Pharmacokinetics of Ivermectin (Ivermic Super®) following Single Dose Subcutaneous Administration in Cattle Calves

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Abstract
The present study was undertaken to evaluate the pharmacokinetics of Ivermectin (Ivermic super®) 0.2 mg/kg b.wt in cattle calves. The study was conducted in four cross-bred male cattle calves (1.0-1.5 yrs in age, weighing 110±5 kg). The plasma concentration of ivermectin was determined by HPLC. The decay in plasma concentration of drug was biexponential in cattle calves. The Cmax value of 28.18 was obtained at Tmax of 4.7 days in cattle calves, following SC administration of Ivermic super®. The elimination half life (Beta HL), volume of distribution (V1_F) and AUC were calculated as 19.18 days, 2.64 L.kg-1, 370.15 day.ng/mL in cattle calves, following SC administration of Ivermic super®. A dosage regimen of 0.2 mg/kg at 14 days interval is recommended in cattle.

Key words: Cattle calf, Ivermectin, Pharmacokinetics, Subcutaneous

Introduction
Ivermectin was the world’s first endectocide, forerunner of a completely new class of antiparasitic agents, potently active against a wide range of internal and external nematodes and arthropods. It is a semisynthetic derivative of avermectin B1a and consists of an 80:20 mixture of the equipotent homologous 22, 23dehydro B1a and B1b. This antiparasitcagent, developed by Merck & Co., is frequently used in veterinary medicine, due to its broad spectrum of activity, high efficacy and wide margin of safety (1, 2). It is a highly lipophilic substance that dissolves in most Organic solvents, but is practically insoluble in water (0.0004% m/v). Ivermectin was first marketed in 1981 by Merck Sharp and Dohme as an antiparasitic agent (2), and it remains the leading worldwide antiparasitic agent for livestock.

At a dosage of 0.2 mg/kg, Ivermectin has been shown to be highly effective against at least seven spe-cies of gastrointestinal nematodes including the adult and larval stages of Ostertagiaspp, Trichostrongylus spp. (3) Oesophagostomumspp. (4), Haemonchusspp. (3), as well as the lung worm Dictyocaulus viviparus (5). Minimum effective concentration- In cattle, plasma concentrations of 0.5-1 ng/mL are required for optimal anthelmintic activity against most gastrointestinal and lung nematodes (6) plasma concentrations of 0.5 ng/mL also control Hypoderma spp. Flies (7). Maximum effective concentration: Single subcutaneous injection up to 6 mg/kg. Neurological symptoms at 8mg/kg body weight in cattle calves (8)

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 ruminal-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 (9). All important gastrointesti-nal and lung nematodes are susceptible to the drug, including sensitive mites (10), ticks (11), biting flies, and parasitic dipteran larvae (12).

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 present study was undertaken to elucidate disposition kinetics and dose regimen of Ivermectin in cattle calves. 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 cross-bred male cattle calves (1.0-1.5 yrs in age, weighing 110±5 kg). Cross-bred male cattle calves for this study were procured from Instructional Dairy Farm (IDF), 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-10AXL, LC10AT) comprised of double plunger pump, Rheodyne injector with 20 µl loop, Fluorescence detector, C18 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 (LobaChemie®), Acetic acid (HPLC grade)

Estimation of Ivermectin: Injectable formulation of Ivermectin super® (M/s Montajat Vet. Pharmaceuticals 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 cattle. 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,
Pharmacokinetics of Ivermectin

1h, 3h, 6h, 12h, 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.* (13) and Na-Bangchang *et al.* (14) 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.* (15). 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 into 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 1 ng.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 (Fig. 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. The method was linear in the range of 1-100 ng/ml with correlation of coefficient of 0.995. The intra and inter coefficient of variations were 6.5 and 7.2%, respectively.

