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Integrated assessment of ivermectin pharmacokinetics, efficacy against resistant *Haemonchus contortus* and P-glycoprotein expression in lambs treated at three different dosage levels

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ABSTRACT

The main goals of the current work were: (a) to assess the ivermectin (IVM) systemic exposure and plasma disposition kinetics after its administration at the recommended dose, x5 and x10 doses to lambs, (b) to compare the clinical efficacy of the same IVM dosages in lambs infected with an IVM-resistant isolate of *Haemonchus contortus*, and (c) to assess the expression of the transporter protein P-glycoprotein (P-gp) in *H. contortus* recovered at 14 days after administration of the IVM dose regimens. There were two separated trials where IVM was administered either subcutaneously (SC, Experiment I) or intraruminally (IR, Experiment II). Each experiment involved twenty-four (24) lambs artificially infected with a highly resistant *H. contortus* isolate. Animals were allocated into 4 groups ($n = 6$) and treated with IVM at either 0.2 (IVM_{x1}), 1 (IVM_{x5}) or 2 mg/kg (IVM_{x10}). Plasma samples were collected up to 12 days post-treatment and analysed by HPLC. An untreated-control Group was included to assess the comparative anthelmintic efficacy of the different treatments. The level of expression of Pgp in *H. contortus* specimens obtained from lambs both untreated and IR treated with the different IVM doses was quantified by real time PCR. Parametric and non-parametric tests were used to compare the statistical significance of the results ($P < 0.05$). After the SC treatment, the IVM plasma area under the concentration–time curve (AUC_{0-LOQ}) increased from 41.9 (IVM_{SCx1}) up to 221 (IVM_{SCx5}) and 287 (IVM_{SCx10})ng.day/mL and after the IR treatment from 20.8 (IVM_{IRx1}) up to 121 (IVM_{IRx5}) and 323 (IVM_{IRx10})ng.day/mL. Dose-adjusted AUC_{0-LOQ} and Cmax were similar among doses, demonstrating dose proportionality for IVM after both SC and IR administration at the three different doses. The efficacies against resistant *H. contortus* after the SC treatment were 42% (IVM_{SC1}), 75% (IVM_{SCx5}) and 75% (IVM_{SCx10}). However, the IR IVM treatment reached clinical efficacies ranging from 48% (IVM_{IRx1}) up to 96% (IVM_{IRx5}) and 98% (IVM_{IRx10}). None of the IR IVM treatments increased the expression of P-gp in adult *H. contortus* at 14 days post-treatment compared to samples collected from the untreated control group. An enhanced

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parasite exposure of the drug at the abomasum may explain the improved efficacy against this recalcitrant *H. contortus* isolate observed only after the IR administration at 5- and 10-fold the IVM therapeutic dosage.

1. Introduction

Ivermectin (IVM) was the first macrocyclic lactone introduced as an antiparasitic drug in 1981 (Campbell, 1989). IVM is effective against endo- and ecto-parasites in a wide variety of hosts, including cattle, sheep, goats, dogs, swine and horses, as well as domesticated wild animals. A large body of scientific literature has been devoted to the pharmacology properties of the macrocyclic lactones, including detailed pharmacokinetic data (Campbell, 1989; Vercruysse and Rew, 2002) in different animals species. However, there is a lack of basic pharmacokinetic information related to drug absorption and dose proportionality studies in target animal species. Dose proportionality occurs when increments in the administered dose are accompanied by a proportional increment in exposure, measured as either the area under the concentration vs time curve (AUC) or the peak plasma concentration (Cmax). Dose proportionality is a desirable property as it makes predicting the effects of dose adjustments easier, although in practice this is not a global property as it usually applies only to a certain dose range (Hummel et al., 2008). The assessment of dose proportionality is of great clinical importance for predicting the consequences of rational dose adjustments. Recent work demonstrated a non dose-proportionality on the gastrointestinal absorption of albendazole (ABZ) in nematode infected lambs, where increasing the ABZ dose (5- to 9-fold) was clearly associated with enhanced plasma ABZ metabolites exposure (Alvarez et al., 2012), which cannot be explained only by a dose proportionality relationship. The enhanced systemic exposure achieved after ABZ treatments at the highest doses correlated with significant increment in drug efficacy against a resistant *Haemonchus contortus* isolate (Barrère et al., 2012). As far as we know, there are no IVM dose proportionality studies undertaken in sheep.

