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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.

The peak areas were used to calculate drug concentrations in spiked and experimental plasma and tissue samples. The solvents (Baker, Phillipsburg, NJ, USA) used for the extraction and drug analysis were HPLC grade. Calibration curves in the range of 4 and 100 ng/ml (plasma) and 40–4000 ng/g (GI tissues and contents) were prepared. Calibration curves were established using least squares linear regression analysis and correlation coefficients (r) were calculated. Linearity was established to determine the MNP and $MNPSO_2$ concentration/detector response relationships. The precision of the extraction and chromatography procedures were estimated calculating the coefficient of variations (CV) of plasma and tissue samples containing known MNP and $MNPSO_2$ concentrations. The linear regression lines for MNP and $MNPSO_2$ showed correlation coefficients $\geq$0.99. The repeatability of the analytical procedures obtained after HPLC analysis showed CV <10% in plasma and different GI content and tissue samples.

4. Pharmacokinetic analysis of the data

The plasma concentration versus time curves obtained after treatment of each individual animal were fitted with the PK Solutions 2.0 (Ashland, OH, USA) computer software. Pharmacokinetic parameters were determined using a non-compartmental model method. The peak concentration (C_{max}) was read from the plotted concentration-time curve of each individual animal. The area under the concentration vs. time curves (AUC) were calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (λz). The terminal (elimination) half-life ($t_{1/2el}$) was calculated as In2/λz. MNP and $MNPSO_2$ plasma and GI concentrations and all the estimated pharmacokinetic parameters are reported as mean ± SD.

5. Statistical analysis

Mean pharmacokinetic parameters for MNP and $MNPSO_2$ were statistically compared using Student's t-test. The assumption that the data obtained after treatments have the same variance was assessed. A non-parametric Mann–Whitney test was used where significant differences among standard deviations were observed. A similar procedure was used to compare MNP and $MNPSO_2$ concentrations measured in plasma and in different GI contents and tissues. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, CA, USA). A value of $P < 0.05$ was considered statistically significant.

6. Results

Low concentrations of MNP were measured in plasma up to 48 h post-administration. A significantly higher concentration profile was observed for the $MNPSO_2$ derivative in the bloodstream compared to that of the parent compound. The persistence of the sulphone metabolite was present longer in the bloodstream; up to 216 h (9 days) after the oral administration of MNP to sheep. The comparative MNP and $MNPSO_2$ plasma concentration profiles are shown in Fig. 1. The observed differences were reflected in the values obtained for the main pharmacokinetic parameters. Higher C_{max} and greater AUC values were obtained in plasma for $MNPSO_2$ compared to MNP. The sulphone/parent drug systemic availability ratio reached a value of 12. The main plasma pharmacokinetic parameters for both molecules are summarized in Table 1.

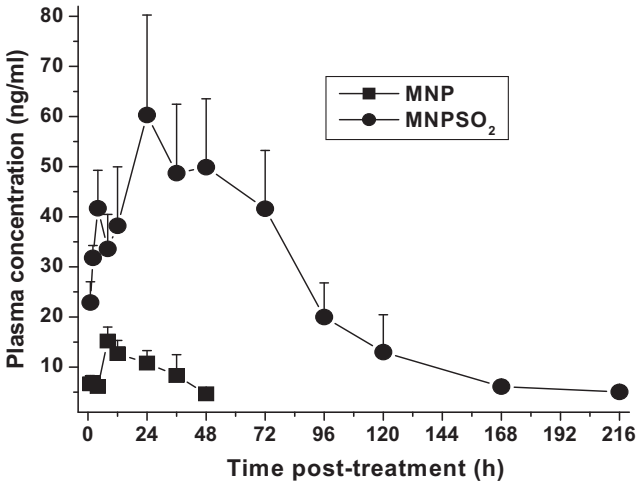

Fig. 1. Plasma concentration profiles of monepantel (MNP) and monepantel sulphone ($MNPSO_2$) (n = 6) obtained after the oral administration of monepantel to sheep (2.5 mg/kg).

