Research in Veterinary Science 88 (2010) 315–320

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Combined subcutaneous administration of ivermectin and nitroxynil in sheep: Age/body weight related changes to the kinetic disposition of both compounds

L. Moreno, L. Ceballos, A. Lifschitz, M. Bistoletti, L. Alvarez, C. Lanusse *

Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Campus Universitario, 7000 Tandil, Argentina
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Accepted 2 October 2009

Keywords:
Ivermectin
Nitroxynil
Disposition kinetics
Age/body weight
Sheep

ABSTRACT

The effect of age/body weight in the plasma disposition kinetics of ivermectin (IVM) and nitroxynil (NTX) after their co-administration as a combined formulation to sheep was studied. Sixteen (16) male sheep were allocated into two experimental groups ($n = 8$ each): (a) high body weight (high bw) (18–20 months old), and (b) low body weight (low bw) (6–8 months old). Animals in both groups were subcutaneously (sc) treated with IVM (200 μg/kg) and NTX (10 mg/kg) using a commercially available combined formulation (*Nitromectin®*, Lab. Ovejero, Spain). Blood samples were taken by jugular venopuncture before (time 0), at 2, 4, 8, 12 h and at 1, 2, 3, 5, 7, 10, 15, 20, 25, 35, 40, 50 and 60 days after administration. Recovered plasma was analysed to quantify IVM and NTX by HPLC. Higher IVM plasma concentrations were measured until 20 days post-administration in "low bw" compared to "high bw" animals, where IVM was recovered up to 35 days post-treatment. The IVM absorption process greatly differed between experimental groups. A significantly higher ($p < 0.01$) C_{max} (36.7 ± 7.52 ng/ml) value was obtained at a delayed ($p < 0.05$) T_{max} (48.0 ± 0.0 h) in light compared to heavy (C_{max}: 8.0 ± 0.80 ng/ml; at 34.0 h) body weight sheep. IVM elimination half-life and mean residence time were significantly shorter in light compared to heavy (older) sheep. NTX mean plasma concentrations were lower in "low bw" compared to those measured in "high bw" sheep, with elimination phases declining up to 60 d post-administration in both experimental groups. The NTX AUC value in "low bw" (1188.5 ± 122.6 μg day/ml) was significantly lower ($p < 0.05$) than that obtained in the "high bw" (oldest) animals (1735.0 ± 155.8 μg day/ml). Shorter NTX elimination half-life and mean residence time ($p < 0.01$) were obtained in the youngest ("low bw") compared to the oldest (high bw) sheep. The work reported here assessed for the first time the disposition of IVM and NTX after their combinated injection to sheep, demonstrating that animal body weight/development greatly affects the kinetic behaviour of both anthelmintic drugs.

1. Introduction

Different pharmaceutical strategies have been developed to broaden the anthelmintic spectrum, particularly in geographic areas where multiple parasitic infections affect livestock production. Combination of drug molecules with different mode of action and complementary spectrum of activity is a modern and challenging approach in parasite control. However, the development of drug combinations requires sound pharmacotechnical support to assure proper drug delivery at the site of injection and adequate absorption patterns. It has been shown that a combined ivermectin (IVM) and nitroxynil (NTX) preparation exerts a broad spectrum of anthelmintic action. IVM is a widely used broad-spectrum macrocyclic lactone (ML) compound from the avermectin family. IVM is extremely effective against adult and larval stages of most gastrointestinal nematodes, lungworms and a variety of ectoparasites in sheep and cattle (Egerton et al., 1979). NTX (4-hydroxy-3-iodo-5-nitrobenzonitrile) is a trematodicidal compound highly effective against adult stages (from 8 weeks post-infection) of the liver fluke *Fasciola hepatica* (Boray and Happich, 1968), which also holds nematotocidal activity against adult and larval stages of *Haemonchus contortus* in sheep and *Haemonchus placei, Oesophagostomum radiatum and Bunostomun phlebotomum in* cattle (Martin, 1997).

Once absorbed, drug molecules are distributed throughout the body in the circulating blood and must diffuse to the different tissues to exert systemic pharmacological effects. Concentrations attained at the tissues depend on the ability of the drug to penetrate capillary endothelium and diffuse across cell membranes (Baggot,

* Corresponding author. Address: Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Campus Universitario, 7000 Tandil, Argentina. Tel./fax: +54 2293 439850.
E-mail address: clanusse@vet.unicen.edu.ar (C. Lanusse).

0034-5288/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2009.10.002

1977), where lipophilicity plays a critical role. Therefore, the tissue distribution process varies widely for different drug molecules (Eichler and Műller, 1998). The pharmacokinetics of the ML is characterized by a long residence of the drug in the animal (Lanusse et al., 1997) due to their extensive tissue distribution, gastrointestinal recycling and low metabolism rate. The host's body condition has been shown to influence the tissue persistence of the ML compounds. A more rapid absorption of IVM and doramectin (DRM) and a lower area under the concentration–time curve (AUC) of both drugs have been reported in steers compared to heifers following their subcutaneous (sc) administration (Toutain et al., 1997). Similar results have also been observed in parasitised sheep with poor body condition compared to healthy uninfected controls (Echeverría et al., 2000). Additionally, the IVM persistence was reduced in pigs fed a restrictive diet compared to those fed a normal grower ration following its sc administration (Craven et al., 2002). This reduced drug persistence was correlated with the smaller fat depot in the animals in poor body condition. Finally, important breed differences have been observed in the absorption pattern and systemic availability of the ML in cattle (Sallovitz et al., 2002; Bengone Ndong et al., 2005; Vercruysse et al., 2008).

