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The pharmacokinetics of eprinomectin in sheep following subcutaneous administration

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ABSTRACT

The pharmacokinetics of eprinomectin was determined in lactating sheep following subcutaneous administration at a dose rate of 0.2 mg kg^{-1}. The eprinomectin concentration in plasma was determined using High Performance Liquid Chromatography (HPLC) with a fluorescence detector. The kinetics of plasma concentrations were analysed using the non-compartment model. The maximum plasma concentration of 24.44 ng/mL occurred 2 days post-administration. Following subcutaneous administration of eprinomectin in sheep, the value of plasma elimination half-life, the area under the plasma concentration time curve (AUC) and mean residence time (MRT) were 388.52 ± 0.25 h. 4282.10 ng.h/mL and 374.50 h, respectively. The values of $Vd_{(area)}$, $Vd_{(ss)}$ and $Cl_{(B)}$ were 20.50 mL/kg, 13.70 mL/kg and 0.04 mL/h/kg, respectively. This study demonstrates that subcutaneous administration of eprinomectin led to higher bioavailability and longer mean residence time with a lower dose than a pour-on application, and that an injectable formulation may be applied in lactating sheep with zero withdrawal period.

Key words: eprinomectin, pharmacokinetics, subcutaneous administration, lactating sheep

Introduction

Eprinomectin is widely used anthelmintic drug belonging to avermectins of macrocyclic lactones. Eprinomectin is a relatively new endectocide, produced from *Streptomyces avermitilis* fermentation. Eprinomectin provides a higher potency against endoparasite than abamectin, ivermectin, doramectin and moxidectin (SHOOP et al., 2001). Eprinomectin is a novel avermectin, selected for development as a topical and injectable

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endectocide in animals. It was selected after an examination of several hundred analogues, because it possessed the most potent broad-spectrum activity against nematodes (SHOOP et al., 1996a). Eprinomectin is the only endectocide approved for use during lactation with a zero milk-withdrawal period in cattle. Furthermore, eprinomectin exhibits a very favorable milk-to-plasma partition ratio (M/P = 0.17), resulting in low residue in milk (SHOOP et al., 1996b). SHOOP et al. (2001) have reported that eprinomectin has full efficacy against mature and immature nematodes in beef cattle by subcutaneous administration. The higher bioavailability of eprinomectin by subcutaneous administration than by a pour on application has recently been demonstrated in small ruminants (ALVINERIE et al., 1999b; LESPINE et al., 2003).

The pharmacokinetics of eprinomectin following topical administration have been described in both lactating and non-lactating bovine species or breeds, such as Prim Holstein (ALVINERIE et al., 1999a), Indian Zebu cattle (BENGONE-NDONG et al., 2006) Water Buffaloes (DUPUY et al., 2008) and Aubrac cattle (non-lactating, LUMARET et al., 2005). Pharmacokinetic parameters varied in different bovine species or breeds. The pharmacokinetics of eprinomectin following subcutaneous administration in plasma and milk in lactating cows have also been reported (BAOLIANG et al., 2006) and in goats (LESPINE et al., 2003) but there have been no reports of the pharmacokinetics of eprinomectin in plasma following subcutaneous administration in lactating sheep. The present study was planned to investigate the plasma pharmacokinetics of eprinomectin (0.2 mg/kg body mass, single dose, s/c administration) in lactating sheep.

Materials and methods

Experimental animal. Animals of the Patanwadi breed of sheep from the Sheep and Goat Research Station, SDAU, Sardarkrushinagar, were included in the present study. Six healthy lactating sheep (*Ovis aries*) having body weight between 25-35 kg and 2-4 years of age were randomly selected for the study. The sheep were housed in a loose housing shed system with a sandy floor in the Sheep and Goat Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The sheep were maintained as per the Sheep and Goat Research Station maintenance schedule. Water was made available ad libitum and free from any contaminants. The animals were kept under constant observation for two weeks prior to the beginning of the experiment. All necessary management practices were followed so the sheep remained free from stress and diseases. In this period they were subjected to clinical examinations in order to exclude the possibility of any disease. Six lactating sheep were utilized for the single dose subcutaneous administration pharmacokinetic study of eprinomectin.

Chemicals. Eprinomectin injectable solution and Eprinomectin pure powder 10 gram (Intas Pharmaceuticals Ltd., Ahmedabad, India) were utilized in the study.