In addition to the plasma pharmacokinetics, the current study also assessed the process of drug accumulation and disposition in tissues of target nematodes habitation. In the abomasal content, MNP concentrations were recovered in a range between 2000 and 4000 ng/g during the first 48 h post-treatment. Interestingly, $MNPSO_2$ was also detected in the abomasal content but the concentrations were significantly lower compared to those of MNP ($P < 0.05$) (Fig. 2). The ratios of the abomasum and plasma concentration profiles of both parent and metabolite molecules were estimated at different times post-administration. These ratios ranged between 200 and 400 (MNP) and only between 1 and 5 for $MNPSO_2$. The abomasal content/plasma concentration ratios for both MNP and $MNPSO_2$ are shown in Fig. 3.

Table 1
Mean (±SD) (n = 6) kinetic parameters for monepantel (MNP) and monepantel sulphone ($MNPSO_2$) in plasma obtained after the oral administration of monepantel (2.5 mg/kg) to sheep.

Kinetic parameters	MNP alone	$MNPSO_2$
AUC_{0-t} (ng h/ml)	438 ± 119	5203 ± 1333*
C_{max} (ng/ml)	15.1 ± 2.83	61.4 ± 18.2*
T_{max} (h)	8.00 ± 0.00	28.0 ± 6.20*
$T^{½}$ el (h)	23.6 ± 8.24	48.7 ± 4.54*
MRT (h)	35.9 ± 10.7	79.3 ± 5.39*
Ratio of the AUC MNPSO/MNP	–	12.0 ± 1.80

AUC_{0-t}: area under the concentration vs. time curve. C_{max}: peak plasma concentration. T_{max}: time to peak plasma concentration. $T^{½}$ el: elimination half-life. MRT: mean residence time.

* Mean kinetic parameters are significantly different from those obtained for MNP at $P < 0.05$.

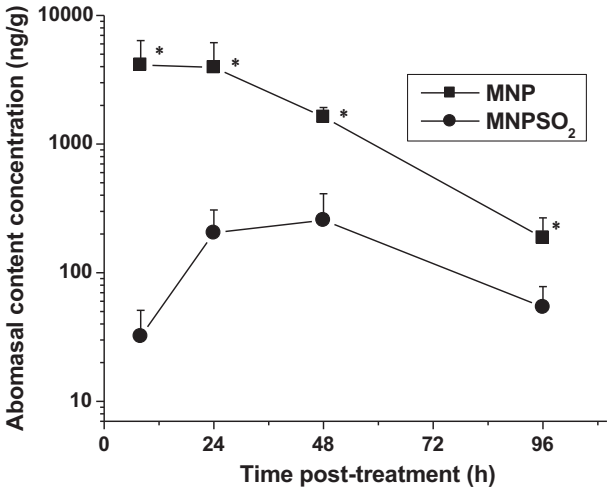

Fig. 2. Mean (±SD) monepantel (MNP) and monepantel sulphone ($MNPSO_2$) concentrations obtained in abosamal fluid content after the oral administration of monepantel to sheep (2.5 mg/kg). * Mean values are significantly different from those obtained for $MNPSO_2$ at $P < 0.05$.

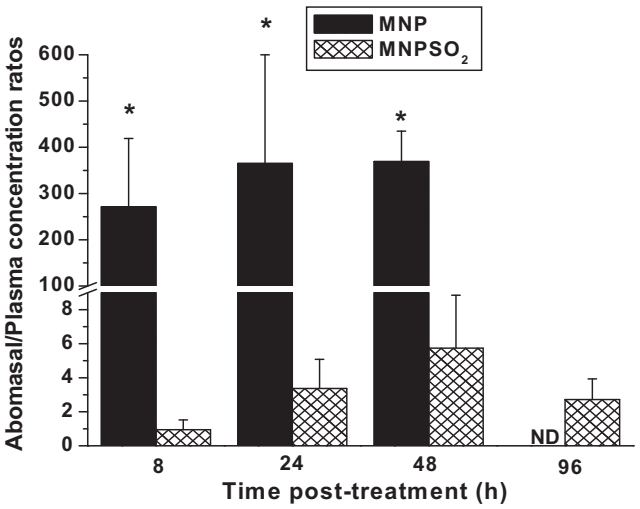

Fig. 3. Relationship between monepantel (MNP) and monepantel sulphone ($MNPSO_2$) concentrations measured in abomasal content and plasma (mean ± SD) after the oral administration of monepantel to sheep (2.5 mg/kg). * Mean values are significantly different from those obtained for $MNPSO_2$ at $P < 0.05$.

