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

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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,

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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