'' owing to their antiparasite activity against endo- and ectoparasites and they are used worldwide for parasite control in livestock (McKellar & Benchaoui, 1996). Plasma pharmacokinetics of ivermectin (IVM, an avermectin) and moxidectin (MXD, a milbemycin) have been extensively investigated in different animal species. Disposition kinetics of endectocides in livestock animals differ according to the animal species (Alvinerie & Galtier, 1997), animal breed (Sallovitz et al. 2002), dietary management and nutritional condition (Ali et al. 1996; Lifschitz et al. 1997), type of drug formulation (Lo et al. 1985) and route of administration (Alvinerie et al. 1998; Escudero et al. 1999), among many other factors.

IVM and MXD, as other avermectin and milbemycin-type compounds, are highly lipophilic. However, MXD differs structurally from IVM, which gives rise to some physicochemical and pharmacological differences between them. MXD is more lipophilic than IVM and its fat storage persists for an extended period after treatment in different animal species (Zulalian et al. 1994; Lifschitz et al. 2000b). Both endectocide compounds distribute extensively from plasma to different tissues, particularly those with the highest fat content (Lifschitz et al. 2000a, b),

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which determines the persistence of these compounds in the body for long periods (Zulalian et al. 1994).

In recent years, intensification of milk production in small ruminants has been associated with enhanced vulnerability to parasitic infections. Gastrointestinal parasites (Hoste & Chartier, 1998) and sarcoptic mange infections (Fthenakis et al. 2000) reduce milk yield in lactating dairy animals. Although different management strategies are used to prevent or minimize production losses, the use of antiparasitic drugs is still the main control measure against parasites in lactating dairy sheep. The use of strategic anthelmintic treatments during lactation is correlated with significant enhancement of milk yield in dairy sheep (Juste Jordán & García Pérez, 1991; Fthenakis et al. 2000) and cattle (Ploeger et al. 1989).

Patterns of excretion in milk for IVM (Toutain et al. 1988; Cerkvenik et al. 2002) and MXD (Alvinerie et al. 1996; Carceles et al. 2001) have been characterized in different species. Pour-on formulations (0·5%) of eprinomectin and MXD are the only preparations approved in some countries for use in lactating dairy cows with zero milk withdrawal time. Commercially available injectable and oral formulations of IVM and MXD are used in dairy animals, however, even though their administration to lactating animals has not been authorized. It is clear that in intensive production systems such as those used for dairy sheep, a possible unapproved use of these compounds to obtain the benefits of controlling endo- and ectoparasites should be considered. This use should be careful and compatible with the production of high quality milk and consumer safety. Knowledge of the pharmacological behaviour of these compounds that dictates their excretion in milk, is relevant to achieving this goal.

The work reported here was designed to assess the comparative pattern of IVM and MXD excretion in lactating dairy sheep, and to elucidate how route of administration might affect the relationship between plasma kinetics and depletion of milk residual concentrations for both compounds.

Materials and Methods

Experimental animals, treatments and sampling

Twenty Pampinta dairy ewes (crossbreed 3/4 Milchschaf and 1/4 Corriedale) weighing 50–86 kg were used. They were clinically healthy, free of parasites as indicated by faecal eggs examination, and in mid to late lactation. They were kept under field conditions, grazing on pasture and had free access to drinking water throughout the experiment. The health of the animals was closely monitored prior to and throughout the trial. Ewes were milked twice a day with a milking machine and milk production was measured prior to and throughout the trial. Daily milk production during the trial averaged 687 ± 222 ml.

