Disposition of Doramectin Milk Residues in Lactating Dairy Sheep

Fernanda A. Imperiale, Lourdes Mottier, Juan M. Sallovitz,
Adrián L. Lifschitz, and Carlos E. Lanusse*

Laboratorio de Farmacología, Núcleo FISFARVET, Departamento de Fisiopatología,
Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires,
Campus Universitario, 7000 Tandil, Argentina

Doramectin (DRM) is a broad spectrum macrocyclic lactone antiparasitic drug not approved for use in dairy animals. However, DRM and other endectocide compounds are widely used extra-label to control endo- and ectoparasites in dairy sheep. The plasma disposition kinetics and the pattern of DRM excretion in milk were characterized following its subcutaneous administration to lactating dairy sheep. DRM concentration profiles were measured in plasma and milk samples after validation of a specific HPLC-based methodology. DRM was detected between 1 h and 30 days post-treatment. DRM concentrations of 0.48 ng·mL^{-1} (plasma) and 1.03 ng·mL^{-1} (milk) were measured at 30 days post-treatment. DRM was extensively distributed from the bloodstream to the mammary gland, and large concentrations were excreted in milk. The peak concentrations and total amount of DRM recovered in milk (expressed as area under the concentration versus time curve) were 3-fold higher than those measured in plasma; 2.44% of the total DRM dose was excreted in milk. The long persistence of DRM milk residues should be seriously considered before its extra-label use in dairy animals is recommended.

KEYWORDS: Endectocides; doramectin; milk residues disposition; milk/plasma ratios; dairy sheep

INTRODUCTION

The avermectins and milbemycins (16-membered macrocyclic lactones) are naturally occurring compounds produced by fermentation of soil-dwelling actinomycetes. The presence of a cyclohexyl group at the C_{25} position characterizes doramectin (DRM), an avermectin-type compound obtained by mutational biosynthesis of *Streptomyces avermitilis* (**Figure 1**). DRM is an endectocide compound with exceptional potency and a broad antiparasitic spectrum (nematodes and arthropods) of activity (*1*). This compound is largely used worldwide to control endo- and ectoparasites in livestock animals (*2*).

The plasma pharmacokinetic behavior of different endectocide molecules in different animal species has been extensively investigated. Animal species (*3*), animal breeds (*4*), nutritional condition and dietary management (*5*, *6*), type of drug formulation (*7*, *8*), and route of administration (*9*, *10*), among many other factors, have been shown to affect the kinetic disposition of endectocides in livestock animals. More recently, the reversible exchange of ivermectin, moxidectin, and DRM between the bloodstream and different tissues has been shown in cattle. DRM is a highly lipophilic compound, which has been shown to extensively distribute from plasma to different tissues, particularly those with the highest fat content (*11*). Furthermore,

evidence of the important recycling of DRM between the bloodstream and the digestive tract in sheep has been reported (*12*).

Endectocides are distributed throughout the body by the circulating blood and diffuse to target tissues to exert systemic antiparasitic effects, reaching other nontarget tissues such as the mammary gland. Drug concentrations attained in different tissues depend on the ability of the drug to penetrate the capillary endothelium and diffuse across biological membranes of lipid nature (*13*). The relationship between drug concentrations attained in the mammary gland and those in the bloodstream will depend on the degree of ionization of the drug in both milk and plasma, its lipid solubility, and the extent to which the drug binds to the milk and plasma proteins. In general, only the unbound, nonionized lipid-soluble molecules reach the mammary gland and are excreted in milk (*13*, *14*).

Dairy sheep in intensive milking systems are subjected to high production pressure, which is associated with an enhanced vulnerability to parasitic infection (*15*). Although different management strategies are used to prevent or minimize production losses, the use of antiparasitic drugs is still the main control measure available against parasitism in lactating dairy sheep. The use of strategic anthelmintic treatments in dairy animals has been correlated with a significant enhancement in milk production in both dairy sheep and cattle (*15*–*17*). However, the implementation of anthelmintic treatments in lactating

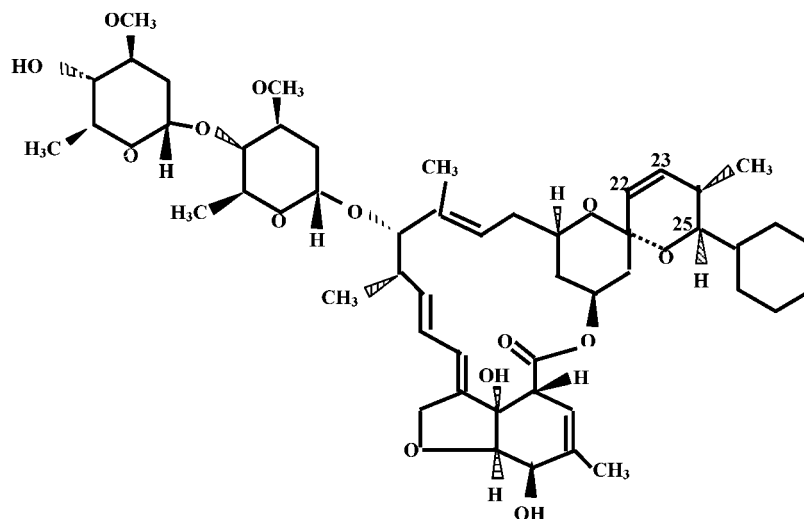* Corresponding author (telephone 54-2293-426667; fax 54-2293-422357; e-mail clanusse@vet.unicen.edu.ar).