NTX is well absorbed after its sc administration to sheep and binds strongly to plasma proteins (~98% of the absorbed fraction), mainly to albumin (Alvinerie et al., 1991a). NTX parent compound is the main analyte recovered in treated animals. Studies carried out in different animal species demonstrated that NTX plasma concentrations attained in the bloodstream are higher than those measured in tissues (EMEA, 1998). The high NTX affinity for plasma proteins may account for a restricted (low) distribution to tissues. Thus, it is expected that body condition and development, among many other host-related factors, would influence the pharmacokinetic behaviour of NTX in a different manner compared to a drug with high volume of distribution such as IVM.

The aim of the present work was to evaluate the plasma disposition kinetics of IVM (model drug with extensive tissue distribution) and NTX (model drug with restricted tissue distribution) after their subcutaneous administration as a combined formulation to sheep of different age/body weight/corporal development. Understanding the influence of host-related factors on the kinetic behaviour of anthelmintic drugs used in combination will be relevant to optimise therapeutic activity (anthelmintic effect) and to assess the impact on tissue residue profiles and withdrawal times.

2. Materials and methods

2.1. Experimental animals and treatment

Sixteen (16) healthy male Corriedale sheep were used. Animals were allocated into two (2) groups ($n = 8$) according to their body weight: low body weight ("Low bw") (28.1 ± 3.2 kg, 6–8 months old) (Group I); and high body weight ("High bw") (51.0 ± 8.0 kg, 18–20 months old) (Group II). The mean body weight between Group I and II were statistically different ($p \leqslant 0.001$). Animals were kept indoor with food and water *ad libitum* during the whole experimental period. All animal procedures and management protocols were approved by the Ethics Committee under approved Animal Welfare policy (act 087/02) from the Veterinary Faculty of the Universidad Nacional del Centro, Tandil, Argentina (http://www.vet.unicen.edu.ar). Animals on both groups were treated with the combined anthelmintic formulation *Nitromectin®* (*Laboratorios Ovejero S.A., León, Spain*) at 200 μg/kg (IVM) and 10 mg/kg (NTX) (maximum volume injected: 2.42 ml) by the sc route (internal face of thigh). The anthelmintic doses were calculated individually according to body weight. Blood samples were taken by jugular venipuncture before (time 0), at 2, 4, 8, 12 h and at 1, 2, 3, 5, 7, 10, 15, 20, 25, 35, 40, 50 and 60 d after administration using 10 ml heparinized Vacutainers® tubes (Becton Dickinson, NJ, USA). Plasma was separated by centrifugation at 2000*g* for 15 min, divided in two aliquots and transferred to plastic tubes and frozen at −20 °C until analyzed by high performance liquid chromatography (HPLC).

2.2. Chemicals

Pure analytical standard of IVM was purchased from Sigma Chemical Company (Saint Louis, MO, USA). The NTX pure standard and the commercial formulation of NTX and IVM (N*itromectin®*) were kindly provided by *Laboratorios Ovejero S.A.*, (*León, Spain*). Acetonitrile and methanol (HPLC grade) were from Sintorgan S.A. (Argentina). Potassium phosphate (HPLC grade) was from Baker (Phillipsburg, USA).

2.3. Analytical procedures

2.3.1. IVM analysis

2.3.1.1. Chromatographic system. IVM was analyzed in plasma by high performance liquid chromatography (HPLC) with fluorescence detection according with the previously described methodology (Lifschitz et al., 2000). A mobile phase of water–methanol–acetonitrile (6:40:54, v/v/v) was pumped in an isocratic way (1 ml/min) into a Shimadzu Chromatography system (Shimadzu Corporation, Kyoto, Japan) through a C_{18} column (BDS Hypersil Thermo, 5 μm, 4.6 mm × 250 mm) placed in an oven at 30 °C. Fluorescence detection (spectrofluorometric detector RF 10, Shimadzu, Kyoto, Japan) was performed at 365 nm excitation and 475 nm emission wavelength.

2.3.1.2. Sample preparation. Plasma samples (1 ml) were placed into a 5 ml plastic tube and spiked with 50 μl of the internal standard (IS) abamectin (2 ng/10 μl). Drug molecules were extracted by addition of 0.5 ml acetonitrile for ten minutes under a high speed vortexing shaker (Multi-tube Vortexer, VWR Scientific Products, West Chester, PA, US). After mixing, the sample was sonicated (Ultrasound Bath, Lab-Line Instrument, Inc., Melrose Park, OL, US) and centrifuged (BR 4i Centrifuge, Jouan®, Saint Herblain, France) at 2000*g* for 10 min at 5 °C. The clear supernatant was transferred to a tube, and the procedure repeated. The total supernatant was transferred to C_{18} cartridges (100 mg, 1 ml, Lichrolut®, Merck, Darmstadt, Germany) using a manifold vacuum (Baker spe-24G, Phillipsburg, US). The cartridges were previously conditioned with 2 ml of methanol, followed by 2 ml of water. All samples were applied and then sequentially washed with 1 ml of water, 1 ml methanol:water (1:4), dried with air for 5 min and eluted with 1.5 ml of methanol. The eluted volume was evaporated (60 °C) to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, CA, US), derivatised (De Montigny et al., 1990) and an aliquot of 100 μl was injected in the chromatographic system.