**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 “PhasightWinNonlin” version 5.3.

**Results**

The plasma concentration-time profile following single dose (0.2 mg.kg⁻¹) subcutaneous administration of Ivermectin(Ivermic super®) in male cattle calves is depicted in Table-1 and Fig. 2. The plasma samples were collected up to 42 days. The concentration of Ivermectin could be detected only up to 33 days. The mean peak plasma concentration was 28.188±0.5 ng.ml⁻¹.
attained at 4.7 days post administration which decreased slowly to a minimum of 2.110±0.03 ng.ml⁻¹ at 33th day. The observed Cmax value mentioned in table 1 is 41.05 ng/ml whereas the value mentioned in text is 28.18 ng/ml which is the calculated value as indicated in table 2. The observed Tmax value mentioned in table 1 is 6 th day , whereas the value mentioned in text is 4.77 th day which is the calculated value as indicated in table 2.

The pharmacokinetic parameters describing the disposition kinetics of Ivermectin (Ivermicsuper®) following single dose (0.2 mg.kg⁻¹) subcutaneous administration are presented in Table-2. A two-compartment model adequately (r= 0.89) described the plasma concentration-time profile of Ivermectin in male cattle calves 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 3465.89±509 ng.mL⁻¹ and 0.315±0.04 ng.ml⁻¹, respectively. The elimination rate constant of first phase (K10) and second phase (Beta) were 0.203±0.001 and 0.037±0.004 day⁻¹, respectively, with an elimination half-life of first phase (K10_HL) and second phase (Beta_HL) calculated as 3.394±0.01 and 19.180±2.71 day, respectively. The transfer rate constant from central to peripheral compartment (K12) and from peripheral to central compartment (K21) were 0.002±0.0006 and 0.038±0.004 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 2648.6±47.01 and 212.999±77.9 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 540.834±9.7 and 7.188±1.65 ml.kg⁻¹.day⁻¹ respectively. The rate constant of distribution phase (a) was 0.207±0.007 day⁻¹ with distribution half-life (Alpha_HL) of 3.34±0.01 day. The rate constant of absorption phase (K01) was 0.212±0.001 day⁻¹ with absorption half-life (K01_HL) of 3.262±0.01 day. The mean area under curve (AUC) was 370.15±6.6 ng.ml⁻¹ day. AUC values, the time interval was o-“

**Discussion**

A two-compartment model adequately described the plasma concentration-time profile of Ivermicsuper® in cattle calves following single dose (0.2 mg.kg⁻¹) SC administration in the present study. The values of Cmax in the present study were 28.18 ng.ml⁻¹ in cattle calves following SC administration of Ivermicsuper®. These findings could be well corroborated with Cmax(33.1 ng/mL) in cattle (16), 32.7 ng/mL in cattle (propylene glycol: glycerol-formal vehicle 60:40 v/v) following SC route of administration (17, 18) have also reported Cmax of 28.5 ng/mL in cattle by intraruminal route of administration. The Cmax in the present study could also be compared with other species viz sheep (32.2 and 30 ng/ml (19 and (20), respectively) and pigs (28.4 ng/mL (21).

A lower peak plasma concentration (Cmax) as compared to the present study has been observed by other workers in cattle using different formulations (22.6, 12.2 and 16 ng/mL (6, 22, 23), respectively). Sheep (24.1, 25.8 and 12.5 ng/mL (24, 25 and 26 respectively). Goats (21.8 and 9.3 ng/mL (27) and (28) respectively). However, higher peak plasma concentration (Cmax) level compared to present study has been reported in cattle (42.8, 133.2, 40 and 39 ng/mL (29, 30, 31 and 18 respectively). Pigs (39.6 ng/mL (32), horses (51.3 ng/mL (33) and dogs (44.3ng/mL(34)). The higher peak plasma concentration (Cmax) in the present study may be attributed to the formulation (propylene glycol: glycerol-formal 60:40 v/v) as a vehicle in the injectable product (Ivermicsuper®). 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
Pharmacokinetics of Ivermectin

administered and may have greater activity against some intestinal nematodes.