Animals were allocated to four groups of five animals. Each animal in each group was treated with either IVM or MXD orally or subcutaneously (s.c.) immediately after milking. IVM was given by s.c. injection in the shoulder area (group A) as a 1% injectable solution (Ivomec, MSD AGVET Division of Merck & Co. Inc. Sao Paulo, Brazil, Lot PR 108/97) or oral drench (group B) as a 0·08% solution (Ivomec, MSD AGVET Division of Merck Frosst Canada Inc., Quebec, Canada, Lot HE 08020). MXD was given by s.c. injection in the shoulder area (group C) as a 1% injectable solution (Cydectin, Fort Dodge, Sanidad Animal S.A. Argentina, Lot 205) or oral drench (group D) as a 0·2% solution (Cydectin, Fort Dodge, Sanidad Animal S.A. Argentina, Lot 09). All formulations used were commercially available for sheep in Argentina and they were given at a dosage of 200 µg/kg body weight. Neither pain nor irritation was observed at the site of injection at any time after treatment. Blood samples were taken from the jugular vein into heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) prior to treatment, at 0·5, 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30 and 35 d post treatment. Milk samples were collected prior to treatment and at 1, 4, 8 and 12 h and at 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30 and 35 d post treatment. At each time point, an aliquot of 30 ml milk sample was collected by hand milking after discarding 30–50 ml and before the complete mechanical milking of each sheep. Blood samples were centrifuged at 2000 ***g*** for 20 min and the recovered plasma was transferred to vials. Milk and plasma samples were frozen at −20 °C until analysed.

Analytical procedures

Extraction procedures and chromatographic conditions to quantify IVM and MXD in fortified and experimental samples (milk and plasma) were carried out following modifications of the technique of Alvinerie et al. (1993). Detailed information on the chromatographic procedures, including the use of abamectin (ABM) as internal standard to quantify IVM and MXD in milk and plasma, and the extraction of both analytes from these biological matrices are described below.

Drug extraction and derivatization. Pure reference standards of IVM (97·5%, purity), ABM (97·4%, purity) and MXD (91·8%, purity) were used to validate the HPLC method. Standard solutions of IVM and MXD were prepared by successive dilutions in methanol from the parent stock solution (1 mg/ml) and stored at 4 °C. The fortified and experimental milk and plasma samples were added with 100 µl of ABM as internal standard (100 ng/ml). Acetonitrile (1 ml) and deionized water (0·25 ml) were added to each tube containing 1 ml of milk or plasma sample. After mixing thoroughly for 15 min, the batch of tubes containing the milk sample was put into an ultrasonic bath for 8 min. After that the batch of tubes (milk and plasma) was centrifuged at 2000 ***g*** for 20 min. Precipitates obtained from the milk samples with MXD were re-extracted as described above with 1 ml of

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acetonitrile. The supernatant was applied to a conditioned Supelclean LC 18 cartridge (Supelco, Bellefonte, PA, USA). After washing with 1 ml water followed by 1 ml water-methanol (4 : 1, v/v), the cartridges were dried off for 5 min and the sample was eluted with 1·5 ml methanol and collected. The eluate was evaporated to dryness under a gentle stream of nitrogen at 60 °C in a water bath, and the dry residue of the eluate was dissolved with 100 µl of *N*-methylimidazole (Sigma, St. Louis, MO, USA) solution in acetonitrile (1 : 1, v/v) and 150 µl of trifluoracetic anhydride (Sigma, St. Louis, MO, USA) solution in acetonitrile (1 : 2, v/v) (De Montigny et al. 1990). After the reaction took place, an aliquot (100 µl) of this solution was injected directly into the chromatographic system.

Chromatographic conditions. Milk and plasma concentrations of IVM and MXD were analysed using a Shimadzu LC-10 AS HPLC system (Shimadzu Corporation, Kyoto, Japan), which included a fluorescence detector (Shimadzu, RF 551, Shimadzu Corporation, Kyoto, Japan) set at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. The mobile phase of acetic acid (0·2% in water, v/v), methanol and acetonitrile (4 : 40 : 56 v/v/v for IVM and 5 : 40 : 55 v/v/v for MXD) was pumped at a flow rate of 1·5 ml/min through a Selectosil C_{18} (5 µm, 250 × 4·60 mm) reversed phase column (Phenomenex, St. Torrance, CA, USA). Compounds were identified by comparison with retention times of pure reference standards. The area under the peaks was calculated using the integrator software (Class LC 10 Software 1.2, Shimadzu Corporation, Kyoto, Japan) of the HPLC system.