The parent drug and the sulphone metabolite were recovered in the different segments of the sheep intestine. MNP concentrations ranged between 56 and 4200 ng/g in the contents of the duodenum, ileum and cecum. $MNPSO_2$ concentrations were significantly higher compared to those observed in abomasum (from 199 to 677 ng/g). As a result of an efficient metabolism of MNP, significantly higher concentrations of the sulphone metabolite were measured in liver tissue and bile. The concentration profiles of both compounds in liver and bile are shown in Fig. 4. Fig. 5 shows the MNP and $MNPSO_2$ concentrations measured in the different intestinal segments.

MNP and $MNPSO_2$ were recovered from the different mucosal tissues along the GI tract of treated sheep. Mucosal concentrations of both molecules (parent and metabolite) were lower than those measured in the corresponding GI fluid contents. MNP and $MNPSO_2$ concentrations from the abomasal and duodenal mucosa were higher compared to

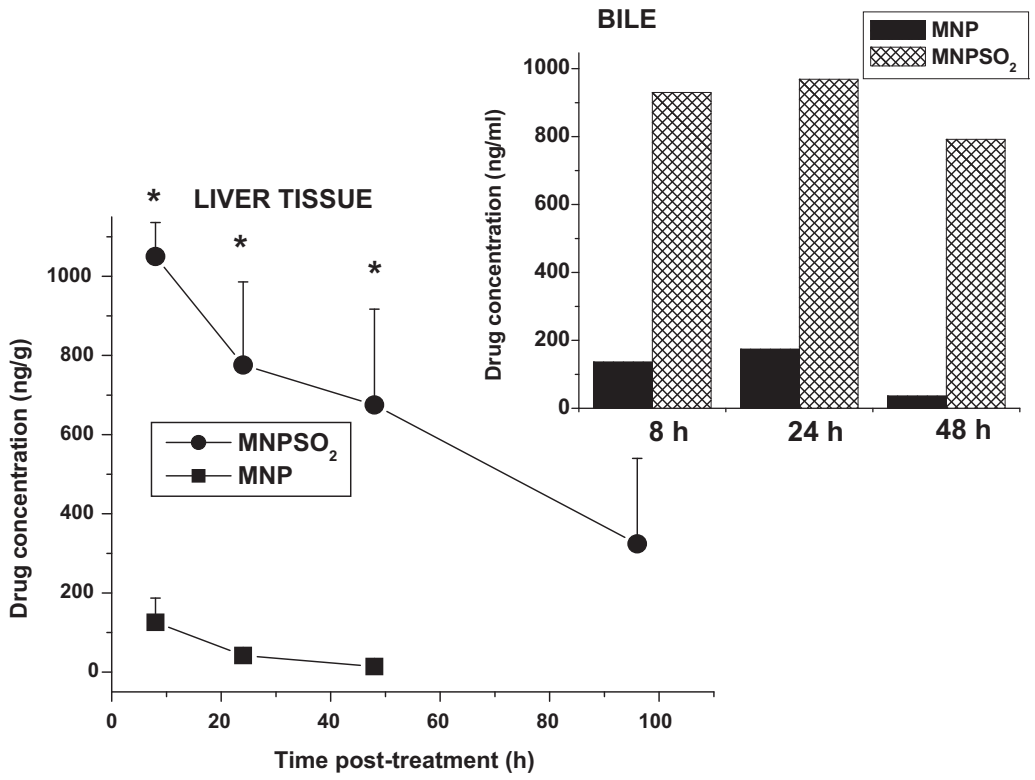

Fig. 4. Mean (±SD) monepantel (MNP) and monepantel sulphone ($MNPSO_2$) concentrations obtained in liver after the oral administration of monepantel to sheep (2.5 mg/kg). The insert shows the mean concentrations of both compounds in bile. * Mean values are significantly different from those obtained for MNP at $P < 0.05$.