2.3.1.3. Method validation. IVM was identified with the retention times of 97–99% of a pure reference standard. A complete validation of the analytical procedure for the extraction and quantification of IVM in plasma was performed before the analysis of the experimental samples. The linearity of the method was tested after elaboration of analytical calibration curves for the compound in plasma in two ranges of calibration: 0.1–5 ng/ml and 5–100 ng/ml. The linearity was determined by the lack of fit test (GraphPad InStat, Version 3.00, GraphPad Software, San Diego, CA, US), giving determination coefficients of 0.999. The mean recovery was ⩾70%. Precision was expressed as coefficient of variation (% CV), with mean values ⩽3.0 or ⩽8.1% intra- or inter- assay, respectively. Accuracy of the method expressed as relative error (% RE), had a

mean value ⩽13.2%. The theoretical LOD was defined as the mean baseline noise/IS peak area ratio plus three standard deviations (SD) and the estimated value was 0.03 ng/ml. The limit of quantification (LOQ) was calculated as the lowest drug concentration ($n = 6$) on the standard curve that could be quantitated with precision not exceeding 20% and accuracy within 20% of nominal, and the obtained value was 0.10 ng/ml.

2.3.2. NTX analysis

2.3.2.1. Chromatographic system. Experimental and fortified plasma samples were analyzed for NTX by HPLC with UV detection. A Shimadzu 10 HPLC System (Kyoto, Japan) with a pump, a UV detector set at 270 nm, an autosampler and a controller (Shimadzu Class LC10, Kyoto, Japan) were used. An isocratic mobile phase composed of acetonitrile:potassium phosphate buffer (0.025 mol/l, pH 4) (25:75) at a flow of 1 ml/min, was used to elute the analyte from the stationary phase (5 μm, 250 mm × 4.6 mm, Selectosil C_{18} column, Phenomenex®, USA).

2.3.2.2. Sample preparation. The drug was extracted from an aliquot of plasma (1 ml) by addition of 1.5 ml of acetonitrile. After vortexing for fifteen minutes under a high speed shaker (Multi-tube Vortexer, VWR Scientific Products, West Chester, PA, US), the sample was centrifuged (BR 4i Centrifuge, Jouan®, Saint Herblain, France) at 2000*g* for 10 min at 4 °C. The clear supernatant was transferred to a tube, and the procedure of sample extraction repeated, afterwards the total volume was evaporated (40 °C) to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, CA, US). In order to avoid the saturation of signal detector, the dry extract was reconstituted in different volumes of mobile phase according of the range of concentrations: 300 μl (<2.5 μg/ml), 600 μl (2.5–5 μg/ml), 1.2 ml (5–25 μg/ml) and 1.8 ml (⩾25 μg/ml). Fifty (50) microliters of the reconstituted extract were injected in the chromatographic system.

2.3.2.3. Method validation. The validation of the analytical procedure for the extraction and quantification of NTX in plasma was performed. The validation parameters were calculated similarly to the methodology described for IVM. Plasma calibration curves for NTX were constructed in two ranges of calibration: 0.1–5 μg/ml and 5–130 μg/ml, with determination coefficients (r) ⩾0.997. The extraction efficiency of the analyte was determined at three concentration levels (0.5, 5 and 50 μg/ml) ($n = 6$) with mean absolute recovery ranging between 88.4 and 94.2%. The CV for the intra and inter assay precision (0.5, 5 and 50 μg/ml concentrations, $n = 6$) were ⩽8.49 and 8.87%, respectively. Accuracy of the method was ⩽12.3% (RE). The LOD and LOQ were 0.002 and 0.1 μg/ml, respectively.

2.4. Pharmacokinetic analysis of the data

The concentration versus time curves for IVM and NTX in plasma for each individual animal were fitted with the PKSolutions™ computer program (Summit Research Service, Ashland, USA). The following equation (Notari, 1987) was used to describe the biexponential concentration–time curves for both drugs after the sc treatment:

$$C_p = Be^{-\beta t} - Be^{-kt}$$

where: C_p = concentration (μg/ml) in plasma at time t after administration; B = concentration at time zero extrapolated from the elimination phase (μg/ml); e = base of the natural logarithm; β = terminal slope (h^{-1}); and k is the slope obtained by feathering which represents either the first order absorption rate constant (k_{ab}) (h^{-1}). The peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration–time curve of each analyte. The area under the concentration–time curve (AUC) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (β). Statistical moment theory was applied to calculate the mean residence time (MRT) for IVM and NTX in plasma as follows (Perrier and Mayersohn, 1982):

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}}$$

where AUC is defined previously and AUMC is the area under the curve of the product of time and the plasma drug concentration vs. time from zero to infinity (Gibaldi and Perrier, 1982).