Water, methanol, acetonitrile, acetic acid (glacial 100%), trifluoroacetic anhydride and N-methylimidazole of analytical- grade reagent were purchased from S. D. Fine Chem. Ltd, India.

Drug administration and sample collection. Eprinomectin (INTAS Pharmaceutical Ltd, Gujarat, India) was injected at a dose rate of 7.5 mg/kg b.w. by subcutaneous routes in each lactating sheep. 4-5 mL of blood samples were collected from the jugular vein with the help of an intravenous catheter in test tubes containing heparin (anticoagulant) at 0 min (pre-administration), 4 and 8 h and 2, 3, 4, 5, 6, 7, 8, 13, 15, 20 and 24 days post administration of the drug. All the samples were centrifuged at 2000 rpm for 15 min and plasma stored at −20 °C until analysis.

Analytical method. The plasma samples were analysed for eprinomectin concentration using the method described by SUTRA et al. (1998). Briefly, 0.75 mL of acetonitrile and 0.25 mL of water were added to 1 mL of plasma. After mixing for 20 min, the samples were centrifuged at 2000 rpm for 2 min. The supernatant was then applied to a Supelco C18 cartridge after washing with water (2 mL), followed by 1 mL of water: methanol (75: 25; v/v), and the eprinomectin eluted with 1.0 mL of methanol. The elute was evaporated to dryness under a gentle nitrogen stream. The dry extract was dissolved in 100 µL of 1-N-methylimidazole solution in acetonitrile (1:1; v/v) and 150 µl of trifluoroacetic anhydride in acetonitrile (1:2; v/v) were added to initiate the derivatization. After mixing, an aliquot (100 µL) was injected directly into the chromatographic system. Mobile phase A was prepared by mixing 4 mL glacial acetic acid in 1000 mL distilled water. Mobile phase B was acetonitrile. The mobile phase was filtered by a 0.22 µ filter and degassed by sonicator and then pumped into the column at a flow rate of 1.0 mL/min at ambient temperature. Detection was performed at an excitation wavelength of 355 nm and an emission wavelength of 465 nm.

The gradient profile of the mobile phase from mobile phase A and mobile phase B are given below:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	60	40
5	20	80
6	20	80
8	60	40
10	60	40

Calibration graphs in the range 2 to 100 ng/mL were prepared for eprinomectin using drug-free plasma. The spiked samples were taken throughout the procedure, and calibration graphs were constructed using the peak area as a function of analyte concentration. The

correlation coefficients (R2) for the calibration curve in the plasma were 0.999 in sheep. The limit of detection (LOD) of the method for plasma was 0.93 ng/mL.

Data analysis. The plasma concentration-time curves for each individual animal were created with PK Solution 2.0 computer software (Summit Research Services, Ashland, OH, USA). Pharmacokinetic parameters were determined using a non-compartmental method. The peak concentration (C_{max}) and the time to the maximum concentration (Tmax) were read from the plotted concentration-time curve for each 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 with the terminal slope (λz). The mean plasma residence time (MRT) of eprinomectin was calculated using the arithmetic trapezoidal rule (GIBALDI and PERRIER, 1982). Pharmacokinetic values are presented as mean ± standard error (SE).

Results

Table 1. Pharmacokinetic parameters of eprinomectin in plasma after single dose subcutaneous administration (0.2 mg/kg body mass) in lactating sheep (Mean ± SE, n = 6)

Pharmacokinetic parameters [a]	Unit	Mean ± SE
B	ng/mL	5.92 ± 0.16
Ka	/h	0.06 ± 0.00
$t_{1/2ka}$	h	12.11 ± 0.26
$t_{1/2\beta}$	h	388.52 ± 0.25
AUC	ng.h/mL	4282.10 ± 23.58
$Vd_{(area)}$	mL/kg	20.50 ± 0.42
$Vd_{(ss)}$	mL/kg	13.70 ± 0.28
C_{max}	ng/mL	24.44 ± 0.08
T_{max}	H	48.00 ± 0.00
Cl_B	mL/h/kg	0.04 ± 0.004
MRT	h	374.50 ± 0.47

[a] Kinetic parameters as described by Gibaldi and Perrier (1982). T_{max}, the time to reach peak or maximum plasma concentration; $t_{1/2ka}$, distribution half life; $t_{½\beta}$, elimination half life; AUC, total area under plasma concentration - time curve; MRT, mean resident time; $Cl_{(B)}$, total body clearance of drug; $Vd_{(area)}$, volume of distribution; $Vd_{(ss)}$, volume of distribution of drug at steady-state; C_{max}, the peak or maximum plasma concentration.