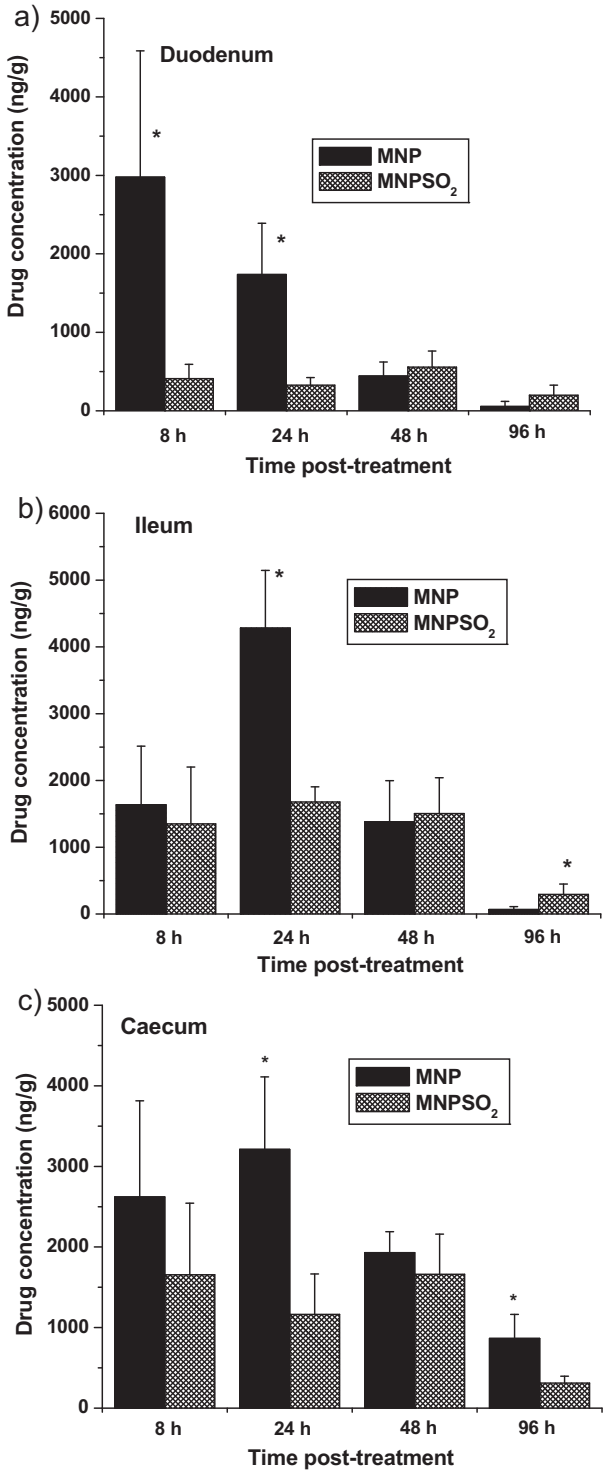

Fig. 5. Mean (±SD) monepantel (MNP) and monepantel sulphone ($MNPSO_2$) concentrations obtained at the intestinal contents in (a) duodenum, (b) ileum and (c) cecum after the oral administration of monepantel to sheep (2.5 mg/kg). * Mean values are significantly different at $P < 0.05$.

those measured in the mucosa of both ileum and cecum. MNP mucosal accumulation ranged from 74 to 513 ng/g (abomasum and duodenum) and from 44 to172 ng/g (ileum and cecum). A similar pattern was observed for the sulphone metabolite with higher concentrations in the proximal segments (279–1018 ng/g) compared to the distal segments of the intestine (69–624 ng/g). MNP and $MNPSO_2$ concentrations measured in mucosal tissues of the different portions of the GI tract at 24 h post-administration are compared in Table 2.

