Original Research Article

Pharmacokinetics of Ivermectin (Noromectin®) Following Single Dose Subcutaneous Administration in Sheep

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A B S T R A C T

The present study was undertaken to evaluate the pharmacokinetics of Ivermectin (Noromectin®) 0.2 mg/kg b.wt in Sheep. The study was conducted in four adult, indigenous male muzzafarnagari sheep (1.5- 2.5 yrs in age, weighing 23±2 kg). The plasma concentration of ivermectin was determined by HPLC. The decay in plasma concentration of drug was biexponential in sheep. The C_{max} value of 17.5 ng/ml was obtained at T_{max} of 3.3 days in sheep, following SC administration of Noromectin®. The elimination half life (β_HL), volume of distribution (V1_F) and AUC were calculated as 6.4 days, 4.19 L.kg^{-1}, 159.3 day.ng/mL in sheep , following SC administration of Noromectin®. A dosage regimen of 0.2 mg/kg at 7 days interval is recommended in sheep.

Introduction

Macrolide endectocides have revolutionized chemotherapy, made an effective contribution to agriculture [1,2] and benefited both animals and humans. Anthelmintic drugs such as the Benzimidazoles, Levamisole, Pyrantel and Morantel had changed management strategies for optimizing nematode control in livestock and companion-animal veterinary practice since the 1960s. The introduction of Ivermectin in 1981 elevated worm control to new levels.

Ivermectin was first marketed in 1981 by Merck Sharp and Dohme as an antiparasitic agent [3], and it remains the leading worldwide antiparasitic agent for livestock.

It has exceptional potency against endo- and ectoparasites at extremely low doses (doses recommended are expressed as µg/kg); this accounts for its large margin of safety. Toxicity to ivermectin is rare across animal species. The signs of toxicosis are mydriasis and depression, followed by ataxia, recumbency, and death. It has no adverse effects on breeding performance. Many rumino-reticular delivery systems, as well as oral, topical, and injectable formulations of

ivermectin, are currently available at the dosage recommended by manufacturers, namely, 200 µg/kg in ruminants (500 µg/kg for topical application) and equines, 300 µg/kg in pigs, and 6 µg/kg in dogs. Its use has revolutionized the treatment of nematode and arthropod parasites in animals and has provided hope for the control or even eradication of filariases in humans [4]. All important gastrointestinal and lung nematodes are susceptible to the drug, including sensitive mites [5], ticks [6], biting flies, and parasitic dipteran larvae [7].

The pharmacokinetic parameters of Ivermectin vary extensively and in accordance with many factors that can all influence the drug's plasma concentration. These factors, which include the species, route of administration, vehicle used in the commercial formulation, bodyweight, body condition, physiological status, and amount and type of nutrition, create difficulties when extrapolating data from one species to another and should be considered in clinical practice in order to achieve effective levels that will last as long as possible. The purpose of present study was undertaken to elucidate disposition kinetics and dose regimen of Ivermectin in sheep. The purpose of the present study was to determine the pharmacokinetics and dosage regimen of Ivermectin following single dose subcutaneous (SC) administration.

Materials and Methods

Experimental animals

The present study was conducted in four adult, indigenous male muzzaffarnagari sheep (1.5-2.5 yrs in age, weighing 23±2 kg). Sheep for this study were procured from Department of Livestock production and Management of college of veterinary and animal sciences, Pantnagar. All these animals were housed in animal house of department of Veterinary

Pharmacology and Toxicology and kept on pre-experimental period for one month before the commencement of experiment to acclimatize them to new environment. Physical and clinical examination was done before the start of experiment. The animals were reared under uniform management and husbandry conditions, maintained on standard ration and water provided *ad libitum*. The animals were kept under constant observation before the commencement of the experiment.

Ethical approval

Institutional Animal ethics committee principles were followed strictly throughout the course of this study. Animals were handled gently and carefully. Deworming was done one month before the start of experimentation with the help of fenbendazole which was given at the rate 5mg/kg body weight.