Figure 1. Chemical structure of doramectin [25-cyclohexyl-5-*O* demethyl-25-de (1-methylpropyl) avermectin B1].

animals is still controversial, largely due to the potential negative impact of drug/metabolites excreted in milk destined for human consumption.

The patterns of milk excretion for ivermectin (*18, 19*) and moxidectin (*20, 21*) have been characterized in different ruminant species. Pour-on formulations of eprinomectin and moxidectin are currently approved for use in dairy cattle in some countries. Injectable and oral preparations of ivermectin and DRM are used in an extra-label mode in dairy animals, although their administration to lactating animals has not been authorized. The extra-label use of different endectocide compounds in dairy sheep is well-known. Nevertheless, a possible unapproved use should be considered to take advantage of the benefits obtained in controlling endo- and ectoparasites, particularly mange infections that represent one the most serious health concerns for dairy sheep farmers (*22*). However, the use of unapproved endectocides in dairy animals should be made with care and compatible with the production of high-quality milk and, more importantly, with consumer health.

Antiparasitic drugs are required to achieve an acceptable parasite control in dairy animals. Thus, there is a need for further investigation on drug distribution and elimination by milk to implement rational and pharmacokinetic-based antiparasitic treatments in dairy animals. Such information should be helpful to recommend parasite control strategies compatible with the safety of the produced milk to the consumer. The relationship between plasma disposition and the pattern of DRM excretion in milk in lactating dairy sheep is reported here.

MATERIALS AND METHODS

Experimental Animals, Treatments, and Sampling. Five female Pampinta dairy sheep (cross-breed 3/4 Milchschaf and 1/4 Corriedale) with an average weight of 92 kg were used. The animals were kept under field conditions during the whole experimental period. The health of the animals was closely monitored prior to and throughout the trial. Dairy sheep were milked twice a day with a milking machine, and milk production was measured prior to and throughout the trial. The average milk production during the trial was 640 mL·day^{-1}.

Doramectin was given by subcutaneous (sc) injection in the shoulder area at 200 μg·kg^{-1} using a commercially available formulation (Dectomax 1%, Pfizer, Sanidad Animal, Buenos Aires, Argentina; lot 704-54004 B) for use in cattle. Neither pain nor irritation was observed at the site of injection at any time after treatment.

Blood samples were taken from the jugular vein in heparinized vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) prior to treatment and at 12 h and 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30, and 35 days post-treatment. Milk samples were collected in vials from each sheep prior to treatment and at 1, 4, 8, and 12 h and 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30, and 35 days post-treatment. The blood samples were centrifuged at 2000g for 20 min, and the recovered plasma was transferred to plastic vials. Plasma and milk samples were frozen at −20 °C until analyzed.

Analytical Procedures. The extraction procedures and chromatographic conditions to quantify DRM in fortified and experimental samples (plasma and milk) were carried out following modifications of a previously described method (*23*). Detailed information on the chromatographic procedures, including the use of abamectin (ABM) as internal standard to quantify DRM in plasma and milk, is described below.

Drug Extraction and Derivatization. Drug-free plasma and milk samples (1 mL) were fortified with DRM (lot 4E139-54QCS-06) to reach the following final concentrations: 0.1, 0.25, 1, 2, 5, 10, 20, 50, and 100 ng·mL^{-1}. Standard solutions of DRM were prepared by successive dilutions in methanol from the parent stock solution (1 mg·mL^{-1}) and stored at 4 °C. The fortified and experimental plasma and milk samples were added with 100 μL of ABM as internal standard (100 ng·mL^{-1}). Acetonitrile (1 mL) was added to each tube containing 1 mL of plasma or milk sample. After thorough mixing for 15 min, the batch of tubes was centrifuged at 2000g for 20 min. The supernatant was collected, and the extraction and cleanup processes were performed manually using a Lichrolut vacuum manifold (Merck, Nogent-Sur-Marne Cedex, France) and Supelclean LC 18 cartridges (Supelco, Bellefonte, PA). The cartridges were previously conditioned with 2 mL of methanol and 2 mL of water, and the plasma or milk sample (supernatant) was passed through them. Cartridges were washed with 1 mL of water followed by 1 mL of water/methanol (4:1) and then dried off for 5 min, and the sample was eluted with 1.5 mL of methanol and collected. The elution was evaporated to dryness under a gentle stream of nitrogen at 60 °C in a water bath.