Method validation. A complete validation of analytical procedures for extraction and quantification of IVM and MXD was performed before starting the analysis of experimental samples from the pharmacokinetic trial. Calibration lines in the ranges 0·1–5 ng/ml and 5–100 ng/ml were plotted using the peak area ratios between each analyte and the internal standard. The data were analysed for linearity using a linear least-squares regression analysis, and using the Run Test and ANOVA to determine whether the data differed from a straight line. Absolute recovery of the drugs under study was measured by comparison of the peak areas from spiked milk and plasma samples with the peak areas resulting from direct injections of standards in methanol. The inter-day precision of the extraction and chromatographic procedures was evaluated by processing four replicate aliquots of pooled sheep plasma and milk samples containing known amounts of IVM or MXD (2 and 20 ng/ml) on different working days. The CV for recovery and inter-day precision of the method were calculated (Bolton, 1984). The limit of detection (LOD) was established by HPLC analysis of blank milk and plasma samples ($n=5$) fortified with the internal standard and measurement of the baseline noise at the time of retention of the IVM and MXD peak. The mean ratio between the baseline noise and ABM area plus three standard deviations was defined as the theoretical detection limit. The limit of quantification (LOQ) was defined as the lowest concentration that could be measured with acceptable precision (CV <20%) (Snyder et al. 1997).

Drug quantification, pharmacokinetic and statistical analyses of the data

Drug concentrations in experimental samples (milk and plasma) were determined by HPLC, calculating the ratio between the areas under the peaks of IVM or MXD and ABM using the CR10 software and interpolating these areas on the calibration lines prepared for each biological matrix (milk and plasma). The statistical program (Instat 3.0, Graph Pad Software Inc., San Diego, CA, USA) was used for linear regression analyses and linearity tests.

Milk and plasma concentration *v.* time curves obtained after treatment in each individual animal were analysed with the PK Solution 2.0. (Ashland, OH, USA) computer program. Pharmacokinetic variables were determined using a non-compartmental method. Peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration-time curves for each individual animal. Terminal half-life ($T_{1/2el}$) was calculated as $\ln 2/\lambda_z$, where λ_z is the elimination rate constant. The λ_z was determined by performing regression analysis using at least five points of the terminal phase of the concentration-time plot. Areas under the concentration-time curves (AUC) were calculated by the trapezoidal rule (Gibaldi & Perrier, 1982) without extrapolation to infinity. $AUC_{milk/plasma}$ ratios were estimated for each drug treatment by using the partial AUC values obtained between 0·5 d and the time at which the last quantifiable concentration was measured, since the first plasma data point was obtained at 0·5 d. IVM and MXD milk and plasma estimated kinetic variables are reported as mean ± SEM. The Mann-Whitney test was used to estimate the differences between kinetic parameters obtained in milk and plasma; probabilities lower than $P<0·05$ were considered significant.

Results

Analytical procedures, including chemical extraction, derivatization and HPLC analysis of IVM and MXD in sheep milk and plasma were validated appropriately. Linear regression lines for IVM and MXD in milk and plasma showed correlation coefficients between 0·998 and 0·999 and the departure from linearity was not statistically significant.

Mean extraction absolute recoveries from milk were between 65·0% (IVM) and 76·0% (MXD). Recovery from plasma varied between 76·5% (MXD) and 81·6% (IVM). Estimated LOD in milk was 0·033 ng/ml for both analytes. LOQ in milk and plasma was 0·10 ng/ml. Inter-day precision of the analytical procedures, obtained after HPLC analysis of IVM- and MXD-spiked blank samples (2 and

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Table 1. Mean (±SEM) kinetic variables describing the disposition of ivermectin and moxidectin from milk and plasma following their subcutaneous and oral administration at 200 µg/kg body weight in lactating dairy sheep ($n=5$)