The value of $T_{max}$ in the present study was 4.77 days in cattle calves following SC administration of Ivermic super®. These findings could be well corroborated with $T_{max}(4$ days) in cattle (29). A lower value of $T_{max}$ compared to the present study has been reported in cattle (2.25 and 2.32 days (6) and(16), respectively), sheep (1.24 days (35and26) respectively) and goats (3 and 2.85 days (27)and (36), respectively). However, higher level of $T_{max}(15.1$ day) compared to present study has been reported in cattle by sustained release bolus through intraruminal route (18).

The mean elimination half-lives in the present study were 19.18 days in cattle calves following SC route of administration of Ivermic super®. These findings could be well corroborated with mean elimination half-life of 17.2 days in cattle by subcutaneous route(29). However, lower mean elimination half-lives compared to present study has been reported in sheep (9.6 days (37), goats (7.4 days (27)and pigs (1.18 days) (38). The higher mean elimination half-life in the present study could be due to low water solubility of Ivermectin and its precipitation in SC tissues favour slow absorption from the injection site, resulting in a prolonged presence in the bloodstream. Retention in the body is also increased due to slow absorption from the injection site.

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 ($V_{1F}$) was 2.64 L.kg⁻¹ in cattle calves following SC administration of Ivermic super®. These findings could be quite similar with volume of distribution (2.7 L.kg⁻¹) in cattle(39), goats (2.8 L.kg⁻¹) (27) and pigs(2.7, L.kg⁻¹)(38). Due to its high lipophilic nature, Ivermectin is extensively distributed with wide volume of distribution ($V_d$) in all species. Inter-individual variation can also be attributed to differences in body condition, age, sex, and physiological status (35). A lower Volume of distribution (1.2 L.kg⁻¹) compared to present study has been reported in cattle (16). However, higher volume of distribution (3.4 L.kg⁻¹) compared to present study has been reported in cattle (29), sheep (5.3, 3 and 12.8 L.kg⁻¹) (40,37 and(35) respectively).

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 (41). The area under curve (AUC) in the present study was 370.15 ng.ml⁻¹ day in cattle calves following SC administration of Ivermic super® respectively. These findings could be well corroborated with AUC(328.8 and 381.1 ng.ml⁻¹ day (16) and (23) respectively) in cattle. However, higher AUC compared to present study has been reported in cattle (459 and 595.1ng.ml⁻¹ day (29) and(23) respectively), sheep (440 ng.ml⁻¹ day (40), horse (550.4 ng.ml⁻¹ day(11). A lower area under curve (AUC) compared to present study has been reported in cattle (189 and 278 ng.ml⁻¹ day; (6) and (31) respectively).

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 (10). The value of clearance in this study was 0.54 L.kg⁻¹ day⁻¹ in cattle calves following SC administration of Ivermic super®. These findings could be well corroborated with plasma clearance (0.48 L.kg⁻¹.day⁻¹) in cattle (29) and in sheep (0.56 L.kg⁻¹.day⁻¹(40). However, higher plasma clearance compared to present study has been reported in sheep (1.11 and 3.24 L.kg⁻¹.day⁻¹(37) and (35) respectively), goats (1.56 L.kg⁻¹.day⁻¹(27) and pigs (4.15 L.kg⁻¹.day⁻¹(38). A lower plasma clearance compared to present study has been reported in cattle (0.27 and 0.35 L.kg⁻¹.day⁻¹(23) and (39) respectively).

Pharmacokinetics of Ivermectin
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(9).

**Conclusion**

A dosage regimen based on the pharmacokinetic data obtained following SC administration of Ivermectin (Ivermic super®) in adult male cattle was calculated with therapeutic concentration of 1 ng.ml⁻¹ at dosing intervals of 7, 14 and 21 days. Priming doses of 0.18928, 0.24523, and 0.31773 mg.kg⁻¹ and maintenance doses of 0.04318, 0.09915 and 0.1715 mg.kg⁻¹ were calculated (Cₘᵦₚ₈ₑₙ₅₃₈ = 46.30, 20.17 and 11.66 ng.ml⁻¹ respectively, Cₘ₈₃₈ₐₓ₃₈ = 59.99, 33.86 and 25.34 ng.ml⁻¹, respectively) at 7, 14 and 21 days interval, respectively.

**References**


Sakthi Karthikeyan et al