Doramectin is a nonfluorescent molecule; therefore, a derivatization process is needed to make it fluorescent. The dry residue of the elution was dissolved with 100 μL of *N*-methylimidazole (Sigma, St. Louis, MO) solution in acetonitrile (1:1) and 150 μL of trifluoroacetic anhydride (Sigma) solution in acetonitrile (1:2) (*24*). After the reaction took place, an aliquot (100 μL) of this solution was injected directly into the chromatographic system.

Chromatographic Conditions. One hundred microliters of each sample was injected into the Shimadzu LC-10 AS HPLC system (Shimadzu Corp., Kyoto, Japan) fitted with a Selectosil C$_{18}$ (5 μm, 250 × 4.60 mm) reverse phase column (Phenomenex, Torrance, CA) and a fluorescence detector (Shimadzu, RF 551) set at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. The mobile phase was composed of acetic acid (0.2%), methanol, and acetonitrile 6:40:54 v/v/v at a flow rate of 1.5 mL·min^{-1}. DRM and

Disposition of Doramectin Residues in Sheep's Milk

J. Agric. Food Chem., Vol. 51, No. 10, 2003 **3187**

ABM retention times were determined after chromatographic analysis of pure reference standards. The areas under the peaks were calculated using the integrator software (class LC 10 software 1.2, Shimadzu) of the HPLC system. The solvents used for sample extraction and drug analysis were of HPLC grade (Baker, Phillipsburg, NJ).

Method Validation. A complete validation of analytical procedures for the extraction and quantification of DRM was performed before the start of the analysis of experimental samples from the pharmacokinetic trial. Calibration lines for DRM in the ranges of 0.1–5 and 5–100 ng·mL^{-1} were prepared using pooled drug-free plasma and milk. Calibration lines were plotted using the peak area ratios between each analyte and the internal standard. The data were analyzed for linearity using a linear least-squares regression analysis, using the Run Test and ANOVA to detemine if the data differed from a straight line. The extraction efficiency (recovery) of the drug under study was measured by comparison of the peak areas from spiked plasma and milk samples with the peak areas resulting from direct injections of standards in methanol, carried through the derivatization procedure. The recoveries of DRM from sheep plasma and milk samples were obtained at 0.1, 0.5, 10, and 50 ng.mL^{-1}, using three replicates for each drug concentration. The interday precision of the extraction and chromatography procedures was evaluated by processing four replicate aliquots of pooled sheep plasma and milk samples containing known amounts of DRM (2 and 20 ng·mL^{-1}) on different working days. The accuracy of the analytical method was estimated both in plasma and in milk at DRM concentrations of 0.1, 10, and 50 ng·mL^{-1}. The coefficient of variation (CV) for recovery and interday precision of the method were calculated (*25*). The limit of detection (LOD) was established by HPLC analysis of blank plasma and milk samples ($n = 5$) fortified with the internal standard and measurement of the baseline noise at the time of retention of the DRM peak. The mean baseline noise plus 3 standard deviations was defined as the theoretical LOD. The limit of quantification (LOQ) was defined as the lowest concentration that can be measured with acceptable precision (CV < 20%) and accuracy ($\pm20\%$) (*26*).

Drug Quantification. Pharmacokinetic and Statistical Analyses of the Data. Drug concentrations in experimental samples (plasma and milk) were determined by HPLC calculating the ratio between the areas under the peaks of DRM and ABM (internal standard) using the CR10 software (Shimadzu) and interpolating these areas on the calibration lines prepared for each biological matrix (plasma and milk). The statistical program (Instat 3.0, GraphPad Software Inc., San Diego, CA) was used for linear regression analyses and linearity tests.

The plasma and milk concentration versus time curves obtained after treatment in each individual animal were fitted with the PK Solution 2.0 (Ashland, OH) computer program. Pharmacokinetic parameters were determined using a noncompartmental method. The peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration–time curves in each individual animal. The terminal half-life ($T_{1/2\ el}$) and absorption half-life ($T_{1/2\ ab}$) were calculated as ln $2/\lambda_z$ and ln $2/k_{ab}$, respectively, where λ_z is the elimination rate constant and k_{ab} represents the first-order absorption rate constant. The appearance half-life ($T_{1/2\ app}$) for DRM in milk was estimated as ln $2/k_{app}$, k_{app} being the rate constant for the appearance of the drug in milk. The k_{ab} and k_{app} rate constants were estimated using the method of residuals. λ_z was determined by performing regression analysis using at least five points of the terminal phase of the concentration–time plot. The areas under the concentration–time curves (AUC) were calculated by the trapezoidal rule (*27*) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (λ_z). Statistical moment theory was applied to calculate the mean residence times (MRT) for DRM as follows: MRT = AUMC/AUC, where AUC is as defined previously and AUMC is the area under the curve of the product of the time and drug concentration versus time from zero to infinity (*27*). The DRM plasma and milk estimated pharmacokinetic parameters are reported as mean $\pm$ standard error of the mean (SEM). The Kolmogorov–Smirnov test was employed to verify the normal distribution of the data, and the unpaired *t* test was used to estimate the differences between kinetic parameters obtained in plasma and milk. Values were considered to be significantly different at $P < 0.01$.
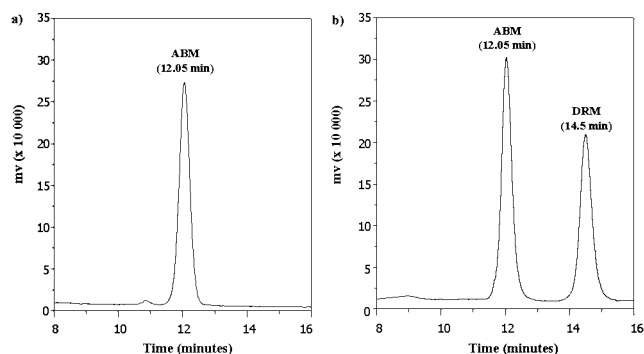

