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Pharmacokinetics of Eprinomectin in Plasma and Milk following Subcutaneous Administration to Lactating Dairy Cattle

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ABSTRACT

Eprinomectin is only available as a topically applied anthelmintic for dairy cattle. To determine whether eprinomectin can be applied as an injectable formulation in dairy cattle, a novel injectable formulation was developed and was subcutaneously delivered to four lactating dairy cattle at a dose rate of 0.2 mg/kg. Plasma and milk samples were collected. The concentrations of eprinomectin in all samples were determined by HPLC. The peak plasma concentration (C_{max}) of 44.0 ± 24.2 ng/ml occurred 39 ± 19.3 h after subcutaneous administration, equivalent to the C_{max} (43.76 ± 18.23 ng/ml) previously reported for dairy cattle after a pour-on administration of 0.5 mg/kg eprinomectin. The area under the plasma concentration–time curve (AUC) after subcutaneous administration was 7354 ± 1861 (ng h)/ml, higher than that obtained after pour-on delivery (5737.68 ± 412.80 (ng h)/ml). The mean residence time (MRT) of the drug in plasma was 211 ± 55.2 h. Eprinomectin was detected in the milk at the second sampling time. The concentration of drug in milk was parallel to that in plasma, with a milk to plasma ratio of 0.16 ± 0.01. The highest detected concentration of eprinomectin in milk was 9.0 ng/ml, below the maximum residue limit (MRL) of eprinomectin in milk established by the Joint FAO/WHO Expert Committee on Food Additives in 2000. The amount of eprinomectin recovered in the milk during this trial was $0.39\% \pm 0.08\%$ of the total administered dose. This study demonstrates that subcutaneous administration of eprinomectin led to higher bioavailability and a lower dose than a pour-on application, and that an injectable formulation of eprinomectin may be applied in dairy cattle with a zero withdrawal period.

Keywords: eprinomectin, injectable formulation, pharmacokinetics, subcutaneous administration, withdrawal period

Abbreviations: AUC, area under plasma or milk concentration–time curve; C_{max}, maximum concentration; HPLC, high-performance liquid chromatography ; MRL, maximum residue limit; MRT, mean residence time; $t_{\frac{1}{2}ab}$, absorption half-life; $t_{\frac{1}{2}el}$, elimination half-life

INTRODUCTION

Eprinomectin (MK-0397) is a novel avermectin developed by Merck & Co., Inc. (Shoop *et al.*, 1996) derived from the natural product avermectin B1 (abamectin). The selection of eprinomectin for the development programme at Merck was the result of a large screening process based on efficacy, pharmacokinetics, and milk-to-plasma partition behaviour (Shoop *et al.*, 1996). Like ivermectin, eprinomectin provides a broad spectrum of activity against nematodes and arthropods (Barth *et al.*, 1997; Pitt *et al.*, 1997; Holste *et al.*, 1998; Chartier *et al.*, 1999), while it exhibits a higher potency against endoparasites than abamectin, ivermectin, doramectin and moxidectin (Shoop *et al.*, 2001). Furthermore, eprinomectin exhibits a very favourable milk-to-plasma partition ratio (M/P = 0.17), resulting in low residue in milk (Shoop *et al.*, 1996).

Eprinomectin has been developed and licensed in the veterinary pharmaceutical market only as a pour-on formulation. Because of low residues, this product has been applied in beef and dairy cattle as a topical endectocide in the United States, New Zealand, Mexico, the European Community, and others, with zero slaughter withdrawal period and zero milk discard. The pharmacokinetics of eprinomectin in lactating dairy cattle have been reported (Alvinerie *et al.*, 1999b).

Although a pour-on administration of avermectins provides some advantage, such as convenient delivery, it also exhibits several disadvantages: the low value in the plasma concentration (McKellar and Benchaoui, 1996), the significant effect of licking behaviour on the pharmacokinetics (Laffont *et al.*, 2001, 2003), and the large dose (0.5 mg/kg). A subcutaneous administration can avoid these disadvantages. Shoop and colleagues (2001) reported that eprinomectin has full efficacy against mature and immature nematodes in beef cattle by subcutaneous administration. Higher bioavailability by subcutaneous administration of eprinomectin than by a pour-on application has recently been demonstrated in the goat (Alvinerie *et al.*, 1999a; Lespine *et al.*, 2003). These results suggest that eprinomectin may be applied as an injectable product. No pharmacokinetics of eprinomectin in lactating dairy cattle following subcutaneous administration have been reported.

