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Pharmacokinetics of injectable long-acting ivermectin aqueous suspension following subcutaneous administration in sheep

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Ivermectin (IVM), a macrocyclic lactone endectocide, is widely used for the treatment and prevention of nematode and arthropod parasites (Geary, 2005). There are many preparations of IVM commercially used worldwide, such as injectable formulations, premxes, tablets, oral pastes, oral solutions and pour-on formulations (Hu *et al.*, 2000). During treatment, animals, such as sheep, continue to ingest parasite larvae and eggs from the contaminated environment or from infested animals. To reduce the risk of reinfestation, in practice, a second administration several days later may be required to eliminate all the parasites (Campbell, 1989; Bates, 1993). This is inconvenient and expensive in labor and drugs. To solve these problems, developing a long-acting preparation seemed to be a favorable approach, although it may have a negative impact on refugia and may cause resistance. Using a long-acting formulation would enhance parasite control in rotationally grazed pastures and also help to eliminate residual parasites from the pastures (Stromberg & Averbeck, 1999).

Some long-acting formulations of IVM, such as a sustained-release bolus (IVOMEC®SR BOLUS, Merial Ltd, Atlanta, GA, USA), a controlled-release capsule (NUMECTIN 100, Merial Ltd, NSW, Australia; IVOMEC® MAXIMIZER™, Merial Ltd), and a long-acting injection (IVOMEC® GOLD, Merial Ltd, Buenos Aires, Argentina) are commercially available. Although the sustained-release bolus and the controlled-release capsule achieve a considerable long-acting time for drugs, they are not easy to operate and some metal boluses stay in the rumen and damage machinery during the slaughtering process. The reported injectable long-acting formulations do not have these disadvantages, but they are nonaqueous. Compared with water, there are costly.

Solid dispersion (SD) is the combination of a group of solid products consisting of at least two components. SD has been widely used in tablets and capsules to achieve a sustained-release effect (Bi, 2000). To our knowledge, there are no reported uses of SD in injectable formulations. We have now combined IVM and hydrogenated castor oil (HCO) to make a SD to be crushed and suspended in water to provide a long-acting injectable aqueous suspension, which is covered by a Chinese patent (Wang *et al.*, 2006). To determine whether this suspension can achieve sustained release, we studied the plasma profiles of SD IVM following its subcutaneous administration to sheep.

Fourteen 2- to 3-year-old Little Tail Han sheep (seven males and seven females), weighing 30–50 kg on Day 0, were used. All animals were healthy and identified with a numbered plastic ear tag. The animals were raised in pens and fed on drug-free balanced rations *ad libitum* with free access to water. The animals were managed under a protocol approved by the Laboratory Animal Institute of China Agricultural University.

The animals were allocated randomly on the basis of bodyweight into the IVM-CONTROL group (four sheep) or the IVM-TEST group (10 sheep). Each group contained equal numbers of female and male sheep. Animals were administrated a single subcutaneous IVM dose (0.6 mg/kg body weight) with the injectable aqueous suspension (IVM-TEST group, 30 mg/mL IVM suspension) or the commercially available 10 mg/mL IVM injection (LG Life Sciences, Ltd, Seoul, Korea) (IVM-CONTROL group). Blood samples (5 mL) were taken from the jugular vein of each sheep prior to injection (0 h), and at 4, 6, 12, 24, 36 h, and at 2, 3, 4, 6, 11, 16, 21, 40 and 60 days afterwards. The plasma was separated by centrifugation at 2000 ***g*** for 15 min and the plasma samples were stored at −20 °C pending analysis.

The extractions and analysis of IVM from plasma samples followed previously published method (Pan *et al.*, 2004, 2006)

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with a little modification. Ten nanograms of doramectin (DOR) were used as internal standard. IVM and DOR in plasma was extracted with methanol, and purified with ODS C18 SPE cartridge (Supelco, Bellfonte, PA, USA). The elution was collected and evaporated to dryness with a stream of nitrogen. Derivatization of IVM and DOR in dried residue was conducted with *N*-methylimidazole, trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) and acetic acid. Then 555 μL mobile phase was added, and 100 μL of the solution was injected into the chromatograph. The concentrations of IVM were determined by HPLC using a previously described Shimadzu HPLC system (Shimadzu, Kyoto, Japan) (Pan *et al.*, 2006). A mobile phase of water/methanol/acetonitrile (4/64/32, v/v/v) was pumped at a flow rate of 1.0 mL/min through a reverse phase C_{18} column (Shimadzu) at room temperature. The excitation wavelength was at 366 nm and an emission wavelength was at 465 nm. The IVM concentrations were calculated by the internal standard method using the Class VP 5.0 software (Shimadzu).