Figure 2. HPLC chromatograms of blank (a) and doramectin (DRM) fortified (10 ng·mL^{-1}) sheep's plasma samples (b). ABM = abamectin (internal standard).
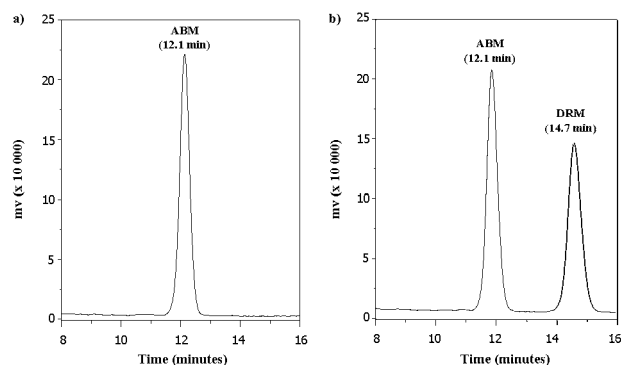

Figure 3. HPLC chromatograms of blank (a) and doramectin (DRM) fortified (10 ng·mL^{-1}) sheep's milk samples (b). ABM = abamectin (internal standard).

RESULTS AND DISCUSSION

The analytical procedures, including chemical extraction, derivatization, and HPLC analysis of DRM in sheep plasma and milk, were validated appropriately. Typical chromatograms of drug-free and DRM-fortified (b) plasma samples are shown in **Figure 2**. Retention times were 12.05 min (ABM, used as internal standard) and 14.5 min (DRM). **Figure 3** shows chromatograms of drug-free (a) and DRM-fortified (b) milk samples, where retention times were 12.1 and 14.7 min for ABM and DRM, respectively. The linear regression lines for DRM showed high correlation coefficients for each concentration range investigated ($r = 0.999$), and the departure from linearity was not statistically significant.

DRM recovery percentages ranged between 72.2 and 98.7% (plasma) and between 68.0 and 83.6% (milk). The estimated LODs were 0.03 ng·mL^{-1} (plasma) and 0.02 ng·mL^{-1} (milk). The LOQ was 0.1 ng·mL^{-1} with a CV $\leq7\%$ and an accuracy $\leq11\%$ in both biological fluids under study. Accuracies were $\leq9\%$ (plasma) and $\leq11\%$ (milk) at the different concentrations analyzed. The interday precision of the analytical procedures, obtained after HPLC analysis of DRM-spiked standards (2 and 20 ng·mL^{-1}) on different working days, showed a CV between 4.15% (milk) and 6.30% (plasma), which is analytically considered as highly satisfactory. The results of the complete validation of the analytical method developed to measure DRM in plasma and milk are summarized in **Table 1**. The results obtained in this validation procedure ensure that a reliable method for the detection of DRM residues in sheep plasma and milk at concentrations as low as 0.1 ng·mL^{-1} is available. The development and testing of an HPLC method to quantify DRM residual concentrations may help the milk industry work with safe residue levels in dairy products, as well as for the

3188 *J. Agric. Food Chem.,* Vol. 51, No. 10, 2003

Imperiale et al.

Table 1. Validation Parameters of the HPLC Method Used To Measure Doramectin (DRM) Concentrations in Milk and Plasma Samples from Dairy Sheep[a]

	milk	plasma
limit of quantification (ng·mL^{-1})	0.1	0.1
accuracy (%)	≤11	≤9
recovery (%)	72.2	80.5
coefficient of variation (%)	10.5	15.3
interday precision (CV %)	4.15	6.30
linearity (*r*)	0.999	0.999

[a] The limit of quantification was defined as the lowest concentration that can be measured with acceptable precision (CV < 20%) and accuracy (±20%). Accuracy was defined as the closeness of the measured value in milk and plasma samples to the true value (concentrations of 0.1, 10, and 50 ng·mL^{-1}). Recovery values are mean percentages of DRM recoveries from fortified sheep's milk and plasma samples (concentrations range between 0.1 and 50 ng·mL^{-1}) (*n* = 3). Coefficient of variation values represent CV for the recovery assays. Interday precision values express the CV for the interday precision studies (*n* = 4). Linearity is the coefficient of correlation obtained from the linear regression lines in the ranges 0.1–5 and 5–100 ng·mL^{-1} in milk and plasma DRM fortified samples.