2.5. Statistical analysis of the data

The pharmacokinetic parameters and concentration data are reported as mean ± SEM. The time-based parameters (MRT, $T_{½abs}$, $T_{½el}$) are expressed as harmonic means. Student t and Mann–Whitney Test were used to compare parameters between groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

The comparative IVM plasma concentration profile measured in "low" and "high" bw sheep after its sc injection are shown in Fig. 1. IVM was rapidly recovered in plasma after its sc administration. In "low bw" sheep, IVM was detected in plasma until 20 days post-administration, while in "high bw" animals, the drug was measured in the bloodstream up to 35 days post-administration. The plasma disposition kinetics data for IVM after sc administration in both groups are summarized in Table 1. The IVM plasma concentration profiles greatly differed between experimental groups. A significantly higher ($p < 0.01$) C_{max} (36.7 ± 7.52 ng/ml) value was obtained at a delayed ($p < 0.05$) T_{max} (48.0 ± 0.0 h) in light compared to heavy body weight (C_{max}: 8.0 ± 0.80 ng/ml; at 34.0 h) animals. The differences observed in concentration vs. time profiles between groups were confirmed by the AUC parameter. The AUC value was 71% higher in "low bw" (121.1 ± 30.7 ng day/ml) than in "high bw" (70.8 ± 15.4 ng day/ml) sheep. However, statistical

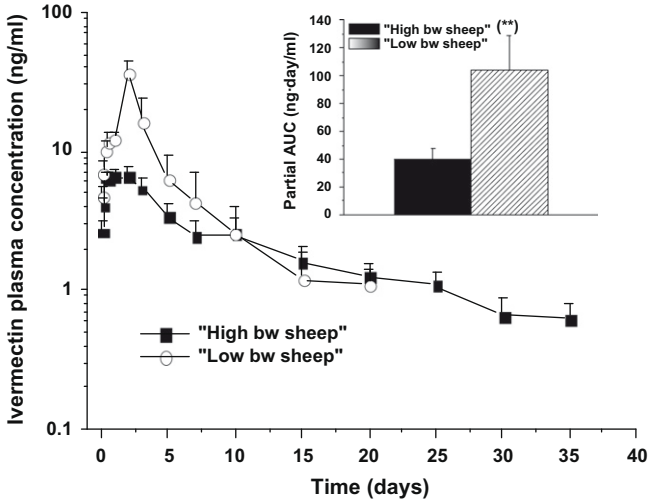

Fig. 1. Mean (±SEM) ivermectin plasma concentration profiles after its subcutaneous administration (200 μg/kg) in combination with nitroxynil (*Nitromectin*®) to sheep with high (18–20 months old) and low (6–8 months old) body weight (bw). The inserted graph represents the mean (±SEM) ivermectin partial AUC values up to 10 days post-treatment in both groups. Statistical differences between groups: $^{*}p < 0.05$, $^{**}p < 0.01$.

Table 1
Mean (±SEM) ivermectin (IVM) pharmacokinetic parameters obtained after its subcutaneous administration (200 μg/kg) in combination with nitroxynil (*Nitromectin*®) in healthy sheep with high (18–20 months old) and low (6–8 months old) body weight.

PK Parameter	Body weight			
	Low (n = 8)		High (n = 8)	
	Mean	SEM	Mean	SEM
$T_{½abs}$ (h)	8.88	4.56	6.48	3.6
C_{max} (ng/ml)	36.7**	7.52	7.98	0.80
T_{max} (days)	2.00	0.00	1.42	0.34
AUC_{total} (ng day/ml)	121.1	30.7	70.8	15.4
$T_{½el}$ (days)	2.61*	0.77	5.07	0.90

$T_{½abs}$: absorption half-life; C_{max}: peak plasma concentration; T_{max}: time to peak plasma concentration; AUC_{total}: area under the concentration vs. time curve extrapolated to infinity; $T_{½el}$: elimination half-life. Statistical differences between groups.
* $p < 0.05$.
** $p < 0.01$.

differences only were found in the partial AUC values (up 10 days) showed in Fig 1. The IVM mean residence time was extended in the heaviest animals, which was reflected in significantly longer $T_{½el}$ (Table 1) and MRT ($p < 0.05$) (see Fig. 3) compared to those obtained in the "low bw" animals.

NTX plasma concentrations were quantified in plasma over 60 days after its sc administration (10 mg/kg) to sheep. The mean plasma concentration vs. time profiles obtained for "high bw" and "low bw" animals are presented in Fig. 2. NTX was measured in plasma as early as 2 h post-injection in animals from both groups. Opposite to that observed for IVM, NTX mean plasma concentrations were higher in "high bw" compared to "low bw" sheep. NTX plasma concentration profiles in animals from both groups declined with high concentrations measured up to 60 days post-administration. The mean pharmacokinetic parameters calculated for NTX in plasma after its sc administration in both experimental groups are summarised in Table 2. The NTX absorption was very rapid (even faster than IVM) in both "low bw" and "high bw" animals. Thus, the C_{max} values (104.4 ± 6.97 and 102.9 ± 3.01 μg/ml) were obtained at an early T_{max} in "low bw" (4.80 ± 0.65 h) and "high bw" sheep (7.20 ± 0.84 h). However, as it can be observed in Fig. 2, the NTX plasma profiles differed between animals with

