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The influence of gastrointestinal parasitism on fecal elimination of doramectin, in lambs

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ABSTRACT

A study was done to investigate the effect of parasitism on patterns of doramectin (DRM) fecal elimination in lambs. Fourteen Suffolk Down parasitized lambs (26.9 ± 1.5 kg body weight: bw) were purposely selected for the study. Seven pairs of lambs were allocated into two experimental groups. Group I (non-parasitized) was pre-treated with 3 repeated administrations of 5 mg/kg bw of fenbendazole to maintain a non-parasitized condition. In Group II (parasitized), the lambs did not receive any anthelmintic treatment. After 85 d of the pre-treatment period, both groups were treated with a subcutaneous injection of 200 μg/kg bw of DRM. Fecal samples were collected at different times between −85 d before and 60 d after the DRM treatment, for both parasitological and chromatographic analysis. Samples were analyzed by high-performance liquid chromatography (HPLC) with fluorescence detection. Data of DRM concentrations were expressed as wet weight. A non-linear pharmacokinetic analysis was performed and results were compared using the Mann Whitney test. Fecal maximum concentrations (C_{max}) of DRM were 1.37 ± 0.19 μg/g (parasitized group) and 0.86 ± 0.15 μg/g (non-parasitized group) observed at the time of the maximum concentration (T_{max}) of 2.1 ± 0.4 and 3.1 ± 0.3 d, respectively. Differences in C_{max} values were significant ($P < 0.05$). The accumulated elimination of DRM in feces, expressed as the percentage of DRM total dose, was 67.1% in the parasitized group, whereas in the non-parasitized group it was 56.5%. Our results showed that gastrointestinal parasitic diseases can modify the patterns of DRM fecal elimination, when the drug is administered by subcutaneous route in lambs.

1. Introduction

Gastrointestinal nematode infection continues to represent a major constraint on animal productivity throughout the world. Such infections may result in alterations in protein, energy, and mineral metabolism, changes in water balance, depression of appetite, and impaired gastrointestinal function. These alterations in turn, can lead to diminution in body composition and carcass quality (Fox, 1997). Hence, sheep's efficient conversion of forage to animal products needs an effective control of parasitism, among other management measures (Sykes, 1978).

The availability of a wide range of anthelmintics and the threat to the productivity posed by nematode infections faced in many sheep farming operations has led to a heavy reliance on their use, particularly in intensive sheep farming. Modern broad-spectrum anthelmintics are highly effective in removing most, if not all, worms present in grazing sheep. However, higher stocking densities frequently force the return of the livestock to the same land from which they acquired their parasite burdens (Taylor, 1999).

Macrocyclic lactones, including doramectin (DRM), ivermectin, and moxidectin, are potent endectocides widely used for control of internal and external parasites in domestic animals and livestock (McKellar and Benchaoui, 1996). These compounds are hydrophobic molecules characterized by a broad-spectrum activity with remarkable long-lasting efficacy (Hennessy and Alvinerie, 2002). DRM is a fermentation-derived antiparasitic agent, which after parenteral administration exhibits potent and persistent activity against nematodes in cattle (Goudie et al., 1993). The persistent anthelmintic efficacy has been attributed to the combination of inherent potency and an extended plasma concentration profile (Owens and Schneider, 2000). Metabolism of DRM following parenteral administration in cattle is limited to the unchanged drug, which account for the major portion of DRM's derived activity recovered from liver, fat, and feces (Owens and Schneider, 2000). Due to its lipophilic nature, DRM is excreted in bile and eliminated unaltered in the feces (Hennessy et al., 2000). The concentration and excretion profile of DRM in feces, have been reported in sheep by Kozuh-Erzen et al. (2005) and Kolar et al. (2006, 2008), and in cattle and horses by Suarez et al. (2009) and Pérez et al. (2010).

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It has been shown that fecal residues from animals treated with avermectins, reduces insect activity of the dung pat and, may thereby, slow dung degradation (Herd, 1995; Strong et al., 1996; Kolar et al., 2008, Suarez et al., 2009). Because undegradated pats in pastures represent losses in grazing areas and soil nitrogen (Fincher, 1981), the studies on the potential ecotoxic effects of endectocides have raised the interest of elucidating the fate of avermectins in the feces of treated animals (Wall and Strong, 1987; Steel and Wardhaugh, 2002).