	Ivermectin		Moxidectin	
Kinetic variables	Subcutaneous	Oral	Subcutaneous	Oral
Milk				
T_{max} (d)	1·70±0·30	0·50±0·00	3·00±0·84	0·70±0·12*
C_{max} (ng/ml)	28·1±6·92	26·0±6·09	134±18·4	173±16·7
$T_{½\ el}$ (d)	2·46±0·45	1·90±0·20	22·8±4·86	16·6±2·64
AUC (ng.d/ml)	127·6±21·2	35·0±8·47*	1753±239·5	478·2±45·0**
Ratio $AUC_{milk/plasma}$	1·78±0·12	2·64±0·20	10·7±1·35	14·3±1·88
Dose fraction recovered in milk (%)	0·77±0·21	0·18±0·04*	6·51±0·95	2·09±0·33**
Plasma				
T_{max} (d)	1·70±0·44	—	1·40±0·90	—
C_{max} (ng/ml)	15·0±3·82	—	12·2±1·51	—
$T_{½\ el}$ (d)	1·99±0·36	2·58±0·50	14·5±2·16	8·60±0·87
AUC (ng.d/ml)	74·4±14·2	13·4±3·57**	166±15·5	37·0±6·10**

Mean kinetic variables obtained for subcutaneous treatments are statistically different at $P<0·05$ (*) or $P<0·01$ (**) from those obtained after oral administration of the same endectocide compound

T_{max}: time to peak concentration; C_{max}: peak milk or plasma concentration; $T_{½\ el}$: elimination half-life; AUC: area under the concentration *v.* time curve from 0 h to the last sampling where the drug was measurable (without extrapolation to infinity); Ratio $AUC_{milk/plasma}$: the ratio between the AUC values obtained in milk and plasma from 0·5 d up to the last sampling at which the drug was measurable

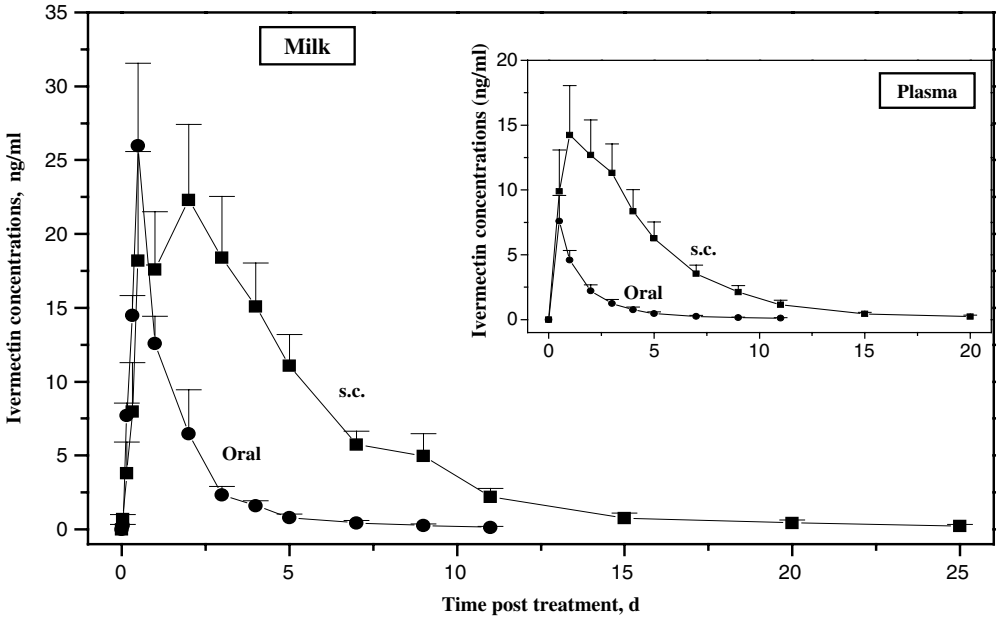

Fig. 1. Comparative mean (±SEM) ($n=5$) ivermectin milk concentration profiles obtained after its subcutaneous and oral administration (200 µg/kg body weight) to lactating dairy sheep. The insert graph shows the mean (±SEM) ivermectin plasma concentration profiles after the same treatments.

20 ng/ml) on different working days, showed a CV between 1·91% (plasma) and 3·09% (milk) for IVM and between 4·81% (plasma) and 6·71% (milk) for MXD.