To determine whether eprinomectin can be administered in dairy cattle as an injectable formulation, a novel injectable formulation was developed and was subcutaneously delivered to four lactating dairy cattle at a dose of 0.2 mg/kg. The present study was carried out to determine the plasma and milk profiles of eprinomectin in lactating dairy cattle following its subcutaneous administration and to find out whether this injectable formulation can be applied in dairy cattle with zero withdrawal period.

MATERIALS AND METHODS

Animals, treatment and sampling

Four healthy Black-and-White Holstein lactating dairy cattle, weighing between 600 and 750 kg and 4–6 years old, were used in this study. The animals were kept in a

cowshed in individual stalls and fed on a mixed silage and concentrate diet. No avermectins had previously been administered to these cattle.

Eprinomectin (medicinal grade, provided by Beijing China Agricultural University Biological Technique Limited Corporation, with a purity of 90.5%) was formulated in a glycercol triacetate–glycerol formal (80:20) vehicle as a 1% solution. Eprinomectin was injected subcutaneously to the four dairy cattle at a dose of 0.2 mg/kg of body weight. All the animals were observed for adverse effects and other reactions throughout the experimental period.

Blood samples (10 ml) were collected from the jugular vein into heparinized tubes at 0 (pre-treatment), 7, 16, 30, 40, 55, 64, 80, 90, 105, 114 , 216, 384 and 576 h after treatment. The plasma was separated by centrifugation at 2000*g* for 10 min within 1 h after sampling, and the plasma samples were stored at –20°C until analysis. Milk samples were collected for analysis from each animal at the same time as the blood samples were collected and these were stored at –20°C until analysis. The cows were milked three times by hand each day; the total daily milk production (between 15 kg and 25 kg, with a average of 18.6 ± 6.5 kg) was collected and recorded and an aliquot (15 ml) was stored at –20°C until analysis.

Analytical method

The plasma and milk samples were analysed for eprinomectin concentration by HPLC using a previously published method (Danaher *et al.*, 2001), with the exception of the inclusion of an internal standard. Briefly, 40 μl of an internal standard solution of doramectin at 250 ng/ml was added to 2 ml of plasma or milk. After homogenization, 3.5 ml of methanol (Beijing Chemical Reagent Company, Beijing, China) was added to the sample. After mixing for 15 min, the sample was centrifuged at 3000*g* for 10 min. The supernatant was then applied to an ODS C_{18} SPE cartridge (Dima Technology Inc., Santa Clara, USA) after washing with water (3 ml), followed by 3 ml of methanol–water (70:30, v/v), and the eprinomectin was eluted with 4 ml of methanol. The eluate was collected and evaporated to dryness. The dried extract was dissolved in 200 μl of methylimidazole–acetonitrile (2:7, v/v) (Merck-Schuchardt, Darmstadt, Germany) and 200 μl of triflaoreacetic anhydride–acetonitrile (2:7, v/v) (Merck-Schuchardt) was added. After homogenization, 45 μl of glacial acetic acid (Beijing Chemical Reagent Company) was added. After mixing for 1 min, the extract was incubated in a fan-assisted oven (30 min, 65°C) for derivatization. The sample was then cooled (4°C, 3 min) and left at room temperature (12 min) before an aliquot (100 μl) was injected into the HPLC system. The HPLC system consisted of two Model LC-10ATvp pumps, a DGA-12A degasser, a RF-10Axl detector, a SCL-10Avp system controller and a CTO-10ASvp column oven (Shimadzu, Kyoto, Japan) connected to a data station Class-vp (Shimadzu). The separation was carried out on a stainless-steel analytical column (150×4.6 mm i.d.), packed with ODS C_{18} (5 μm) material (Shimadzu). The mobile phase (methonal–acetonitrile, 2:1, v/v)–water (98:2, v/v) was pumped at a flow rate of 1.0 ml/min. The column temperature was room temperature (20–25°C). The detector was set at an excitation wavelength of 366 nm and an emission

wavelength of 465 nm. Calibration curves for eprinomectin in the range 1–80 ng/ml in plasma and 1–40 ng/ml in milk were prepared using drug-free plasma or milk, respectively. Calibration curves were constructed using the value of eprinomectin peak area divided by doramectin peak area as a function of concentration of eprinomectin. The extraction recovery was 91.4–104.27% in plasma and 83.4–105.65% in milk. The regression coefficients were 0.997 for milk and 0.998 for plasma. The inter-assay precision showed variation coefficients between 6.32% and 9.05% for milk and between 5.48% and 7.62% for plasma. The quantification limit of the method for both milk and plasma was 0.1 ng/ml. The detection limit of the method for both milk and plasma was 0.04 ng/ml.