The plasma concentrations (Mean ± SE) of eprinomectin in relation to time in healthy lactating sheep after single dose (0.2 mg/kg body weight) subcutaneous administration are presented in Fig. 1. The plasma concentrations increased with the advancement of time at the beginning and reached their peak level (24.44 ± 0.08 ng/mL) on day 2. The plasma

concentrations, however, showed a gradual declining trend thereafter reaching the lowest levels (2.12 ± 0.21 ng/mL) on day 24 of the experiment. The pharmacokinetic parameters calculated from plasma concentrations of eprinomectin after single dose subcutaneous administration (0.2 mg/kg body weight) in lactating sheep are presented in Table 1.

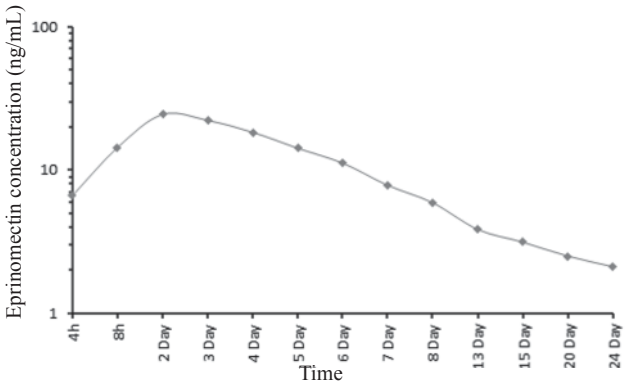

Fig. 1. Semi-logarithmic plot of concentration-time profile of eprinomectin in the plasma of lactating sheep (n = 6), following subcutaneous administration of eprinomectin (0.2 mg/kg)

Discussion

Following single dose, subcutaneous administration of eprinomectin at the rate of 0.2 mg/kg body mass, the mean peak plasma level was 24.44 ± 0.08 ng/mL achieved on day 2 and the lowest detectable plasma level of eprinomectin was 2.12 ± 0.21 ng/mL detected on day 24. Comparatively higher values of peak plasma as 44.0 ng/mL were found at 39 h following eprinomectin administration (0.2 mg/kg body weight) in lactating dairy cattle (BAOLIANG et al., 2006). The plasma eprinomectin concentrations measured at various time intervals in sheep following single dose subcutaneous administration, given at the rate of 0.2 mg/kg body weight, were employed for the calculation of pharmacokinetic parameters viz; elimination half-life, apparent volume of distribution, volume of distribution at steady state, total body clearance and mean residence time. The semi-logarithmic plot of the plasma drug concentration, as a function of time following single dose subcutaneous administration of eprinomectin, was plotted to study the ascending and descending trends of eprinomectin concentration in the body, as well as to construct pharmacokinetic parameters by the ready-to-use computer based non-compartmental technique. This was also used by LESPINE et al. (2003) and BAOLIANG et al. (2006) to calculate pharmacokinetic parameters in goats and lactating dairy cattle, respectively.

The effective dosage of an endectocide depends on the formulation of the compound and its route of administration, pharmacokinetic behavior and pattern of metabolism. These factors determine the plasma concentration-time profile of the drug at the site of action. The influence of these factors varies due to differences in the chemical structure of the drugs and the animal species (McKELLAR and GOKBULUT, 2012). Eprinomectin, doramectin and ivermectin are members of the macrocyclic lactone family of antiparasitic drugs, and they show some similar structural features, as well as an apparent common mode of action and efficacy against the most important parasites of animals. While structural similarities may impart a similar mode of action, structural differences lead to variations in dosages and toxicity, and pharmacokinetic differences may explain certain differences in activity and efficacy (LANUSSE et al., 1997). It is generally accepted that the effect of a drug may be more closely related to the systemic area under the curve (AUC) than to the actual dose administered. The observed area under the plasma concentration curve for eprinomectin (4282.10 ± 23.58 ng.h/mL) in the present study after subcutaneous administration was greater than that of ivermectin (115.50 ± 43.00 ng.d/mL) or doramectin (168.00 ± 41.70 ng.d/mL) in animals that received a dose of 0.5 mg/kg by topical administration (GAYRARD et al., 1999). Greater values of AUC than 7916 ± 1710 ng.h/mL (mean ± SD) were reported by BAOLIANG et al. (2006) in lactating cattle after subcutaneous administration of eprinomectin. The larger AUC observed for eprinomectin may result from the high value of bioavailability of eprinomectin by subcutaneous administration.