Parent IVM was detected in plasma between 12 h and 11 d (oral) or 20 d (s.c.) post treatment, and in milk between 1 h and 11 d (oral) or 25 d (s.c.) post treatment. Milk residues of IVM administrated both s.c. and orally increased progressively to reach peak concentrations of 28·1 and 26·0 ng/ml at 1·7 and 0·5 d post treatment, respectively, as shown in Table 1. Mean milk depletion half-lives for IVM administered s.c. were not statistically different from those observed for IVM given orally. Concentrations of IVM measured in milk for both treatments were higher than those measured in plasma at all sampling times (Fig. 1). AUC for IVM administered orally was 2·64 times higher in milk than in plasma.

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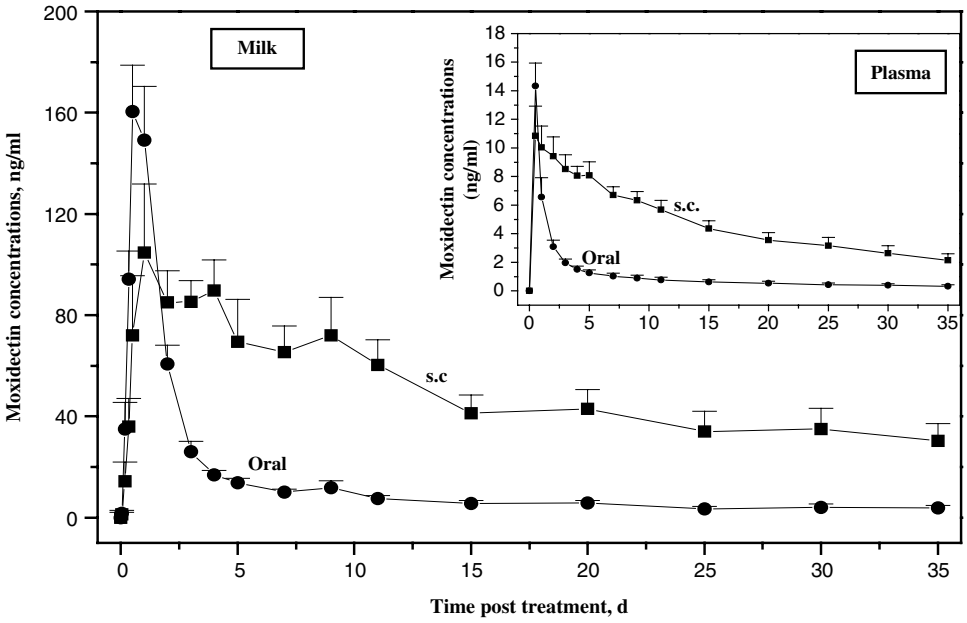

Fig. 2. Comparative mean (±SEM) ($n=5$) moxidectin milk concentration profiles obtained after its subcutaneous and oral administration (200 µg/kg body weight) to lactating dairy sheep. The insert graph represents the mean (±SEM) moxidectin plasma concentration profiles after the same treatments.

MXD was detected in milk and plasma up to the last day of sampling (day 35) after its administration by both routes. Milk residues of MXD administrated both s.c. and orally increased progressively to reach peak concentrations of 134 and 173 ng/ml attained at 3·00 and 0·70 d post treatment, respectively (Table 1). Concentrations of MXD measured in milk (both treatments) were greater than those measured in plasma at all sampling times. Mean MXD milk concentrations as high as 3·77 ng/ml (oral treatment) and 30·3 ng/ml (s.c. treatment) were measured at 35 d post treatment. The peak plasma concentration (12·2 ng/ml) for MXD after s.c. treatment decreased gradually to reach a value of 2·14 ng/ml at 35 d (Fig. 2).

The extensive distribution of both analytes from the bloodstream to milk was clearly reflected in the AUC ratios between milk and plasma (Table 1). The greater endectocide plasma concentrations obtained after s.c. administration accounted for the large AUC values in milk for IVM and MXD, which were between 172·6% (IVM) and 1066% (MXD) of those obtained in plasma in all the animals under assay. However, there was clear individual variation among animals in the concentration profiles of IVM and MXD in plasma and milk after s.c. treatment.