Data analysis

The plasma and milk concentration–time curves obtained after treatment in each individual animal were fitted with the PK Solutions 2.0 (Ashland, OH, USA) computer software. Pharmacokinetic parameters were determined using a non-compartmental method. For the subcutaneous administration of eprinomectin, the peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration–time curve in each individual animal. The area under the concentration–time curve (AUC) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to time infinity by dividing the last experimental concentration by the terminal slope (λ_z). Statistical moment theory was applied to calculate the mean residence time (MRT) for eprinomectin as follows:

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

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). Eprinomectin plasma and milk concentrations are reported as mean $\pm$SD.

RESULTS

No adverse effects were observed in the experimental animals. The plasma and milk concentrations of eprinomectin in lactating dairy cattle (mean$\pm$SD) following the subcutaneous administration are presented in Figure 1. The individual values for plasma and milk pharmacokinetic parameters are reported in Tables I and II, respectively.

In plasma, eprinomectin was detected at the second sampling. The plasma concentrations increased progressively to reach maximum peak concentrations of 44.0$\pm$24.2 ng/ml. The mean time of peak occurrence was 39.0$\pm$19.3 h for plasma. The highest detected maximum peak concentration of eprinomectin was 70.9 ng/ml

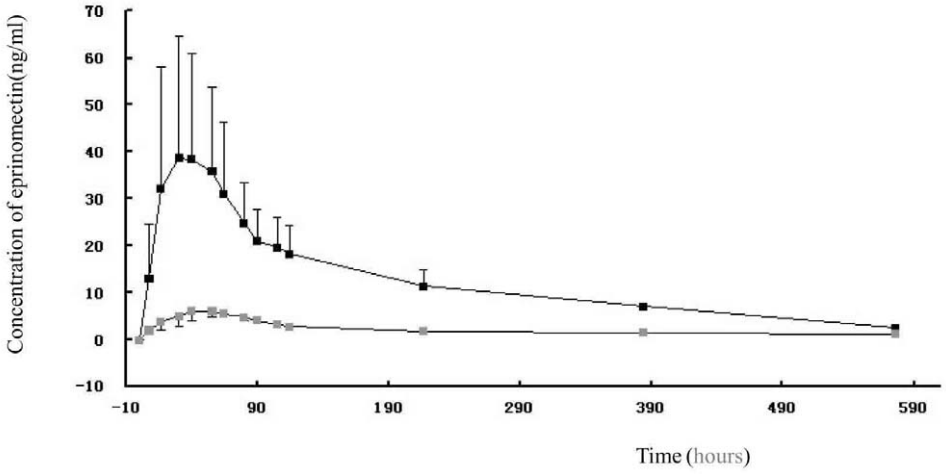

Figure 1. Concentration–time profile of eprinomectin in plasma (black square) and in milk (grey square) of dairy cattle (n = 4) after its subcutaneous administration at 0.2 mg/kg

TABLE I
Pharmacokinetic parameters describing the disposition of eprinomectin in plasma after its subcutaneous administration (0.2 mg/kg) to four lactating dairy cattle

Parameter	Animal number				Mean ± SD
	47	99	200	204	
C_{max} (ng/ml)	22.2	70.9	24.9	58.1	44.0 ± 24.2
T_{max} (h)	55.0	30.0	55.0	16.0	39.0 ± 19.3
$t_{\frac{1}{2}ab}$ (h)	20.0	9.0	12.4	6.4	12.0 ± 5.9
$t_{\frac{1}{2}el}$ (h)	181	122	174	178	164 ± 29.0
$AUC_{0\text{-}576\ h}$ ((ng h)/ml))	6446	10115	6097	6756	7354 ± 1861
$AUC_{0\text{-}inf}$ ((ng h)/ml))	7110	10446	6706	7401	7916 ± 1710
MRT (h)	269	148	258	209	221 ± 55.2

Values are the mean ± standard deviation: C_{max}, observed plasma peak concentration; T_{max}, time to reach C_{max}; $t_{\frac{1}{2}ab}$, half-life of absorption; $t_{\frac{1}{2}el}$, half-life of elimination; MRT, mean residence time; AUC, area under the plasma concentration–time curve