Table 2. Mean (± SEM) Pharmacokinetic Parameters Describing the Disposition of Doramectin from Milk and Plasma Following Its Subcutaneous Administration at 200 μg·kg^{-1} in Lactating Dairy Sheep (*n* = 5)[a]

kinetic parameter	milk	plasma
C_{max} (ng·mL^{-1})	79.8 ± 14.9	25.0 ± 4.03[b]
T_{max} (days)	3.00 ± 0.32	2.20 ± 0.37
$T_{1/2\ app}$ (days)	0.80 ± 0.20	1.17 ± 0.27
$T_{1/2\ el}$ (days)	3.90 ± 0.99	4.06 ± 0.75
MRT (days)	6.70 ± 0.88	7.00 ± 0.87
AUC_{total} (ng·day·mL^{-1})	641 ± 113	217 ± 17.2[b]
ratio $AUC_{total\ milk/plasma}$	2.88 ± 0.30	
dose fraction recovered in milk (%)	2.44 ± 0.44	

[a] C_{max} = peak milk or plasma concentration; T_{max} = time to peak concentration; $T_{1/2\ app}$ = appearance half-life in milk or absorption half-life in plasma ($T_{1/2\ ab}$); $T_{1/2\ el}$ = elimination half-life; AUC_{total} = area under the concentration vs time curve extrapolated to infinity; MRT = mean residence time. [b] Values are statistically different at $P < 0.01$.

application to in vivo pharmacokinetic trials. However, mass spectral analysis may be required to undertake proper drug/metabolite identification in tissue residue analysis.

Using the described methodology, DRM was detected in both plasma and milk samples as early as 1 h after its sc administration to lactating dairy sheep. Mean DRM concentrations greater than 0.45 ng·mL^{-1} (plasma) and 1.00 ng·mL^{-1} (milk) were measured at 30 days post-treatment. The plasma and milk concentration profiles measured after sc administration of DRM are compared in **Figure 4**. A delayed absorption of DRM was reflected in the T_{max} values obtained in plasma, which were attained at 2 days post-treatment. The low water solubility of DRM, its formulation in nonaqueous formulation, and its deposition in subcutaneous tissue favor a slow absorption from the site of injection, which accounts for its prolonged residence in the bloodstream and the detection of sustained high tissue concentrations in cattle (*11*). The oil-based formulation of DRM and, perhaps, a slow metabolic rate due to the presence of the cyclohexyl group at C25 (*1*) may contribute to the high plasma availability (AUC) and large excretion in milk observed in the current trial. The kinetic parameters summarizing the disposition of DRM from plasma and milk are presented in **Table 2**.

The plasma availability of DRM obtained after its sc administration in lactating dairy sheep differs from that reported in cattle (*23, 28*) nondairy sheep breeds (*29*), and goats (*30*). The plasma AUC value (217 ± 17.2 ng·day·mL^{-1}) was lower than those reported for DRM in cattle (between 511 and 627 ng·day·mL^{-1}) and sheep (404 ng·day·mL^{-1}) and higher than that reported in goats (102 ng·day·mL^{-1}). These differences among species are not surprising considering the number of factors that have been shown to affect the disposition kinetics of endectocide compounds in ruminants. The larger size, greater body weight, and higher fat content of the dairy sheep used in this trial compared to other sheep breeds (*29*) may account for the differences observed in drug availability and/or disposition from the bloodstream. Similarly, a prolonged detection of higher moxidectin tissue residue levels was observed in sheep with higher fat content and greater body weight (*31*).

The milk residues of DRM increased progressively to reach a peak concentration of 79.8 ± 14.9 ng·mL^{-1} at 3.0 days post-treatment. The concentrations of DRM measured in milk were greater than those recovered in plasma at all of the sampling times. The kinetics of DRM excretion in milk of sheep differs from that reported in goats (*21*). Total DRM availability in milk (AUC = 641 ± 113 ng·day·mL^{-1}) was higher than that reported in goats (144 ng·day·mL^{-1}). Similarly, the peak concentration
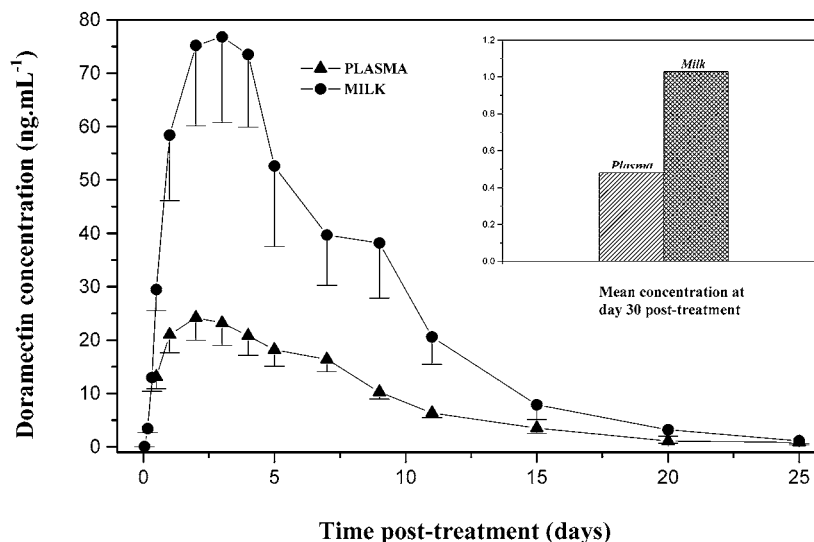
Figure 4. Comparative mean (± SEM) (*n* = 5) plasma and milk concentration profiles of doramectin (DRM) obtained after its subcutaneous administration (200 μg·kg^{-1}) to lactating dairy sheep. The insert represents the mean concentration values measured in plasma and milk at 30 days post-treatment.