The sustained presence of high concentrations of MXD in milk over 35 d after s.c. treatment accounted for the prolonged milk depletion half-life of the drug (22·8 d). Mean MXD milk depletion half-life, as well as that in the bloodstream, were significantly longer ($P<0·01$) than those observed for IVM after both treatments. MXD delivered by both routes of administration had mean milk depletion half-lives 8·74–9·27 times longer than those of IVM administered by the same routes (Table 1).

A higher percentage of MXD was excreted in milk (2·09–6·51%). Marked differences in the total amount of IVM ($P<0·05$) and MXD ($P<0·01$) excreted in milk were observed between routes of administration. Both endectocides showed the highest percentage of dose eliminated in milk after the s.c. treatment. Percentages of IVM and MXD excreted in milk after s.c. treatment were, respectively, 4·27 and 3·11 times higher than for oral treatment owing to the enhanced bioavailability of the drug after s.c. administration. The total percentage of dose recovered in milk for MXD after s.c. and oral administration was significantly higher ($P<0·01$) than the corresponding values for IVM. MXD showed the highest percentage of the dose excreted in milk after its s.c. administration (Table 1), which was consistent with the plasma kinetic results.

Discussion

Plasma kinetics of endectocide compounds in ruminant species, particularly in non-dairy animals, is well documented for both IVM and MXD. The present results corroborated the relevance of the mammary gland as a route of elimination for IVM, and particularly for MXD, which was due to the lipophilic properties of these compounds. Much progress has been achieved in characterizing the plasma and milk concentration profiles of endectocide compounds in different animal species (Alvinerie et al. 1993; Escudero et al. 1999; Oukessou et al. 1999; Carceles et al. 2001; Anastasio et al. 2002). However, only limited information is available on the milk disposition of these drugs in lactating dairy sheep (Cerkvenik

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et al. 2002; Imperiale et al. 2003). Previously available data on the disposition kinetics of endectocides in ruminant species differ greatly from the findings reported here for lactating dairy sheep.

Milk kinetics after s.c. and oral administrations of IVM (Table 1) differed from the disposition variables reported for cows (Toutain et al. 1988), goats (Alvinerie et al. 1993) and camels (Oukessou et al. 1999). The total IVM AUC value in milk after s.c. treatment was higher than that reported in goats (60·7 ng.d/ml) and camels (37·5 ng.d/ml). Similarly, the milk kinetics of MXD after s.c. and oral administration to lactating dairy ewes differed from that reported in goats (Carceles et al. 2001) and camels (Oukessou et al. 1999). The MXD AUC values in milk of dairy sheep were 1·7 times higher than those reported in goats and 6 times higher than for camels.

These differences among species are not surprising considering the number of factors known to affect the disposition kinetics of endectocide compounds in ruminants (Lanusse, 2003). The higher body weight and the fat content of the dairy sheep used in the present trial as well as differences in metabolism and drug elimination may have accounted for the differences observed in the pattern of MXD and IVM milk excretion compared with the earlier work referred to above.

Comparison of the mean depletion half-lives of MXD and IVM after s.c. administration indicates that MXD persisted for 9·3 times longer than IVM in milk. A prolonged detection of higher MXD tissue residues is reported in sheep with higher fat content and greater body weight (Lifschitz et al. 2000a). The chemical structure of MXD contributes to the extreme lipophilicity of this compound, it being more lipophilic than IVM (Shoop et al. 1995), which would explain the longer persistence and the greater excretion of MXD in milk.

The percentage of total IVM dose excreted in milk after s.c. treatment was 0·77%, which was similar to the 0·7% reported previously for dairy sheep (Cerkvenik et al. 2002), intermediate between the values described in buffaloes (0·94%; Anastasio et al. 2002) and in goats (0·31%; Alvinerie et al. 1993) and lower than that reported for dairy cows (5·46%; Toutain et al. 1988). The fraction of the MXD dose eliminated in milk after s.c. (6·51%) and oral (2·09%) treatments was lower than those reported for goats (22·5% after s.c. and 5·71% after oral treatment; Carceles et al. 2001), but higher than that estimated in cows, where a sucking calf received about 5% of the dose excreted in milk after s.c. injection (Alvinerie et al. 1996).