A calibration curve for IVM in the range 0.5–80 ng/mL was prepared using drug-free plasma and the prepared stock solution (200 ng/mL). The linear regression line had a regression coefficient of 0.9995. The extraction recoveries ($n = 4$) of IVM were 98.2% ± 4.0, 93.2% ± 6.1, and 95.1% ± 4.6, intra-assay coefficients of variation ($n = 4$) were 7.1, 7.8, and 5.2%; and inter-assay coefficients of variation ($n = 4$) were 4.1, 6.5, and 4.8%, respectively. The quantification limit and detection limit for IVM were 0.5 and 0.1 ng/mL ($n = 4$), respectively.

The plasma concentrations vs. time curves obtained after treatment in each individual animal were fitted with the PK Solutions 2.0 (Ashland, Ohio, USA, L& RN PKSW 2449) computer software. Pharmacokinetic parameters were determined using a noncompartmental method following a previously reported method (Pan *et al.*, 2006). Mean plasma concentrations and mean pharmacokinetic parameters of IVM obtained following subcutaneous injection of the two formulations were statistically compared by using unpaired, two-sided *t*-test by statistical software SPSS 10.0 (SPSS Inc., Chicago, IL, USA). The value of $P < 0.05$ was considered significant.

Plasma concentrations of IVM were detected between 4 h and 21 days in the IVM-CONTROL group and between 4 h and 60 days in the IVM-TEST group. The plot of mean plasma concentration vs. time and the comparative results of mean plasma concentrations between the two groups are presented in Fig. 1. The mean values for plasma pharmacokinetic parameters are shown in Table 1. The number of days post-treatment in which plasma concentrations of IVM were >0.5 and 1 ng/mL after the administration of the two formulations are shown in Table 2.

The results presented in Fig. 1 show that following administration of the IVM-TEST formulation, plasma drug concentrations rose slowly but were sustained significantly longer than those obtained using the control preparation. Following injection of 0.6 mg/kg of the IVM-CONTROL formulation, plasma concentrations increased rapidly and with an average of 230.8 ± 109.1 ng/mL at 0.45 days. Then, they declined quickly and were below 0.5 ng/mL by 21 days postadministration. In contrast, the IVM plasma concentrations for IVM-TEST rose more slowly in sheep; with maximum concentrations of about 87.8 ± 46.1 ng/mL being detected at the 1.1 day time point. From the Fig. 1 profile, the plasma drug concentrations of the IVM-TEST formulation were significantly lower ($P < 0.05$) than those of the IVM-CONTROL formulation between 0.17 and 0.5 days following administration but significantly higher at all

Table 1. Comparison of pharmacokinetic parameters for ivermectin (IVM) obtained after subcutaneous administration of the IVM-TEST and IVM-CONTROL formulations to sheep at 0.6 mg/kg

Parameter	IVM-TEST ($n = 10$)	IVM-Control ($n = 4$)	*P*-value
C_{max} (ng/mL)	87.8 ± 46.1	230.8 ± 109.1	0.004
T_{max}(day)	1.1 ± 0.3	0.45 ± 0.10	0.002
$T_{1/2}$ ab	12.6 ± 10.3	0.67 ± 0.39	0.043
$T_{1/2}$ el	18.5 ± 11.5	4.8 ± 3.1	0.041
AUC_{0-t}	644.3 ± 249.1	267.7 ± 111.3	0.014
MRT	21.5 ± 6.6	2.0 ± 1.0	0.000

C_{max}, maximum plasma concentration; T_{max}, time to peak plasma concentration; $T_{1/2}$ ab, absorption half-life; $T_{1/2}$ el, elimination half-life; AUC_{0-t}, area under the concentration vs. time curve between drug administration and 60 days (IVM-TEST) or 21 days (IVM-CONTROL); MRT, mean residence time.