Disposition of Doramectin Residues in Sheep's Milk

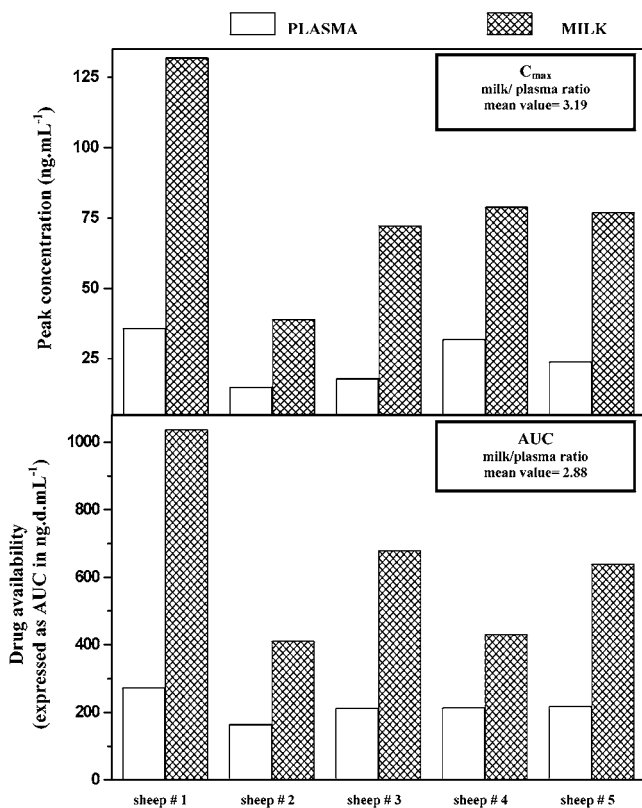*J. Agric. Food Chem.,* Vol. 51, No. 10, 2003 **3189**

Figure 5. Comparative peak concentrations (C_{max}) and drug availability [expressed as area under the curve values (AUC_{total})] obtained in individual lactating dairy sheep after subcutaneous administration of doramectin (200 $\mu g \cdot kg^{-1}$).

obtained in milk (79.8 ng·mL^{-1}) was higher than that obtained in goats (22.8 ng·mL^{-1}) and the T_{max} occurred later (3.00 ± 0.32 days) than in goats (1.65 days). The values of C_{max} and AUC for DRM in milk were 3-fold higher than those obtained in plasma (**Figure 5**). DRM MRT in milk (6.7 days) was similar to that obtained in plasma (7 days), being longer than that reported in goats (4.68 days). A large milk–plasma partitioning value was obtained for DRM (2.88) in dairy sheep (**Table 2**). The kinetic results obtained here indicate that DRM is excreted in milk in dairy sheep. These lactating animals, with a milk production of 640 mL·day^{-1}, excreted 2.44% of the total DRM dose in milk.

Different drugs extensively used in veterinary therapeutics are organic bases with high lipid solubility. In milk with pH values between 6.5 and 6.8, large milk excretion and milk/plasma ratios between 2 and 3.5 have been reported for different antibacterial drugs in sheep (*14*) and cattle (*32*). DRM, as other avermectin-type compounds, is highly lipophilic (*1, 33*) and presents an exceptional ability to penetrate into the mammary gland and, thus, to be excreted in milk. The excretion of high levels of residues of DRM in milk has been clearly demonstrated in the work reported here. Milk-to-plasma ratios close to 3 were observed for DRM in lactating dairy sheep, which are larger than those obtained for DRM in goats (1.4) (*21*) and for ivermectin subcutaneously administered in different species including sheep, goats, camels, and buffaloes, for which milk/plasma ratios close to 1 have been reported (*19, 34–36*).

The high lipophilicity of endectocide drugs and the high fat content of sheep's milk (7.8%) compared to that of other dairy animal species account for this large milk excretion pattern. A positive relationship between the milk–plasma concentration ratios and milk fat content has been shown for ivermectin (*19*).

A similar correlation may be expected for DRM, considering both the observed milk disposition pattern and steady increment of fat content during the lactation period. Several factors including interspecies' differences, physiological status of the treated animals, climatic conditions, and differences in volume and fat contents in the milk of different animal species may drastically affect the resultant plasma–milk distribution of endectocides in lactating ruminants and the pattern of milk residue elimination.