The systemic exposure measured as the plasma AUC values for both IVM and MXD given orally at the same dose rate was lower than that obtained following s.c. treatment. The lower plasma profiles attained after the oral treatment were reflected in the drug concentrations measured in the milk of dairy sheep treated with both compounds. Milk AUC values and consequently the total dose excreted in milk for both IVM and MXD were markedly lower after oral than for s.c. treatment. However, a similar ratio $AUC_{milk/plasma}$ was obtained for both routes of administration.

The level of milk production is another key issue to be considered. The extent of MXD exchange from plasma to milk, expressed as the ratio of $AUC_{milk/plasma}$, after its s.c. and oral administrations in the current experiments was higher than that described for MXD in goats (7·5; Carceles et al. 2001). However, the average milk production of the sheep in the current trials was markedly lower than the value reported by Carceles et al. (2001) in goats. Differences in milk production, among other factors, may account for the differential pattern of exchange between plasma and milk observed for MXD, given at the same dose and by the same route, in dairy animals from both small ruminant species.

The present results indicate that excretion in milk is an important route of elimination for lipophilic drugs such as IVM and, particularly, MXD. Differences among species might relate to milk yield and the high fat content of sheep milk (7·8%) compared with other dairy species. Milk-plasma concentration ratios and milk fat content are positively related (Cerkvenik et al. 2002). A similar correlation may be expected for MXD, considering the observed milk disposition pattern and steady increment of fat content during the lactation period.

Milk maximum residue limits (MRL) for endectocide compounds in dairy sheep have not been established. MRL values for eprinomectin and MXD in dairy cattle were determined by the EU and included in Annex I of Council Regulation (EEC) No 2377/90. A provisional MRL value (0·01 mg/kg) for IVM in bovine milk has been suggested (Baynes et al. 1999), which is consistent with the value established by the New Zealand Mandatory Food Standard (1999). In conclusion, the high concentration profiles and the long persistence, particularly of MXD residues in sheep should be considered when establishing suitable withdrawal times, to ensure the quality of dairy products and the safety of consumers. Concentrations of IVM in milk decreased from 28·1 ng/ml to 0·23 ng/ml (s.c.) and from 26·0 ng/ml to 0·14 ng/ml (oral) over the time period studied. However, after MXD administration by both routes the highest concentration profiles were detected in milk. Residues of MXD in milk decreased from 134 ng/ml to 30·3 ng/ml after s.c. treatment and from 173 ng/ml to 3·77 ng/ml after oral treatment. Mean milk depletion half-lives varied between 1·90 and 2·46 d (IVM) and from 17 up to 22·8 d (MXD). It is suggested (Baynes et al. 2000), that 10 half-lives may be required to achieve 99% excretion of a given dose, which would indicate that between 19 (oral) and 24 (s.c.) d would be required to excrete IVM in such a proportion via milk. However, withdrawal times as long as 170 d (oral) and 230 d (s.c.) may be required to eliminate 99% of the administered dose of MXD in dairy sheep.

In cheese made from milk of IVM-treated buffaloes (Anastasio et al. 2002) collected 3 d after treatment, IVM residual concentrations in the cheese are up to 4 times

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higher than in the original milk. Since most of the dairy sheep milk production is used for cheesemaking and since a higher concentration of the drug is recovered in the cheese because of water loss, the results reported here for IVM and MXD milk residual concentrations are particularly relevant. Again, because optimal parasite control is required to reach adequate milk production levels in dairy ewes, special emphasis should be given to the information reported here on the pattern of milk excretion in relation to the safety of the consumer, if the extra-label use of endectocide compounds is to be recommended. The present results provide useful information to establish safe withdrawal periods in dairy sheep.

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