The impact of increasing the IVM dosage levels on its systemic exposure and in the resultant efficacy against IVM-resistant nematodes in ruminants remains unclear. However, it seems that the transcuticular diffusion is the main route of access for different lipophillic substances, including IVM in gastrointestinal nematodes (Cross et al., 1998; Alvarez et al., 2007). Two major determinants of the rate of transfer across the nematode cuticle are drug lipophilicity (Mottier et al., 2003) and concentration gradient (Mottier et al., 2006). Thus, a higher parasite drug exposure induced by a dose increment, may be assessed as a strategy to kill resistant parasites. For instance, Várady et al. (1996) reported efficacies for IVM against *Oesophagostomum dentatum* in pigs of 88.7, 96.1 and 99.6% after its administration at 0.15, 0.3 or 0.6 mg/kg, respectively. This upward trend in favour of the highest IVM doses could be explained by a higher parasite drug exposure related to the administered dose.

The therapeutic response to an increased anthelmintic dose depends on the genetic diversity in the parasite population being exposed to the drug (Prichard, 2001). Evidence derived from parasitic nematodes and the free living nematode *Caenorhabditis elegans* suggest that the permeability glycoprotein (P-gp) and other ATP-binding cassette (ABC) transporters may be involved on IVM resistance (Molento and Prichard, 1999; Kerboeuf et al., 2003; Prichard and Roulet, 2007; James and Davey, 2009; Ardelli and Prichard, 2013). P-gp is a multidrug membrane transporter which acts as an efflux pump to transport hydrophobic compounds, such as drugs/metabolites, across cell membranes (Higgins, 1992). The activation of this defense mechanism in mammals and nematodes is often observed as changes in expression levels (Chin et al., 1990). Thus, the expression level could be used as an indicator of treatment response of a resistant nematode isolate.

The main goals of the current work were: (a) to assess the IVM systemic exposure and disposition kinetics after its subcutaneous (SC) and intraruminal (IR) administrations at the recommended, x5 and x10 doses to lambs, (b) to compare the clinical efficacy of the same IVM dosages in lambs infected with an IVM-resistant isolate of *H. contortus*, and (c) to assess the expression of P-gp in *H. contortus* collected at 14 days after the IR IVM treatments.

2. Materials and methods

2.1. Animals

Parasite free Corriedale lambs (6–7 months old, 25.2 ± 5.6 kg), artificially infected (trial day −41) with an IVM-resistant *H. contortus* isolate (7.000 L_3/animal) were involved in the current trial. The isolate was from a sheep Experimental Unit (Reserva 8, Instituto Nacional de Tecnología Agropecuaria, Balcarce, Argentina) with a parasite control programme based on the intensive use of anthelmintics over many years. The use of IVM several times a year over many years had been documented until 1997 (Entrocasso C., personal communication). It had been previously found that IVM failed to control this *H. contortus* strain. Efficacies of 80% (FECRT, SC treatment, Entrocasso et al., 2008), 0% (controlled test, SC treatment, Lifschitz et al., 2010), 40% (controlled test, IR treatment, Lloberas et al., 2012), 20% (controlled test, IR treatment, Lloberas et al., 2013) and 0% (controlled test, IR treatment, Lloberas et al., in press), were reported for this isolate after IVM treatment (0.2 mg/kg). Forty days after infection (trial day −1), all lambs were checked for faecal egg counts (epg), ear tagged and their individual body weights were