TABLE II
Selected pharmacokinetic parameters of eprinomectin in milk after its subcutaneous administration (0.2 mg/kg) to four lactating dairy cattle

Parameter	Animal number				Mean ± SD
	47	99	200	204	
C_{max} (ng/ml)	5.1	9.0	5.7	5.7	6.4 ± 1.8
T_{max} (h)	55.0	40.0	64.0	40.0	49.8 ± 11.8
$AUC_{0\text{-}576\ h}$ ((ng h)/ml))	1111	1468	1105	1090	1194 ± 183
MRT (h)	571	358	752	1379	765 ± 439
Milk-to-plasma ratio	0.17	0.15	0.16	0.15	0.16 ± 0.01
Dose fraction recovered in milk (%)	0.30	0.48	0.36	0.44	0.39 ± 0.08

(animal no. 99 at 30 h). After reaching the peak values, the plasma concentrations decreased progressively with apparent half-life of 164 ± 29.0 h. The MRT value was 221 ± 55.2 h for plasma. The AUC value was 7354 ± 1861 (ng h)/ml.

Eprinomectin was detected in the milk at the second sampling time. The drug in the milk was parallel to plasma, the highest detected concentration of eprinomectin in milk was 9.0 ng/ml (animal no. 99 at 40 h). The mean peak concentration in milk was 6.39 ± 1.77 ng/ml; this occurred 49.8 ± 11.8 h after administration. After reaching the peak values, the milk concentrations decreased progressively with a mean half-life of 164 ± 29.0h. The MRT value was 765 ± 439 for milk. The AUC value was 1194 ± 183 (ng h)/ml.

There was a significant positive correlation between the eprinomectin concentration profiles in plasma and those obtained in milk. The high correlation coefficient of 0.95 is a good indicator that the plasma concentration reflects the fate of the drug in other compartments. The C_{max} and AUC of eprinomectin in milk in four dairy cattle were significantly lower than those obtained in plasma ($p < 0.05$) (Figure 1). The AUC milk/plasma ratio was 0.16 ± 0.01.

DISCUSSION

The formulation of the compound and the route of administration have a significant effect on the pharmacokinetics of avermectins (McKellar and Benchaoui, 1996; Lo *et al.*, 1985; Lanusse *et al.*, 1997). The pharmacokinetics of eprinomectin in dairy cattle following topical administration have been reported (Alvinerie *et al.*, 1999b). In that study, eprinomectin was topically administered at a dose of 0.5 mg/kg. In the present study, eprinomectin was administered subcutaneously at a dose of 0.2 mg/kg. The

C_{max} in plasma (44.0 ± 24.2 ng/ml) obtained in this study was equivalent to that (43.76 ± 18.23 ng/ml) for pour-on administration. The AUC values obtained in the present study were higher: 7354 (ng h)/ml compared with 5736 (ng h)/ml (Alvinerie *et al.*, 1999b). These results demonstrate that subcutaneous administration of eprinomectin is 2.5-fold more efficacious than topical administration in dairy cattle, in terms of bioavailability. These results are in good agreement with those obtained in goats, which showed a higher C_{max} and AUC of eprinomectin following subcutaneous administration at a dose of 0.2 mg/kg than after topical administration at a dose of 0.5 mg/kg (Dupuy, 2001; Lespine *et al.*, 2003).

The MRT of eprinomectin in plasma was 221 ± 55.2 h after subcutaneous administration. The efficacious subcutaneous dose for eprinomectin necessary to kill 95% of mature and immature nematodes in cattle was 0.056 mg/kg or even lower (Shoop *et al.*, 2001). This long presence of eprinomectin in plasma after the subcutaneous administration would contribute to long persistence of efficacy against endo/ectoparasites.

It is very crucial to ensure that the concentrations of eprinomectin in milk after subcutaneous administration are below the established maximum residue limit (MRL) to attain a zero withdrawal period in lactating dairy cattle. In the present study, the highest detected concentration of eprinomectin in milk was 9.0 ng/ml (animal no. 99). This value is below the MRL for milk (20 ng/ml), established by FAO/WHO (2000). The result indicates that this injectable formulation can be applied to dairy cattle with zero withdrawal.

