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Accumulation of monepantel and its sulphone derivative in tissues of nematode location in sheep: Pharmacokinetic support to its excellent nematodicidal activity

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ABSTRACT

The amino-acetonitrile derivatives (AADs) are a new class of anthelmintic molecules active against a wide range of sheep gastrointestinal (GI) nematodes including those that are resistant to other anthelmintic families. The plasma disposition of monepantel (MNP) has been previously characterized in sheep. However, information on drug concentration profiles attained at tissues of parasite location is necessary to fully understand the pharmacological action of this novel compound. The current work aimed to study the relationship between the concentrations of MNP parent drug and its main metabolite monepantel sulphone ($MNPSO_2$), measured in the bloodstream and in different GI tissues of parasite location in sheep. Twenty two (22) uninfected healthy Romney Marsh lambs received MNP (Zolvix®, Novartis Animal Health) orally administered at 2.5 mg/kg. Blood samples were collected from six animals between 0 and 14 days post-treatment to characterize the drug/metabolite plasma disposition kinetics. Additionally, 16 lambs were sacrificed at 8, 24, 48 and 96 h post-administration to assess the drug concentrations in the GI fluid contents and tissues. MNP and $MNPSO_2$ concentrations were determined by HPLC. MNP parent compound was rapidly oxidized into $MNPSO_2$. MNP systemic availability was significantly lower than that observed for $MNPSO_2$. The peak plasma concentrations were 15.1 (MNP) and 61.4 ng/ml ($MNPSO_2$). The $MNPSO_2$ to MNP plasma concentration profile ratio (values expressed in AUC) reached a value of 12. Markedly higher concentrations of MNP and $MNPSO_2$ were measured in both abomasal and duodenal fluid contents, and mucosal tissues compared to those recovered from the bloodstream. A great MNP availability was measured in the abomasal content with concentration values ranging between 2000 and 4000 ng/g during the first 48 h post-treatment. Interestingly, the metabolite $MNPSO_2$ was also recovered in abomasal content but its concentrations were significantly lower compared to MNP. The parent drug and its sulphone metabolite were detected in the different segments of the sheep intestine. $MNPSO_2$ concentrations in the different intestine sections sampled were significantly higher compared to those measured in the abomasum. Although MNP is metabolized to $MNPSO_2$ in the liver, the large concentrations of both anthelmintically active molecules recovered during the first 48 h post-treatment from the abomasum and small intestine may greatly contribute to the well-established pharmacological activity of MNP against GI nematodes.

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1. Introduction

Gastrointestinal nematodes control programs are mainly based on a combination of animal management practices and the use of antiparasitic drugs. During the last 30 years, antiparasitic treatment has been mainly restricted to three anthelmintic groups: the imidazothiazoles, the benzimidazoles and the macrocyclic lactones. After years of intensive use to optimize animal productivity, the widespread appearance of resistant parasites in different areas of the world was inevitable. In this context, the need of novel drugs acting at novel target sites has been highlighted on many occasions.

The amino-acetonitrile derivatives (AADs) represent one of the newest anthelmintic classes (Kaminsky et al., 2008) introduced in veterinary medicine. From many compounds evaluated, the racemic molecule AAD 96, was selected and the active s-enantiomer of this molecule, named monepantel (MNP), was launched into the veterinary pharmaceutical market for oral administration to sheep in 2009 (Hosking et al., 2010). MNP acts at a new target as a positive allosteric modulator of the nematode specific receptor MPTL-1, which belongs to the DEG-3 subfamily of acetylcholine receptors (Rufener et al., 2010). MNP binding to receptor accounts for an alteration in ion flux and leads to the paralysis of nematodes (Epe and Kaminsky, 2013). This new mechanism of action explains the high efficacy of MNP against nematodes resistant to other anthelmintic classes (Baker et al., 2012).