Instruments used

HPLC system (Shimadzu Corporation, Kyoto, Japan, Model RF-10A$_{XL}$, LC10AT) comprised of double plunger pump, Rheodyne injector with 20 µl loop, Fluorescence detector, C$_{18}$ reverse phase column (Lichrospher 100 RP-18, 5µm (125 mm x 4 mm) with a guard column (Lichrospher 100 RP-18e (5µm), Merck Kga A, 64271 Darmstadt, Germany, Hamilton Syringe, Manufactured by Hamilton (Co., RE No. Nevada, USA) volume 20µl, to load the sample into the injector, Refrigeration Centrifuge machine

Drugs and chemicals used

Pure technical standard Ivermectin (Sigma Aldrich Ltd), Methanol (HPLC grade), Acetonitrile (HPLC grade), Water (HPLC grade), Trifluoroacetic anhydride (Avra®), 1-methyl imidazole (HIMEDIA®), Heparin (Loba Chemie®), Acetic acid (HPLC grade).

Estimation of ivermectin

Injectable formulation of Noromectin® (M/s Norbrook Laboratories Ltd.,) was used in the study. Pharmacokinetic study of Ivermectin was conducted following a single dose (0.2 mg kg^{-1}) Subcutaneous (SC) injection in neck region of Sheep. The blood samples were collected from jugular vein of calves in heparinized microcentrifuge tubes by disposable plastic syringes at time interval of 0 min, 15 min, 30 min, 1h, 3 h, 6 h, 12 h, 1 day, 3 day, 6 day, 9 day and up to 42 days. The blood samples collected in heparinized tubes following administration of Ivermectin were centrifuged at 5000 rpm (15 min) for separation of plasma. The plasma thus obtained was collected in micro centrifuge tubes and stored at -20°C till further analysis. An intervening wash out period of one month was given to all the animals before commencement of new experiment.

Extraction and Derivatization of Ivermectin from plasma samples

Extraction of plasma samples was carried out as per the method described by Perez *et al*. (2007) [8] and Na-Bangchang *et al*. (2006) [9] with slight modifications. 1 ml of acetonitrile and 0.25 ml of deionised water was added to 1 ml of plasma sample, vortex mixed for 20-30 seconds and centrifuged at 12,000g for 12 minutes (4°c).

The supernatant was transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 30-40°c. The residue was subjected to derivatization according to the method of De Montigny *et al*. (1990) [10]. The residue was dissolved in 100 µL of 1-methylimidazole solution in acetonitrile (1:2 v/v). To initiate the derivatization, 150 µL of Trifluoroacetic anhydride solution in acetonitrile (1:2 v/v) was added. After completion of the reaction (< 30 s), an aliquot (20 µL) of this solution was injected directly

in to HPLC. The isocratic mobile phase consists of acetic acid (0.2% in water), methanol, and acetonitrile (4:32:64, v/v/v). The flow rate was kept at 0.7 ml.min^{-1} at a temperature of 30°C with fluorescence detection at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. Ivermectin was quantified from its respective retention time.

Preparation of standard curve

The standards for Ivermectin were made by dissolving 1 mg of pure Ivermectin in 1 ml of methanol from which concentrations of 100, 50, 25, 10, 5, 1 ng.ml^{-1} were made in methanol. 20 µl of these concentrations was injected into HPLC system and quantified under the HPLC conditions mentioned above. The standard calibration curve for Ivermectin was obtained by plotting concentrations *versus* mean of the peak areas obtained for their respective standards. The limit of quantification (LOQ) for Ivermectin was 1ng.ml^{-1}. The method for Ivermectin was found to be linear and reproducible in the concentrations ranging 100 to 1 ng.ml^{-1}. A retention time of 24 .1 min for Ivermectin was observed (Figure-1).

The concentrations of the Ivermectin standard were made in drug free plasma as 100, 50, 25, 10, 5, 1 ng.ml^{-1} applying serial ten times dilution (100 µl standard + 900 µl drug free plasma) of 1000, 500, 250, 100, 50, 10 ng.ml^{-1} of standard in methanol, in equal volumes of drug free plasma, each time.

The extraction from plasma was done by the same procedure as mentioned earlier. The areas obtained by chromatography were plotted against concentration in order to get a standard calibration curve. Recovery of the drug was done by deproteinizing the plasma having above mentioned drug concentration. Recovery percent of Ivermectin from plasma was 83.2.