Finally, the levels of milk/plasma partitioning (0.16 ± 0.10) obtained in this study were low; only a small fraction of subcutaneously administered eprinomectin (0.39% of the total administered dose) was excreted in milk. The structure of eprinomectin may contribute to this finding (Shoop *et al.*, 1996).

In conclusion an injectable formulation of eprinomectin has been developed. This preliminary assay indicates that this formulation could be applied to lactating dairy cattle by subcutaneous administration with a zero milk withdrawal period.

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REFERENCES

Alvinerie, M., Lacoste, E., Sutra, J.F. and Chartier, C., 1999a. Some pharmacokinetic parameters of eprinomectin in goats following pour-on administration. *Veterinary Research Communications*, **23**, 449–455

Alvinerie, M., Sutra, J.F., Galtier, P. and Mage, C., 1999b. Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. *Research in Veterinary Science*, **67**, 229–232

Barth, D., Hair, J.A, Kunkle, B.N., Langholff, W.K., Lowenstein, M., Rehbein, S., Smith, L.L., Eagleson, J.S. and Kutzer, E., 1997. Efficacy of eprinomectin against mange mites in cattle. *American Journal of Veterinary Research*, **58**, 1257–1259

Chartier, C., Etter, E. and Pors, I., 1999. Activity of eprinomectin in goats against experimental infections with *Haemontus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Veterinary Record*, **144**, 99–100

Danaher, M., O'Keeffe, M., Glennon, J.D. and Howells, L., 2001. Development and optimisation of an improved derivatisation procedure for the determination of avermectins and milbemycins in bovine liver. *The Analyst*, **126**, 576–580

Dupuy, J., Chartier, C., Sutra, J.F. and Alvinerie, M., 2001. Eprinomectin in dairy goats: dose influence on plasma levels and excretion in milk. *Parasitology Research*, **87**, 294–298

Gibaldi, M. and Perrier, D., 1982. *Pharmacokinetics*, 2nd edn, (revised and expanded), (Marcel Dekker, New York), 45–109

Holste, J.E., Colwell, D.D. and Kumar, R., 1998. Efficacy of eprinomectin against *Hypoderma* spp in cattle. *American Journal of Veterinary Research*, **59**, 56–58

Laffont, C.M., Alvinerie, M., Bousquet-Melou, A. and Toutain, P.L., 2001. Licking behaviour and environmental contamination arising from pour-on ivermectin for cattle. *International Journal of Parasitology*, **31**, 1687–1692

Laffont, C.M., Bousquet-Melou, A., Bralet, D., Alvinerie, M., Fink-Gremmels, J. and Toutain, P.L., 2003. A pharmacokinetic model to document the actual disposition of topical ivermectin in cattle. *Veterinary Research*, **34**, 445–460

Lanusse, C., Lifschitz, A., Virkel, G., Alvarez, L., Sanchez, S., Sutra, J.F., Galtier, P. and Alvinerie, M., 1997. Comparative plasma disposition kinetics of ivermectin, moxidectin and doramectin in cattle. *Journal of Veterinary Pharmacology and Therapeutics*, **20**, 91–99.

Lespine, A., Sutra, J.F., Dupuy, J. and Alvinerie, M., 2003. Eprinomectin in goat: assessment of subcutaneous administration. *Parasitology Research*, **89**, 120–122

Lo, P.K., Fink, D.W., Williams, J.B. and Blodinger, J., 1985. Pharmacokinetic studies of ivermectin: effects of formulation. *Veterinary Research Communications*, **9**, 251–268

McKellar, Q.A. and Benchaoui, H.A., 1996. Avermectins and milbemycins. *Journal of Veterinary Pharmacology and Therapeutics*, **19**, 331–351

Pitt, S.R., Langholff, W.K. and Eagleson, J.S., 1997. The efficacy of eprinomectin against induced infections of immature (fourth larval stage) and adult nematode parasites in cattle. *Veterinary Parasitology*, **73**, 119–128

Shoop, W., Michael, B., Egerton, J., Mrozik, H. and Fisher, M., 2001. Titration of subcutaneously administered eprinomectin against mature and immature nematodes in cattle. *Journal of Parasitology*, **87**, 1466–1469

Shoop, W.L., Egerton, J.R., Eary, C.H, Haines, H.W. and Michael, B.F., 1996. Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. *International Journal of Parasitology*, **26**, 1237–1242

WHO/FAO, 2000. Joint FAO/WHO Expert Committee on Food Additives. *Residues of Some Veterinary Drugs in Animals and Foods*, (WHO, Rome), 118

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