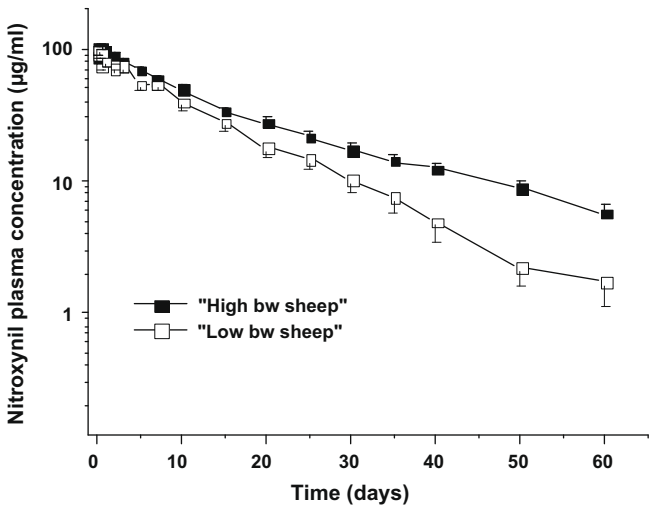

Fig. 2. Mean (±SEM) nitroxynil plasma concentration profiles obtained after its subcutaneous administration (10 mg/kg) combined with ivermectin (*Nitromectin*®) to sheep with high (18–20 months old) and low (6–8 months old) body weight (bw).

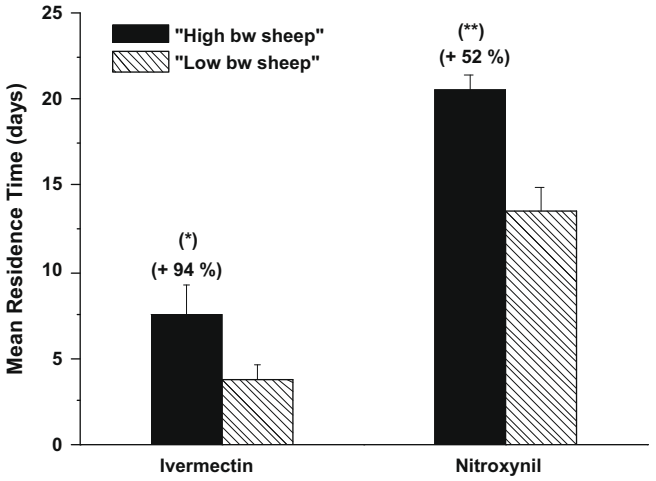

Fig. 3. Comparison of the mean plasma residence time (MRT) for ivermectin and nitroxynil in sheep of different body weight obtained after their combined subcutaneous administration. Values in brackets indicate the percentage of enhancement on the time of drug residence according to the weight of the experimental animals. Statistical differences between groups: * $p < 0.05$, ** $p < 0.01$.

Table 2
Mean (±SEM) nitroxynil (NTX) pharmacokinetic parameters obtained after its subcutaneous administration (10 mg/kg) in combination with ivermectin (*Nitromectin*®) in healthy sheep with high (18–20 months old) and low (6–8 months old) body weight.

PK Parameter	Body weight			
	Low (n = 8)		High (n = 8)	
	Mean	SEM	Mean	SEM
$T_{½abs}$ (h)	3.76*	0.60	9.07	1.94
C_{max} (μg/ml)	104.4	6.97	102.9	3.01
T_{max} (h)	4.80	0.65	7.20	0.84
AUC_{total} (μg d/ml)	1188.5*	122.6	1735.0	155.8
$T_{½el}$ (d)	9.50**	0.94	14.0	0.63

$T_{½abs}$: absorption half-life; C_{max}: peak plasma concentration; T_{max}: time to peak plasma concentration; AUC_{total}: area under the concentration vs. time curve extrapolated to infinity; $T_{½el}$: elimination half-life. Statistical differences between groups.
* $p < 0.05$.
** $p < 0.01$.

different bw. In fact, NTX systemic exposure estimated as plasma AUC values was significantly ($p < 0.05$) lower in "low bw" (1188.5 ± 122.6 μg day/ml) compared to "high bw" (1735.0 ± 155.8 μg day/ml) sheep. Additionally, NTX disposition in the lightest animals was faster, being the $T_{½el}$ (Table 2) and MRT (see Fig. 3) values significantly ($p < 0.01$) shorter (9.50 ± 0.94 and 13.5 ± 1.37 days) than in "high bw" animals (14.0 ± 0.63 and 20.5 ± 0.96 days).

The correlation analysis between individual animalś body weight and AUC values for both IVM and NTX are shown in Fig. 4.