Gastrointestinal parasitic burden is associated with physiopathological changes such as intestinal dysfunction and nutritional stress leading to poor body condition (Holmes, 1987; Fox, 1997). These changes may have a major effect on the plasma, tissue, and gastrointestinal disposition of the anthelmintic drugs, consequently affecting their efficacy (Lespine et al., 2004). As previously shown (Perez et al., 2006), gastrointestinal parasitism induces significant changes in plasma disposition and availability of DRM. Similar effects are described for moxidectin in sheep (Lespine et al., 2004).

The aim of the study was to know the effect of gastrointestinal parasitism on DRM's fecal elimination patterns in parasitized lambs, after subcutaneous administration of the drug.

2. Materials and methods

Animals: The protocol of this study was reviewed and approved by the Animal Care and Use Committee of the Facultad de Ciencias Veterinarias, Universidad de Concepción, Chile.

For the study, 14 Suffolk Down lambs between 3 and 4 months of age and 26.9 ± 1.5 kg bw, were selected. During the experimental period, the lambs were kept outdoors during the day and housed at night. Lambs were fed daily with a mixed rye grass and clover hay and 200 g of supplementary concentrate. Water and hay were provided *ad libitum*. All lambs were weighed before the treatments using a digital scale.

A panel of serum clinical biochemistry tests, including hepatic function tests, was performed to assess the animals' health condition. These tests yielded values within ranges described normal for the ovine species (Meyer et al., 1992).

To identify parasitic natural infection levels, fecal egg counts (FEC) exams were performed in all lambs. Quantitative pre- and post-treatment FEC were done using a modified McMaster technique (Zajac, 1994) during a 90-d period prior to and a 70-d period after the DRM treatment. All fecal samples were obtained from the rectum during the 7-d interval between the two periods. A minimum of 200 eggs per gram (epg) of feces was established for incorporation of lambs into the experimental groups.

Treatments: For the study, seven pairs of lambs were allocated into two groups equally balanced in bw and sex. Once the animal pairs were established, their distribution to the experimental groups was performed according to their nematode FEC. In Group 1 (non-parasitized), the animals were treated three times with an oral dose of 5 mg/kg bw of fenbendazole (FBZ; Panacur®, Intervet) in order to maintain a healthy, parasite-free condition for an 85-d period. Fenbendazole was selected due to its fast elimination rate and short persistence of the active metabolite in plasma, as well as its good efficacy against most gastrointestinal nematodes of sheep (Lanusse et al., 1995). Considering these characteristics, we assumed that fenbendazole did not produce any effect on the disposition of DRM. In Group 2 (parasitized), infection was sustained by oral inoculation with nematode cultures in the infective stage. A mixed larval inoculum containing approximately 5000 third-stage strongyle larvae (40% *Ostertagia*, 28% *Trichostrongylus*, and 22% *Cooperia*) was orally administrated once a week for 3 weeks. These types of larvae are the most common types of gastrointestinal nematodes in Central-southern Chile. Group 2 did not receive any anthelmintic treatment to maintain their parasitized condition during the experimental period.

Following an 85-d period of pre-treatment, lambs of Group 1 (non-parasitized) were treated with 3 doses of FBZ at 21 d intervals; whereas lambs of Group 2 (parasitized) were inoculated with cultures of nematode larvae. Both groups were injected in the shoulder area with a subcutaneous dose of 200 μg/kg bw of DRM. After the DRM treatment both groups were continuously monitored for any sign of adverse reaction during a 4-h period and twice a day during the next 2 d post-treatment.

Sampling: Fecal samples were collected from the rectum prior to DRM treatments and at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 25, 30, 40, 50, and 60 d post-treatment. Collected fecal samples were stored at $-18\ ^{o}$C until analysis.

Analytical procedures: DRM was assayed by HPLC with fluorescence detection after solid phase extraction using procedures described by Perez et al. (2001).