The pattern of eprinomectin absorption expressed by the values of absorption half-life ($t_{1/2ka}$) was calculated to be 12.10 ± 0.26 h. A similar $t_{1/2ka}$ of 12.0 ± 5.9 (mean ± SD) has been reported in lactating cattle after subcutaneous administration of eprinomectin (BAOLIANG et al., 2006). Comparatively slightly higher values of absorption half-lives of eprinomectin were reported in lactating dairy cattle as 0.66 day (ALVINERIE et al., 1999a). The observed peak plasma eprinomectin concentration (C_{max}) was much lower than that obtained in lactating dairy cattle (44.0 ng/mL; BAOLIANG et al., 2006). The value of t_{max} for eprinomectin in the present study was higher (48 h) than that reported in dairy cattle (39.0 h by BAOLIANG et al., 2006).

The elimination half-life ($t_{1/2\beta}$) expresses the overall rate of elimination of a drug and allows prediction of drug retention in the body. The rates of the processes that contribute to the termination of drug action are determined by the chemical and physical properties of the drug and its interaction with the specialized issues responsible for elimination (GOLDSTEIN et al., 1994). In the present experiment, the elimination half-life of eprinomectin (388.52 ± 0.25 h) was greater than the values of $t_{1/2\beta}$ as 164 ± 29.0 h reported in lactating cattle (BAOLING et al., 2006) following subcutaneous administration of eprinomectin. The time required for an intact drug molecule to transit through the body is termed as mean residence time (MRT). The mean residence time of eprinomectin

following its subcutaneous administration in the present study was calculated to be 374.50 ± 0.47 h. Lower values of eprinomectin MRT as 221 ± 55.2 h have been reported by BAOLING et al. (2006) in lactating dairy cattle. Mean residence time integrates all the steps governing the fate of the drug in the body: absorption, distribution and elimination, and it is the most pertinent parameter for the comparison of drug persistence. This long presence of eprinomectin in the plasma of sheep after its subcutaneous administration (0.2 mg/kg) would contribute to long persistence of efficacy against endo-ectoparasites in this animal.

It may be concluded that valuable pharmacokinetic data of eprinomectin in plasma of lactating sheep are generated following its subcutaneous administration. This long presence of eprinomectin in plasma after the subcutaneous administration would contribute to long persistence and higher bioavailability of efficacy against endo/ectoparasites. Eprinomectin is the only endectocide approved for use during lactation, with a zero milk withdrawal period.

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SAŽETAK

Farmakokinetika eprinomektina određivana je u ovaca u laktaciji nakon potkožne primjene u dozi od 0,2 mg/kg. Njegova koncentracija u plazmi utvrđena je visoko učinkovitom tekućinskom kromatografijom s fluorescentnim detektorom. Kinetika koncentracija u plazmi analizirana je na osnovi modela bez odjeljaka. Najveća koncentracija u plazmi od 24,44 ng/mL dokazana je dva dana nakon primjene. Poluživot eliminacije iz plazme iznosio je 388,52 ± 0,25 sati, površina ispod krivulje koncentracije u plazmi (AUC) 4282,10 ng/h/mL, a prosječno vrijeme zadržavanja iznosilo je 374,50 sati. Vrijednost prividnog volumena raspodjele, $Vd_{(area)}$, iznosila je 20,50 mL/kg, prosječni vidljivi volumen raspodjele, $Vd_{(ss)}$, 13,70 mL/kg, a ukupni klirens 0,04 mL/sat/kg. Istraživanje je pokazalo da potkožna primjena eprinomektina pruža veću bioraspoloživost, duže vrijeme zadržavanja s manjom dozom u odnosu na veliku te da se injekcijska formulacija može primijeniti u ovaca u laktaciji.

Ključne riječi: eprinomektin, farmakokinetika, potkožna primjena, laktacija, ovca