The plasma disposition kinetics of MNP has been assessed in sheep after its intravenous and oral administration (Karadzovska et al., 2009). Monepantel sulphone ($MNPSO_2$) was the main metabolite detected in the bloodstream after MNP administration. As this metabolite is also active against nematodes, the pharmacokinetic behavior of $MNPSO_2$ is relevant for the interpretation of residue and efficacy studies (Karadzovska et al., 2009). Although the evaluation of drug concentration profiles in the bloodstream contributed with useful information (Karadzovska et al., 2009), MNP and $MNPSO_2$ exert their anthelmintic effects in some non-vascular target tissues such as the gastrointestinal (GI) tract (Kaminsky et al., 2009), where nematode parasites are located. The characterization of MNP and $MNPSO_2$ concentration profiles attained at specific GI sites of parasite location and the establishment of the relationship between their plasma and gastrointestinal content/tissues availabilities were the main goals of the experiment described here.

2. Material and methods

2.1. Animals

The study was conducted in clinically healthy and parasite-free sheep. Twenty two (22) Romney Marsh (15–20 kg) lambs were used. The animals were kept under field conditions during the experimental period. Their health was monitored prior to and throughout the experiment. Animals were in optimal body condition, grazed on a lucerne/red clover pasture with free access to water during the study. Animal procedures and management protocols were approved by an Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar).

2.2. Experimental design, treatments and samplings

All experimental lambs received MNP (Zolvix®, Novartis Animal Health Inc.) orally at the minimum recommended dose of 2.5 mg/kg. Six lambs were involved in a plasma disposition study. Jugular blood samples (7 ml) from the six lambs were collected into heparinised tubes prior to and at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 216, 264 and 336 h post-treatment. Blood samples were centrifuged at 2000*g* for 20 min and the recovered plasma was stored in labeled vials. To characterize the drug concentration at the GI tissues (target tissue distribution study), 16 lambs treated with MNP were sacrificed at 8, 24, 48 and 96 h (four animals in each experimental slaughter time) by a captive bolt to render them unconscious and immediately exsanguinated according to the institutional and international animal euthanasia guidelines. Samples collected included, blood, liver, bile, mucosal tissue and luminal content of the GI tract. The GI tract samples were collected from the abomasum, duodenum, ileum and cecum. After collection of the intestinal and abomasal contents, the mucosal tissues of each GI section were obtained by scraping. Bile was collected directly from the gall bladder. All samples were transported on ice to the laboratory and stored at −20 °C until HPLC analysis.

3. Analytical procedures

3.1. Monepantel and monepantel sulphone quantitation

The extraction of MNP and $MNPSO_2$ from spiked and experimental plasma and tissue samples was carried out following the technique described by Karadzovska et al. (2009). Briefly, 0.5 ml of plasma and 0.3–0.5 g of GI content/mucosa mixed with 0.5 ml of water and 1.3 ml of acetonitrile. After mixing for 2 min, the solvent-sample mixture was centrifuged at 2000*g* for 15 min. The supernatant was manually transferred into a tube, mixed with 5.0 ml of water and injected onto a polymeric sorbent solid phase extraction cartridge (Strata-X 33 lm Polymeric Sorbent 60 mg, Phenomenex Torrance, CA, USA) conditioned with 1.0 ml acetonitrile and 1.0 ml water. The cartridge was washed with 2.0 ml of acetonitrile:water (30:70, v/v). MNP and $MPNSO_2$ were eluted with 1 ml of acetonitrile and concentrated to dryness under a stream of nitrogen. The resuspension was made with 250 μl of mobile phase (acetonitrile:methanol:water 60:8:32, v/v/v), and 50 μl were injected onto the HPLC (Shimadzu 10 A HPLC system with autosampler, Shimadzu Corporation, Kyoto, Japan). HPLC analysis was done using a reverse phase C_{18} column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm, 4.6 mm × 250 mm) and an acetonitrile:methanol:water 60:8:32, v /v/v mobile phase at a flow rate of 0.8 ml/min at 30 °C. Both analytes were measured by UV detection (SPD-10A; Shimadzu) reading at 230 nm.