In conclusion, the high concentration profiles and the long persistence of DRM residues in sheep's milk are worthy of concern. Milk maximum residues limits (MRL) have been established for ivermectin, eprinomectin, and moxidectin in dairy cattle. In the meantime, the characterization of the relationship between plasma and milk residue disposition of DRM in dairy sheep reported here may be useful in the development of kinetic-based models to optimize the use of these types of drugs for parasite control in dairy animals and to establish suitable withdrawal times to ensure the quality of milk-derived products, which will contribute to consumer safety.

ABBREVIATIONS USED

DRM, doramectin; sc, subcutaneous; ABM, abamectin; HPLC, high-performance liquid chromatography; SEM, standard error of the mean; CV, coefficient of variation.

LITERATURE CITED

(1) Goudie, A.; Evans, N.; Gration, K.; Bishop, B.; Gibson, S.; Holdom, K.; Kaye, B.; Wlicks, S.; Lewis, D.; Weatherley, A.; Bruce, C.; Herbert, A.; Seymour, D. Doramectin, a potent novel endectocide. *Vet. Parasitol.* **1993**, *49*, 5–15.

(2) McKellar, Q. A.; Benchaoui, H. A. Avermectins and milbemycins. *J. Vet. Pharmacol. Ther.* **1996**, *19*, 331–351.

(3) Alvinerie, M.; Galtier, P. Comparative pharmacokinetic properties of moxidectin and ivermectin in different animal species. *J. Vet. Pharmacol. Ther.* **1997**, *20* (Suppl. 1), 74.

(4) Sallovitz, J.; Lifschitz, A.; Imperiale, F.; Pis, A.; Virkel, G.; Lanusse, C. Breed differences on the plasma availability of moxidectin administered pour-on to calves. *Vet. J.* **2002**, *164*, 47–53.

(5) Ali, D.; Hennessy, D. The effect of level of feed intake on the pharmacokinetic disposition and efficacy of ivermectin in sheep. *J. Vet. Pharmacol. Ther.* **1996**, *19*, 89–94.

(6) Lifschitz, A.; Murno, G.; Pis, A.; Sallovitz, J.; Virkel, G.; Lanusse, C. Malnutrition modifies the disposition kinetics of ivermectin in calves. *J. Vet. Pharmacol. Ther.* **1997**, *20* (Suppl. 1), 87–109.

(7) Lo, P. K. A.; Fink, D. W.; Williams, J. B.; Blodinger, J. Pharmacokinetic studies of ivermectin: effects of formulation. *Vet. Res. Commun.* **1985**, *9*, 251–268.

(8) Wicks, S.; Kaye, B.; Weatherley, A.; Lewis, D.; Davison, E.; Gibson, S.; Smith, D. Effect of formulation on the pharmacokinetics and efficacy of doramectin. *Vet. Parasitol.* **1993**, *49*, 17–26.

(9) Nowakowski, M.; Lynch, M.; Smith, D.; Logan, N.; Mouzin, D.; Lukaszewicz, J.; Ryan, N.; Hunter, R.; Jones, R. Pharmacokinetics and bioequivalence of parenterally administered doramectin in cattle. *J. Vet. Pharmacol. Ther.* **1995**, *18*, 290–298.

3190 *J. Agric. Food Chem.,* Vol. 51, No. 10, 2003

Imperiale et al.

(10) Alvinerie, M.; Escudero, E.; Sutra, J. F.; Eeckhoute, C.; Galtier, P. The pharmacokinetics of moxidectin after oral and subcutaneous administration to sheep. *Vet. Res.* **1998**, *29*, 113−118.

(11) Lifschitz, A.; Virkel, G.; Sallovitz, J.; Sutra, J. F.; Galtier, P.; Alvinerie, M.; Lanusse, C. Comparative distribution of ivermectin and doramectin to parasite location tissues in cattle. *Vet. Parasitol.* **2000**, *87*, 327−338.

(12) Hennessy, D.; Page, S.; Gottschall, D. The behaviour of doramectin in the gastrointestinal tract, its secretion in bile and pharmacokinetic disposition in the peripheral circulation after oral and intravenous administration to sheep. *J. Vet. Pharmacol. Ther.* **2000**, *23*, 203−213.

(13) Baggot, J. Principles of drug disposition. In *The Basis of Veterinary Clinical Pharmacology*, 1st ed.; Saunders: Philadelphia, PA, 1997; pp 1−134.

(14) Ziv, G.; Sulman, F. G. Penetration of lincomycin and clindamycin into milk in ewes. *Br. Vet. J.* **1973**, *129*, 83−91.

(15) Juste Jordán, R. A.; García Pérez, A. L. Effect of treatment with netobimin on milk production of sheep. *Vet. Parasitol.* **1991,** *38*, 173−183.