Drug extraction and derivatisation: One-gram of drug-free fecal samples was fortified with DRM to reach final concentrations of 5, 10, 20, 50, 100, 200, and 400 ng/g. Fortified and experimental fecal samples were homogenized and solid phase extraction was performed after 15 min of incubation at room temperature. Briefly, 4 mL of acetonitrile and 2 mL of water were added to 1 g of feces. After mixing for 20 min, samples were centrifuged at 2000*g* for 5 min, and the supernatant was transferred to a Supelco C18 cartridge. After cartridges were washed with water, DRM was eluted with 1.6 mL methanol and solid phase extraction procedures were performed. The eluate was evaporated to dryness under a gentle nitrogen stream, and the residue was dissolved in 100 μL of a N-methylimidazole solution in acetonitrile (1:1 v/v). To initiate the derivatization, 150 μL of trifluoroacetic anhydride solution in acetonitrile (1:2 v/v) was added. Once the reaction was complete, a 100 μL aliquot of the solution was injected directly into the chromatograph.

Chromatographic conditions: The mobile phase consisted of acetic acid (0.2% in water), methanol, and acetonitrile (4:32:64, v/v/v) at a flow rate of 1.5 mL/min through a Supelcosil C18 column (5 μm; 4.6 mm id × 150 mm) with fluorescence detection at an excitation wavelength of 383 nm and at 447 nm emission wavelength (RF .551 Fluorescence detector, Shimadzu).

Method of Calibration: Calibration graphs for DRM in the range of 5–400 ng/g were prepared, using drug-free feces from non-treated lambs. Pooled fecal samples were taken throughout the calibration procedure, and calibration curves were plotted using the peak area as a function of analyte concentration. Linear regression analysis was used to determine the slopes and correlation coefficients of the different calibration curves. The extraction efficiency of DRM was measured by comparing the peak area from the spiked fecal samples with the peak area resulting from direct injections of the standards in methanol. The inter-assay precision was evaluated by processing replicate aliquots of fecal samples containing known amounts of the drug on different days.

The chromatographic analytical method used to quantify fecal concentrations of DRM was validated. The regression lines between peak areas and drug concentrations presented correlation coefficient of 0.9992 ± 0.0007. The mean DRM extraction recovery from feces was $82.2 \pm 2.5\%$ at the spiked concentrations between 5 and 400 ng/g. The inter-assay precision showed variation coefficients of 5.6%. The chromatographic-method's quantification limit was defined as the lowest concentration that would have a coefficient of variation of $< 20\%$, and it was found to be 5.1 ng/g.

Fecal elimination: To estimate the accumulated percentage of DRM's fecal elimination (AF_{el}), 10 lambs were lodged in metabolic cages to measure the total amount of feces eliminated during a 24 h period. The value obtained was divided by the lamb's bw and multiplied by 100. Then, this value was used to estimate the daily fecal shedding of lambs. Thus, the AF_{el} was calculated using the formula

$$AF_{el}(\%) = (Q_{feces}/Dose) \times 100$$

where Q_{feces} was the product of the calculated daily weight of wet feces multiplied by the DRM concentration in feces (μg/g wet feces)

Pharmacokinetic analysis: Data of DRM concentrations were expressed as wet weight. The areas under concentration vs. time curve (AUC) were calculated by the trapezoidal rule using the PK Solutions® software (Farrier, 1997). The peak fecal concentrations (C_{max}) and the peak concentration time (T_{max}) were read from the DRM's fecal concentration–time curve of each animal. The pharmacokinetic parameters were reported as mean ± SEM and were compared using the Mann Whitney U test. Mean values were considered different at $P < 0.05$.

3. Results

Geometric mean of FEC at −85, 0, and 60 d of DRM administration were 300, 29, and 57 and 460, 814, and 200, for the non-parasitized and parasitized lambs groups, respectively. At the beginning of the assays, the FEC was similar in both groups. In non-parasitized group the anthelmintic pre-treatment with fenbendazole reduced significantly the FEC; whereas in the lambs of Group 2 an increase ($P < 0.05$) in its FEC was observed at day 0. Values of FEC were different ($P < 0.05$) at 60 d after DRM administration.

At the beginning of the experimental period, the lamb's bw was 26.9 ± 1.5 kg in Group 1 and 27.2 ± 1.4 kg in Group 2. In lambs of Group 1, the pre-treatment with fenbendazole produced an increase in bw (34.8 ± 1.8 kg); in comparison to the parasitized group, in which the bw was 28.7 ± 1.8 kg ($P < 0.05$). At the end of the period of 60 d after the DRM treatment, we observed a reduction in the differences in bw between both groups. Where, body weights of 44.6 ± 1.6 and 41.6 ± 1.9 kg were observed for the non-parasitized and parasitized groups, respectively.