4. Discussion

Neither irritation at the site of injection nor clinical adverse reactions was observed after the sc injection of the IVM + NTX combined formulation to sheep. Both molecules were rapidly recovered in plasma, being detected as early as 2 h post-treatment. Large variability in the measured IVM concentrations was observed among animals, which is consistent with the large inter- and intra-species variability in the ML pharmacokinetic disposition previously reported (Scott and McKellar, 1992; Andrews et al., 1993; McKellar and Benchaoui, 1996; Lanusse et al., 1997; Cerkvenik

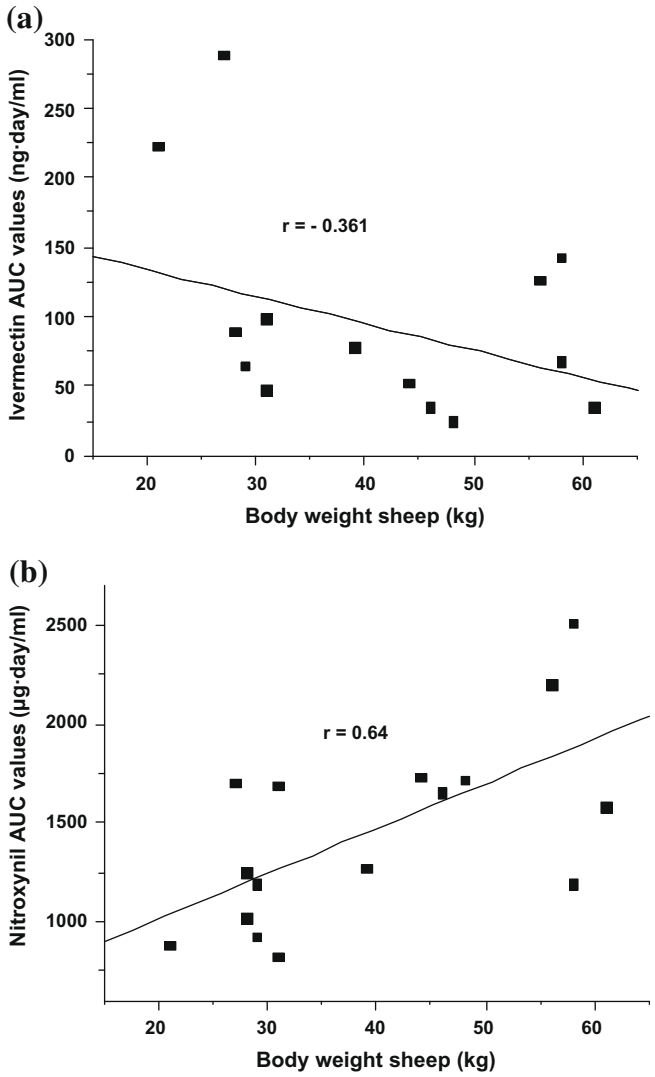

Fig. 4. Relationship between sheep body weight (kg) and drug systemic availability (expressed as AUC values) for ivermectin (a) and nitroxynil (b). *r*: correlation coefficient.

et al., 2002; Moreno et al., 2008). Recent pharmacokinetic studies following sc administration of IVM at the recommended dose (0.2 mg/kg) of the classical formulations have been performed in sheep (Cerkvenik et al., 2002; Barber et al., 2003; Imperiale et al., 2004; El-Banna et al., 2008). Although those kinetic assessments were carried out in sheep of different breed, the IVM AUC values reported were similar to that observed in "high bw" animals in the current work.

Differences on body weight/age influenced the IVM disposition after its sc administration to healthy sheep. Although the total IVM AUC value did not reached statistical differences between experimental groups, the partial AUC0–10 days value estimated in "high bw" sheep was significantly lower than that observed in "low bw" animals (see Fig. 1). A reduced absorption rate may have accounted to explain the lower partial AUC assessed up to 10 days post-treatment in the heaviest animals. A different fat infiltration grade at the site of sc tissue injection may affect the IVM absorption rate. Fatty tissues could constitute a lipophilic drug reservoir, from which IVM may be slowly absorbed. Additionally, a higher tissue fat content in the heaviest animals could facilitate a more extensive distribution process of the lipophilic IVM molecule, which can be observed in the differential shape of the plasma concentration–time curve obtained in "high" compared to "low bw" animals. However, all these speculative arguments should be proven following the intravenous administration of the combined anthelmintic formulation, which was not performed in the experimental work described here.

The extended IVM persistence in the heaviest sheep correlated with the prolonged $T_{½el}$ and MRT values (see Table 1 and Fig. 3, respectively). A significantly longer MRT and a lower C_{max} for IVM was obtained in "high bw" compared to "low bw" sheep (see Table 1 and Fig. 3), which may be explained by an extended tissue distribution in the "high bw" animals, due to their higher fat tissue content. Again, this hypothesis cannot be confirmed due to the lack of a pharmacokinetic study involving the intravenous administration of the combined formulation. However, in agreement with the results reported here, IVM-treated pregnant sheep had significantly extended MRT values than those observed in non pregnant animals with lower body weight (Pérez et al., 2007). An enhanced distribution facilitated by the higher body fat content in pregnant animals may have accounted for such a finding. Similarly, the intravenous administration of IVM in pigs with different body conditions was longer MRT in "fat" compared to "thin" animals (Craven et al., 2001).