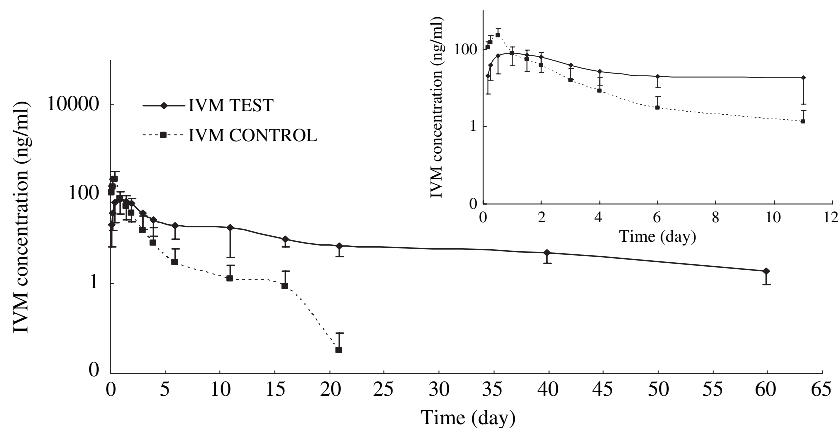

Fig. 1. Mean (±SD) plasma concentrations of ivermectin (IVM) following subcutaneous administration of IVM-TEST ($n = 10$) and IVM-CONTROL ($n = 4$) formulations to sheep (0.6 mg/kg). Error bars indicate SD. Values at all time point except on 1, 1.5, 2 and 3 days are significantly different between the two formulations ($P < 0.05$).

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Table 2. Comparison of the number of days post-treatment during which ivermectin (IVM) plasma concentrations were >0.5 and 1 ng/mL following administration of IVM-TEST and IVM-CONTROL formulation to sheep

	IVM-TEST (n = 10)	IVM-CONTROL (n = 4)	P-value
Days > 0.5 ng/mL	60 ± 0.0	12.3 ± 4.8	<0.001
Days > 1 ng/mL	58 ± 6.3	9.25 ± 5.4	<0.001

points from 4 to 60 days post-treatment. The IVM mean plasma concentrations for IVM-TEST were higher than 1 ng/mL till 60 days but the drug concentrations for IVM-CONTROL formulation were undetectable after 21 days. It has been reported that IVM plasma concentrations between 0.5 and 1 ng/mL could be indicative of the minimal drug level required for optimal anthelmintic activity for most gastrointestinal/lung nematodes (Lifschitz *et al.*, 1999). Following the above results, the estimated persistent period of the IVM-TEST formulation against gastrointestinal/lung nematodes would be near 60 days.

The statistical results revealed that the time to maximum concentration (T_{max}), half-life of absorption ($T_{1/2}$ ab) and half-life of elimination ($T_{1/2}$ el) of IVM-TEST formulation are significantly longer ($P < 0.05$) than those of the IVM-CONTROL formulation. The significantly greater *AUC* indicates that the IVM-TEST formulation had a better bioavailability than the IVM-CONTROL.

The formulation of the compound has a significant effect on the pharmacokinetics of avermectins (Lo *et al.*, 1985; Clark *et al.*, 2004; Lifschitz *et al.*, 2004) and the persistence of the broad-spectrum antiparasitic activity of endectocide compounds relies on their disposition kinetics and pattern of plasma/tissue exchange in the host (Lanusse *et al.*, 1997). Even slight modifications to their plasma/tissue exchange pattern and/or disposition kinetics may result in substantial changes in their concentration and residence time at the site of parasite location which, in turn, would alter the potency and persistence of their antiparasitic activity (Lifschitz *et al.*, 1999). In the experiment reported here, the difference in pharmacokinetic properties between the two IVM formulations confirmed the influence of drug formulation on the disposition and persistence time in plasma.

Therefore, the IVM-HCO solid-dispersion aqueous suspension has considerable potential for the development of an injectable sustained-release formulation which could be subcutaneously administrated to sheep as an endectocide.

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