(16) Ploeger, H. W.; Schoenmaker, G. J. W.; Kloosterman, A.; Borgsteede, F. H. Effect of anthelmintic treatment of dairy cattle on milk production related to some parameters estimating nematodes infection. *Vet. Parasitol.* **1989**, *34*, 239−253.

(17) Fthenakis, G. C.; Papadopulos, E.; Himonas, C.; Leontide, L.; Kritas, S.; Papatsas, J. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. *Vet. Parasitol.* **2000**, *87*, 207−216.

(18) Toutain, P. L.; Campan, M.; Galtier, P.; Alvinerie, M. Kinetic and insecticidal properties of ivermectin residues in milk of dairy cows. *J. Vet. Pharmacol. Ther.* **1988**, *11*, 288−291.

(19) Cerkvenik, V.; Grabnar, I.; Skubic, V.; Doganoc, D.; Beek, W.; Keukens, D.; Kosorok, M.; Pogacnik, M. Ivermectin pharmacokinetics in lactating sheep. *Vet. Parasitol.* **2002**, *104*, 175−185.

(20) Alvinerie, M.; Sutra, J. F.; Lanusse, C.; Galtier, P. Plasma profile study of moxidectin in a cow and its suckling calf. *Vet. Res.* **1996**, *27*, 545−549.

(21) Carceles, C.; Diaz, M.; Vicente, M.; Sutra J.; Alvinerie, M.; Escudero, E. Milk kinetics of moxidectin and doramectin in goats. *Res. Vet. Sci.* **2001**, *68*, 1−5.

(22) O'Brien, D. Treatment of psorotic mange with reference to epidemiology and history. *Vet. Parasitol.* **1999**, *83*, 177−185.

(23) Lanusse, C.; Lifschitz, A.; Virkel, G.; Alvarez L.; Sanchez, S.; Sutra, J. F.; Galtier, P.; Alvinerie, M. Comparative plasma disposition kinetics of ivermectin, moxidectin and doramectin in cattle. *J. Vet. Pharmacol. Ther.* **1997**, *20*, 91−99.

(24) De Montigny, P.; Shim, J. S.; Pivinichny, J. V. Liquid chromatographic determination of ivermectin with trifluoroacetic anhydride and *N*-methylimidazole as the derivatization reagent. *J. Biomed. Anal.* **1990**, *8*, 507−511.

(25) Bolton, S. *Pharmaceutical Statistics. Practical and Clinical Applications*; Swarbrick, J., Ed.; Dekker: New York, 1984; Vol. 25, pp 19−22.

(26) Snyder, L.; Kirkland, J.; Glajch, J. Completing the method: Validation and transfer. In *Practical HPLC Method Development*, 2nd ed.; Wiley: New York, 1997; pp 685−713.

(27) Gibaldi, M.; Perrier, D. *Pharmacokinetics*, 2nd ed.; Dekker: New York, 1982; pp 45−109.

(28) Toutain, P. L.; Upson, D. W.; Terhune, T. N.; McKenzie, M. E. Comparative pharmacokinetics of doramectin and ivermectin in cattle. *Vet. Parasitol.* **1997**, *72*, 3−8.

(29) Atta, A. H.; Abo Shihada, M. N. Comparative pharmacokinetics of doramectin and ivermectin in sheep. *J. Vet. Pharmacol. Ther.* **2000**, *23*, 49−52.

(30) Escudero, E.; Carceles, C.; Diaz, M.; Sutra, J. F.; Galtier, P.; Alvinerie, M. Parmacokinetics of moxidectin and doramectin in goats. *Res. Vet. Sci.* **1999**, *67*, 177−181.

(31) Lifschitz, A.; Imperiale, F.; Virkel, G.; Muñoz Cobeñas, M.; Scherling, N.; DeLay, R.; Lanusse, C. Depletion of moxidectin tissue residues in sheep. *J. Agric. Food Chem.* **2000**, *48*, 6011−6015.

(32) Ziv, G.; Sulman, F. G. Serum and milk concentration of spectinomycin and tylosin in cow and ewes. *Am. J. Vet. Res.* **1973**, *34*, 329−333.

(33) Shoop, W.; Mrozik, H.; Fisher, M. Structure and activity of avermectins and milbemycins in animal health. *Vet. Parasitol.* **1995**, *59*, 139−156.

(34) Alvinerie, M.; Sutra, J. F.; Galtier, P. Ivermectin in goat plasma and milk after subcutaneous injection. *Ann. Rech. Vet.* **1993**, *24*, 417−421.

(35) Oukessou, M.; Berrag, B.; Alvinerie, M. A comparative kinetic study of ivermectin and moxidectin in lactating camels (*Camelus dromedarius*). *Vet. Parasitol.* **1999**, *83*, 151−159.

(36) Anastasio, A.; Esposito, M.; Amorena, M.; Catellani, P.; Serpe, L.; Cortesi, M. Residue study of ivermectin in plasma, milk, and mozzarella cheese following subcutaneous administration to buffalo (*Bubalus bubalis*). *J. Agric. Food Chem.* **2002**, *50*, 5241−5245.

JF026118B