Pharmacokinetic analysis of data

The plasma concentrations and pharmacokinetic variables of Ivermectin were expressed as mean ± S.E. The pharmacokinetic analysis of the plasma concentration obtained following SC administration of Ivermectin in this study was done by pharmacokinetic software "Phasight WinNonlin" version 5.3.

The plasma concentration-time profile following single dose (0.2 mg.kg^{-1}) subcutaneous administration of Ivermectin (Noromectin®) in sheep is depicted in Figure 2.

The plasma samples were collected up to 42 days. The concentration of Ivermectin could be detected only up to 18 days. . The mean peak plasma concentration was 17.5±0.13 ng.ml^{-1} which attained at 3.3 day post administration which decreased slowly to a minimum of 2.076±0.03 ng.ml^{-1} at 18th day.

The pharmacokinetic parameters describing the disposition kinetics of Ivermectin (Noromectin®) following single dose (0.2 mg.kg^{-1}) subcutaneous administration are presented in Table 1.

Results and Discussion

A two-compartment model adequately (r=0.91) described the plasma concentration-time profile of Noromectin® in sheep following single dose subcutaneous administration.

The mean values of zero_time intercept of distribution phase (A) and elimination phase (B) in the present study were calculated to be 4494.1±1097.4 ng.mL^{-1} and 0.3±0.126 ng.ml^{-1}, respectively. The elimination rate constant of first phase (K$_{10}$) and second phase (β) were 0.2±0.003 and 0.1±0.018 day^{-1}, respectively, with an elimination half-life of first phase (K$_{10}$_HL) and second phase (Beta_HL) calculated as 2.3±0.028 and 6.4±1.19 day, respectively. The transfer rate constant from central to peripheral compartment (K$_{12}$) and from peripheral to central compartment (K$_{21}$) were 0.005±0.00 and 0.1±0.01 day^{-1}, respectively. The volume of distribution of central compartment (V1_F; when fraction of drug absorption is not known), and volume of distribution of peripheral compartment (V2_F; when fraction of drug absorption is not known) were 4194.6±38.3 and 124.1±91.14 ml.kg^{-1} respectively. The clearance from central compartment (CL_F; when fraction of drug absorption is not known) and clearance from peripheral compartment (CLD2_F; when fraction of drug absorption is not known) were estimated as 1254.9±5.73 and 10.1±6.45 ml.kg^{-1}.day^{-1} respectively. The rate constant of distribution phase (α) was 0.3±0.001 day^{-1} with distribution half-life (Alpha_HL) of 2.2±0.014 day. The rate constant of absorption phase (K01) was 0.3±0.002 day^{-1} with absorption half-life (K01_HL) of 2.3±0.019 day. The mean area under curve (AUC) was 159.3±0.72 ng.ml^{-1} day.

A two-compartment model adequately described the plasma concentration-time profile of Noromectin® sheep following single dose (0.2 mg.kg^{-1}) SC administration in the present study. The values of C$_{max}$ in the present study were 17.50 ng.ml^{-1} in sheep following SC administration of Noromectin®. These findings could be well corroborated with C$_{max}$ in sheep 16.3 ng/mL[11]

The higher peak plasma C$_{max}$ level compared to present study has been reported in cattle (42.8, 46.4 and 40 ng/mL;[12,13 &14] respectively), sheep (41.2 and 30 ng/mL; [15 & 16]respectively), pigs (39.6;[13]), horses (51.3 ng/mL; [17]) and dogs (44.3 ng/mL; [18]).