High NTX concentrations were rapidly measured in plasma after its sc injection in both experimental groups. Thus, some individual concentration–time profiles looked as a typical intravenous disposition pattern rather than plasma profiles from a sc treatment. The available information on NTX pharmacokinetic properties is scarce. In agreement with the results reported here, high NTX plasma concentrations have been measured in sheep (Alvinerie et al., 1991a), cattle and rabbits. NTX residues in plasma are always higher than those found in tissues and consisted almost entirely of the parent drug (EMEA, 1998). When NTX was administered to sheep, at the same dose and route used in the current trial, shorter $T_{½el}$ (7.4 days) and plasma residence time (48 days) were reported (Alvinerie et al., 1991b). The animal age/body weight affected the NTX disposition after its sc administration to sheep. High NTX mean plasma concentrations were measured in animals from both experimental groups up to 60 days post-administration. However, oppositely to that observed for IVM, the NTX systemic exposure (systemic availability) was higher in "high" than in "low bw" sheep. These differences were reflected in AUC, where a higher value was obtained in the heaviest animals. The assessment of the NTX intravenous kinetics will be required to further interpret the observed changes between animals of different age/body weight.

Age/body weight differences affected the pharmacokinetic behaviour of both IVM and NTX in a different way. While the correlation between body weight and drug available in plasma (systemic exposure) was directly proportional for NTX (see Fig. 4b), this correlation was inversely proportional for IVM (see Fig. 4a).

The successful combination of two powerful active ingredients expands their individual activity resulting in a complementary long persistence broad-spectrum antiparasitic formulation. IVM plasma concentration between 0.5 and 1 ng/ml have been reported as the minimal drug level required for optimal anthelmintic activity for most gastrointestinal/lung nematodes (Lifschitz et al., 1999). Mean IVM concentrations were above 0.5 ng/ml from 2 h up to 20 days post-treatment in "low bw" sheep, and up to 35 days post-injection in "high bw" sheep. Thereafter, some differences on the persistence of the anthelmintic activity could be expected between animals with different age/body weights.

On the other hand, the differences found in IVM and NTX plasma disposition among animals with different body weight/corporal development are probably associated to differences on the patterns of tissue residue depletion. It has been suggested that doramectin withdrawal times estimation may need to be adjusted between

non-parasitised and parasitised lambs with poor body condition (Pérez et al., 2008). Therefore, the results of the current work are useful to show how differences in body condition in healthy animals may influence the estimation of safe withdrawal periods. This could impact on the implementation of residue control strategies for food safety. However, to corroborate this statement, further studies should be carried out to evaluate the tissue depletion of IVM and NTX in animals with different body weight.

In conclusion, the work reported here assessed for the first time the kinetic behaviour of IVM and NTX co-administered in sheep. Interestingly enough, the results demonstrated how the animal body weight/corporal size may affect in a different manner, the disposition kinetics of two anthelmintic drugs with different physico-chemical features. This is a further evidence of a need for deeper investigation before drug combinations are introduced into the pharmaceutical market.

References

Alvinerie, M., Floc'h, R., Galtier, P., 1991a. Plasma protein binding of nitroxynil in several species. Journal of Veterinary Pharmacology and Therapeutic 14, 170–173.

Alvinerie, M., Perez, C., Sutra, J.F., Flor, R., Bayle, R., Galtier, P., 1991b. Disposition of [^{14}C]-nitroxynil in sheep. In: Proceeding of the fifth EAVPT International Congress. Copenhagen, Denmark.

Andrews, S.J., Ferrari, M.M., Pow, J.D.E., Lancaster, M.B., 1993. Nematode egg output and plasma concentration of ivermectin after its administration to reed deer (*Cervus elaphus*). Veterinary Record 132, 161–163.

Baggot, J., 1977. Principles of Drug Disposition: The Basis of Veterinary Clinical Pharmacology. W.B. Saunders, Philadelphia, PA. pp. 1–134.

Barber, S., Bowles, V., Lespine, A., Alvinerie, M., 2003. The comparative serum disposition kinetics of subcutaneous administration of doramectin, ivemectin and moxidectin in the Australian Merino sheep. Journal of Veterinary Pharmacology and Therapeutic 26, 343–348.

Bengone Ndong, T.B., Kane, Y., Ba, M., Sane, I., Sutra, J., Alvinerie, M., 2005. Pharmacokinetics of ivermectin in zebu Gobra (Bos indicus). Veterinary Parasitology 128, 169–173.

Boray, J.C., Happich, A., 1968. Standardised chemotherapeutical tests for immature and mature Fasciola hepatica infections in sheep. Australian Veterinary Journal 44, 72–78.

Cerkvenik, V., Grabnar, I., Skubic, V., Doganoc, D.Z., Beek, W.M.J., Keukens, H.J., Drobnič Košorok, M., Pogačnik, M., 2002. Ivermectin pharmacokinetics in lactating sheep. Veterinary Parasitology 104, 174–185.

Craven, J., Bjorn, H., Hennessy, D.R., Friis, C., Nansen, P., 2001. Pharmacokinetics of moxidectin and ivermectin following intravenous injection in pig with with different body composition. Journal of Veterinary Pharmacology and Therapeutic 24, 99–104.

Craven, J., Bjorn, H., Hennessy, D.R., Friis, C., 2002. The effects of body composition on the pharmacokinetics of subcutaneously injected ivermectin and moxidectin in pigs. Journal of Veterinary Pharmacology and Therapeutic 25, 227–232.