Table.1 Table.1 Pharmacokinetic parameters of Ivermectin (Noromectin®) in plasma following single dose (0.2 mg.kg-1) subcutaneous administration in sheep (n=4)
Pharmacokinetic Parameters: Symbols & Units

Parameters	Units	Sheep Number				Mean±SE
		I	**II**	**III**	**IV**	
V1_F	ml/kg	4298.956	4115.414	4172.299	4191.940	4194.6±38.3
K01	1/day	0.301	0.304	0.293	0.303	0.3±0.002
K10	1/day	0.289	0.305	0.304	0.297	0.2±0.003
K12	1/day	0.006	0.005	0.007	0.002	0.005±0.00
K21	1/day	0.072	0.151	0.140	0.109	0.1±0.01
AUC	day.ng/ml	160.546	158.823	157.584	160.575	159.3±0.72
K01_HL	Day	2.298	2.275	2.360	2.286	2.3±0.019
K10_HL	Day	2.391	2.265	2.278	2.33	2.3±0.028
Alpha	1/day	0.298	0.307	0.304	0.3008	0.3±0.001
Beta	1/day	0.070	0.151	0.140	0.107	0.1±0.018
Alpha_HL	Day	2.321	2.257	2.278	2.304	2.2±0.014
Beta_HL	Day	9.816	4.581	4.943	6.418	6.4±1.19
A	ng/ml	4669.011	5913.479	1337.116	6056.468	4494.1±1097.4
B	ng/ml	0.56	0.336	0.003	0.515	0.3±0.126
CL_F	ml/day/kg	1245.747	1259.263	1269.163	1245.523	1254.9±5.73
V2_F	ml/kg	391.127	15.061	0.208	90.127	124.1±91.14
CLD2_F	ml/day/kg	28.451	2.287	0.029	9.853	10.1±6.45
Tmax	Day	3.353	3.273	3.345	3.322	3.3±0.017
Cmax	ng/ml	17.280	17.824	17.324	17.669	17.5±0.13

Figure.1 Peak of Ivermectin (retention time = 24.1 minutes) in plasma
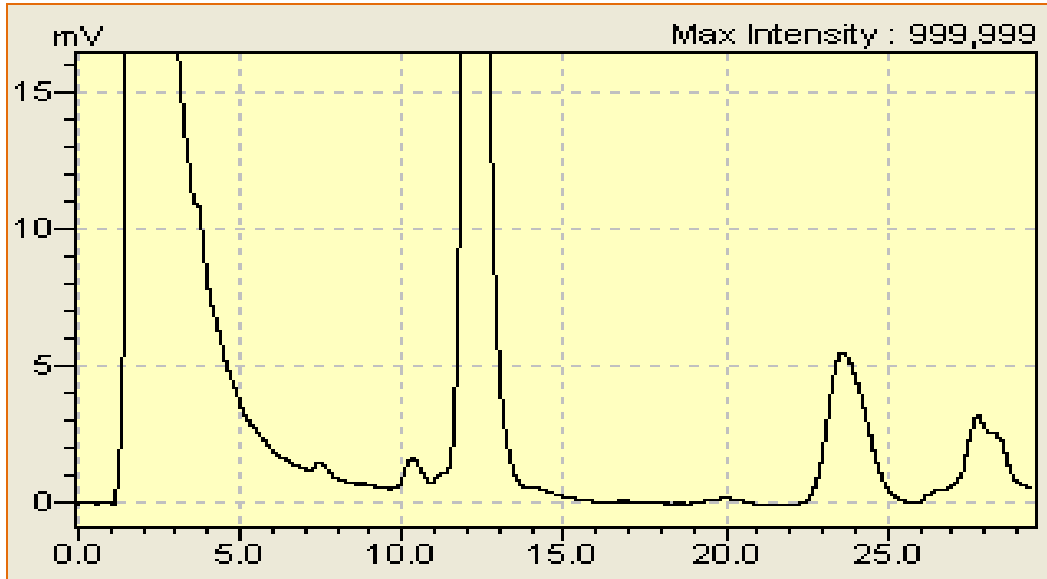
Figure.2 Plasma concentration-time plot of observed concentration (mean) Vs predicted profile of Ivermectin (Noromectin®) following single dose (0.2 mg.kg^{-1}) subcutaneous administration in sheep (n=4)
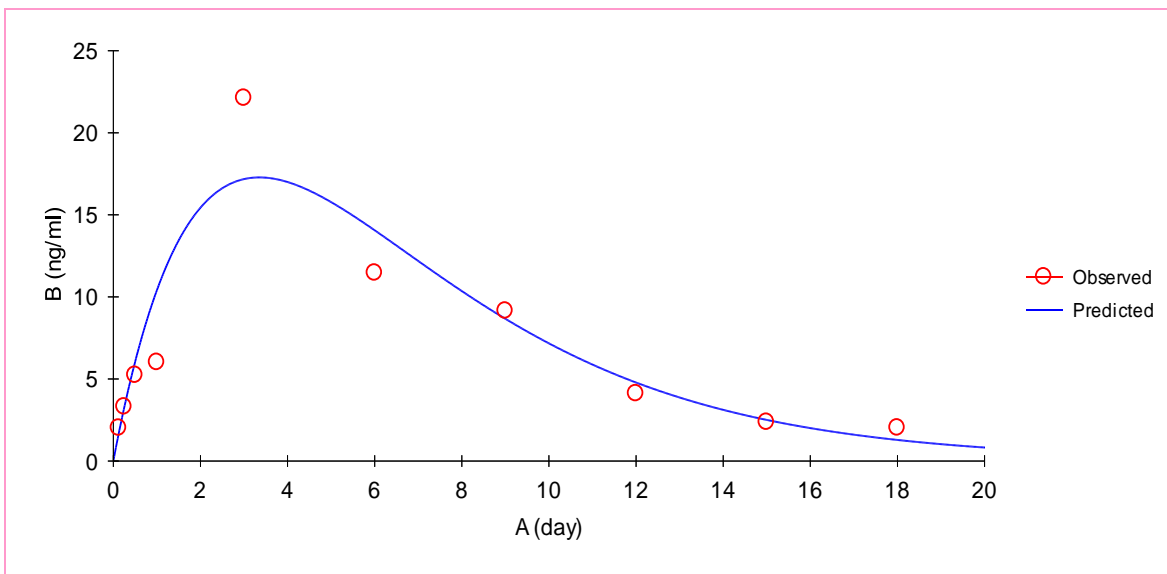
Pharmacokinetic Parameters: Symbols & Units

V1_F	L.Kg-1	Volume of distribution of central compartment when fraction of drug absorption is not known
K01	day-1	Rate constant of absorbtion phase
K10	day-1	Elimination rate constant of first phase
K12	day-1	Transfer rate constant from central to peripheral compartment
K21	day-1	Transfer rate constant from peripheral to central compartment
AUC	ng.day.ml-1	Total area under the curve (from time zero to infinity)
K01_HL	Day	Absorption half life
K10_HL	Day	Elimination half life of first phase
α	day-1	First order rate constant; Regression coefficient for the distribution phase of the disposition curve
β	day-1	Regression coefficient for the elimination phase, it is the terminal slope of the least-squares linear regression line through a plot of the natural logarithm of plasma-serum conc. (lnC) versus time (t)
Alpha_HL	Day	Distribution half-life
Beta_HL	Day	Elimination half-life of second phase
A	ng.ml-1	Zero time intercept of plasma concentration of distribution phase
B	ng.ml-1	Zero-time intercept of plasma concentration of elimination phase
CL_F	L.kg-1.day-1	The clearance from central compartment when fraction of drug absorption is not known
V2_F	L.Kg-1	Volume of distribution of peripheral compartment when fraction of drug absorption is not known
Tmax	Day	Time to reach peak plasma concentration
Cmax	ng.ml-1	Peak plasma concentration

However, lower peak plasma C_{max} level compared to present study has been reported in sheep as (12.2, 16 and 11.9 ng/mL; [19,20 & 21], respectively), goats (9.3 and 6.1 ng/mL; [22,23], respectively).

The lower plasma levels in sheep in the present study may be due to a wider distribution rather than to faster elimination. Injectable product has the advantage that higher maximum plasma concentration are achieved and, thus presumably (by gradient diffusion) greater skin penetration and ectoparasiticidal activity, whereas the oral product is more easily administered and may have greater activity against some intestinal nematodes.

The value of T_{max} in the present study was 3.3 days in sheep following SC administration of Noromectin®. These findings could be well corroborated with T_{max} (3.4 days) in cattle [24] and goats (3 days; [25]).

A lower level of T_{max} (2.25 days) compared to present study has been reported in cattle [26], sheep (1.7 and 1.24 days; [27 & 28], respectively). However, higher level of Tmax (15 days) has been reported in cattle [29]. The difference in the value of T_{max} in the present study could be due to the species variation.