De Montigny, P., Shim, J., Pivinichny, J., 1990. Liquid chromatographic determination of ivermectin with trifluoro acetic anhydride and *N* methylimidazol as the derivatization reagent. Journal of Pharmaceutical and Biomedical Analysis 8, 507–511.

Echeverría, J., Mestorino, N., Turic, E., Pesoa, J., Colantonio, M., Errecalde, J.O., 2000. Bioequivalence of ivermectin in healthy sheep and sheep with mange. In: Proceeding of Eighth European Association for Veterinary Pharmacology and Toxicology (EAVPT) International Congress. Jerusalem, Israel.

Egerton, J.R., Ostlind, D., Blair, L., Eary, C.H., Suhayda, D., Cifelli, S., Rick, R., Campbell, W., 1979. Avermectins, a new family of potent anthelmintic agents: efficacy of the B1a component. Antimicrobial Agents and Chemotherapy 15, 372–378.

Eichler, H.G., Műller, M., 1998. Drug distribution. The forgotten relative in clinical pharmacokinetics. Clinical Pharmacokinetic 34, 95–99.

El-Banna, H.A., Goudah, A., El Zorga, H., Abd-El-Rahman, S., 2008. Comparative pharmacokinetics of ivermectin alone and a novel formulation of ivermectin and rafoxanide in calves and sheep. Parasitology Research 102, 1337–1342.

EMEA/MRL/452/98-Final. Committee for Veterinary Medicinal Products. Nitroxinil. Summary report.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics. Revised and Expanded, second ed. Marcel Dekker, Inc., NY, USA. pp. 45–109.

Imperiale, F., Lifschitz, A., Sallovitz, J., Virkel, G., Lanusse, C., 2004. Comparative depletion of ivermectin and moxidectin milk residues in dairy sheep after oral and subcutaneous administration. Journal of Dairy Research 71, 427–433.

Lanusse, C., Lifschitz, A., Virkel, G., Alvarez, L., Sánchez, S., Sutra, J.F., Galtier, P., Alvinerie, M., 1997. Comparative plasma disposition kinetics of ivermectin, moxidectin and doramectin in cattle. Journal of Veterinary Pharmacology and Therapeutic 20, 91–99.

Lifschitz, A., Virkel, G., Pis, A., Imperiale, F., Sanchez, S., Alvarez, L., Kujanek, R., Lanusse, C., 1999. Ivermectin disposition kinetics after subcutaneous and intramuscular administration of an oil-based formulation to cattle. Veterinary Parasitology 86, 203–215.

Lifschitz, A., Virkel, G., Sallovitz, J., Sutra, J.F., Galtier, P., Alvinerie, M., Lanusse, C., 2000. Comparative distribution of ivermectin and doramectin to parasite location tissues in cattle. Veterinary Parasitology 87, 327–338.

Martin, R.J., 1997. Modes of action of anthelmintic drugs. Veterinary Journal 154, 11–34.

McKellar, Q.A., Benchaoui, H.A., 1996. Avermectins and milbemycins. Journal of Veterinary Pharmacology and Therapeutic 19, 331–351.

Moreno, L., Alvarez, L., Ceballos, L., Sánchez Bruni, S., Lanusse, C., 2008. Pattern of ivermectin (sheep) and doramectin (cattle) residues in muscular tissue from different anatomical locations. Food Additives and Contaminants 25, 406–412.

Notari, R., 1987. Biopharmaceutics and Clinical Pharmacokinetics. Marcel Dekker, New York. pp. 45–129.

Pérez, R., Palma, C., Núñez, M.J., Cox, J., Arboix, M., 2007. Pharmacokinetics of ivermectin in pregnant and nonpregnant sheep. Journal of Veterinary Pharmacology and Therapeutic 31, 71–76.

Pérez, R., Palma, C., Núñez, M.J., Cabezas, I., 2008. Patterns of doramectin tissue residue depletion in parasitized and non-parasitized lambs. Parasitology Research 102 (5), 1051–1057.

Perrier, D., Mayersohn, M., 1982. Non-compartmental determination of the steady-state volume of distribution for any mode of administration. Journal of Pharmaceutical Science 71, 372–373.

Sallovitz, J., Lifschitz, A., Imperiale, F., Pis, A., Virkel, G., Lanusse, C., 2002. Breed differences on the plasma availability of moxidectin administered pour-on to calves. Veterinary Journal 164, 47–53.

Scott, E.W., McKellar, Q.A., 1992. The distribution and some pharmacokinetic parameters of ivermectin in pigs. Veterinary Research Communications 16, 139–146.

Toutain, P.L., Upson, D.W., Terhume, T.N., McKenzie, M.E., 1997. Comparative pharmacokinetics of doramectin and ivermectin in cattle. Veterinary Parasitology 72, 3–8.

Vercruysse, J., Deprez, P., Everaert, D., Bassissi, F., Alvinerie, M., 2008. Breed differences in the pharmacokinetics of ivermectin administered subcutaneously to Holstein and Belgian Blue calves. Veterinary Parasitology 152, 136–140.