The mean elimination half-lives in the present study was 6.4 days in sheep following SC route of administration of Noromectin®. These findings of mean elimination half-lives could be corroborated with (7.4 days) in goats [25].

The higher mean elimination half-lives compared to present study has been reported (17.2 days) in cattle [12], in sheep (9.6 days; [30]). However, lower mean elimination half-lives compared to present study has been reported (1.18 days) in pigs[31]

Volume of distribution is a measure of extravascular distribution of a drug and higher values would always be advantageous for therapeutic purposes indicating excellent tissue penetration. In the present study, the volume of distribution (V1_F) was 4.19 L.kg⁻¹ in sheep following SC administration of Noromectin®.

These findings could be well corroborated with volume of distribution (4.6 L.kg⁻¹) in sheep[32]. Distribution in the sheep is faster and wider than in cattle or dogs [32] due to substantial deposition into adipose tissue, which may act as a drug depot [33]. The larger fat reservoir in sheep compared to cattle could contribute not only to the more extensive distribution but also the greater persistence in plasma at lower concentrations, probably because less blood is supplied to fatty tissues [11].

A lower volume of distribution (1.2 L.kg⁻¹) compared to present study has been reported in cattle[34], goats (2.8 L.kg⁻¹; [25], pigs (2.7 L.kg⁻¹; [31]) and sheep (3 L.kg⁻¹; [30]). However, higher volume of distribution compared to present study has been reported (8.8 L.kg⁻¹) in sheep [15], and goats (12.8 L.kg⁻¹, [25]).

The AUC is the parameter that integrates both time and intensity of drug concentration. The area under the concentration time curve characterizes the relative availability of drug in the body [35]. The area under curve (AUC) in the present study was 159.3 ng.ml⁻¹day in sheep following SC administration of Noromectin® respectively. The findings of the present study could be compared with that reported (153 ng.ml⁻¹day) in goats [25], pigs (165 ng.ml⁻¹day;[13] and cattle (149 ng.ml⁻¹day; [32]).

However, higher area under curve (AUC) compared to present study has been reported in cattle (459, 595.1 and 328.8 ng.ml⁻¹ day; [12],[20 & 34], respectively), horses (550.4 ng.ml⁻¹ day; [6]), sheep (197,175 and 207.5 ng.ml⁻¹day; (30, 16 & 15), respectively). The lower area under curve (AUC) compared to present study has been reported (121.5 ng.ml⁻¹day) in cattle[19], sheep (64, 82.1 and 74.6 ng.ml⁻¹day;[21, 28 & 16] respectively), goats (60 and 34.4 ng.ml⁻¹day; [23 & 22], respectively), pigs (71.41 and 85.7 ng.ml⁻¹day; [36 & 37], respectively) horses (137.1 ng.ml⁻¹day, [17]), donkeys (119.3 ng.ml⁻¹day; [38]) and dogs (4.5 ng.ml⁻¹day; [18]).

Plasma clearance of drug is the volume of the blood or plasma cleared of drug by metabolism and excretion per unit of time. It is a better index of efficiency of drug elimination than half-life as it gives the clearance of drug from blood per unit of time [5].The values of clearance in this study were 1.25 L.kg⁻¹.day⁻¹ in sheep following SC administration of Noromectin®. The findings of the present study could be compared with that plasma clearance observed (1.11 L.kg⁻¹.day⁻¹) in sheep [30] and goats (1.56

L.kg^{-1}.day^{-1};[25]).

The higher plasma clearance compared to present study has been reported (3.24 L.kg^{-1}.day^{-1}) in sheep [27]), and pigs (4.15 L.kg^{-1}.day^{-1}, [31]).

However, lower plasma clearance compared to present study has been reported in cattle (0.27, 0.35 and 0.48 L.kg^{-1}.day^{-1}; [20,39 & 12], respectively) and sheep (0.56 L.kg^{-1}.day^{-1}; [33]).

Ivermectin persists in the body for a prolonged period, not only due to low plasma clearance but also due to the accumulation in fat tissue. Plasma clearance appears to be greater in pigs than in (goats > sheep > cattle) polygastric species (4).

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