Table 2
Mean values ($n = 4$) monepantel (MNP) and monepantel sulphone ($MNPSO_2$) concentrations (ng/g) in mucosal tissues of different portions of the gastrointestinal tract obtained at 24 h post-administration of monepantel by the oral route (2.5 mg/kg) to sheep.

Gastrointestinal segment	Concentrations (ng/g) at 24 h post-treatment	
	MNP	$MNPSO_2$
Abomasum	423	755*
Duodenum	293	1018*
Ileum	172	394*
Cecum	70.9	443*

* Mean values are significantly different from those obtained for MNP at $P < 0.05$.

7. Discussion

Data on plasma kinetic profiles can help to explain the comparative efficacy and persistence of different anthelmintic drugs. However, the characterization of drug concentration profiles at the tissue sites of parasite habitation permits a more direct interpretation and provides a basis for understanding the therapeutic action of nematodicidal compounds. The work described here assessed the accumulation and disposition kinetics of the novel anthelmintic MNP and its active sulphone metabolite in different GI tissues and contents of parasite habitation.

The MNP plasma concentration profiles were previously evaluated after its intravenous and oral administration to sheep at 1, 3 and 10 mg/kg (Karadzovska et al., 2009). In agreement with published data (Karadzovska et al., 2009; Hosking et al., 2010), higher concentration profiles of the metabolite $MNPSO_2$ compared to the parent drug, were measured in the sheep bloodstream in the current study. The C_{max} of $MNPSO_2$ was four fold-higher compared to that measured for the parent compound. MNP is rapidly converted in the liver into different metabolites (Karadzovska et al., 2009). Nine Phase I and Phase II metabolites were described after *in vitro* MNP incubation in primary culture of ovine hepatocytes (Stuchlíková et al., 2013). However, the main metabolite detected *in vivo* in plasma is restricted to $MNPSO_2$. As the persistence of $MNPSO_2$ is significantly longer than MNP, this metabolite is the marker for tissue residue studies. Besides, since a similar *in vitro* anthelmintic activity against nematode larvae has been demonstrated for the sulphone metabolite and the parent drug (Karadzovska et al., 2009), the accumulation and the tissue disposition of $MNPSO_2$ may be relevant to the overall MNP nematodicidal activity.

Under the described circumstances, it is important to understand the fate of the drug within the GI tissues and fluid contents. In the abomasum, MNP parent drug concentrations were much higher than those measured in plasma but $MNPSO_2$ was also recovered. Gastric secretions may be involved in the appearance of MNP sulphone in abomasal content as it was demonstrated for benzimidazole

compounds in sheep (Hennessy, 1993). The characterization of MNP and $MNPSO_2$ accumulation in target digestive tissues provides relevant information on drug exposure for GI nematode parasites. Such a kinetic pattern may support the well established high efficacy of MNP against the abomasal nematode *Haemonchus contortus* (Kaminsky et al., 2009). Both MNP and its sulphone metabolite may reach the target parasite from plasma after oral ingestion. However, considering that they are highly lipophilic compounds (Karadzovska et al., 2009), the great availability of MNP and $MNPSO_2$ in the abomasal content could facilitate accumulation of both active molecules within the parasite through a transcuticular diffusion process.

The rapid *in vivo* metabolism of MNP was clearly reflected in the drug concentration profiles measured in liver tissue and bile. The $MNPSO_2$ liver concentrations were significantly higher compared to those of the parent drug. Mean MNP and $MNPSO_2$ concentrations at 1 day post-administration were 776 and 42 ng/g, respectively. Both compounds were secreted into the bile, where the sulphone metabolite accumulation was 6-fold higher than that of the parent molecule on day 1 post-administration. The mechanism involved in the biliary secretion of MNP and $MNPSO_2$ is unknown, but the potential participation of ABC transporters such as *P*-glycoprotein or breast cancer resistance protein cannot be ruled out. The high $MNPSO_2$ concentrations secreted by bile are a main source of this metabolite to the gut, which would explain its recovery in the contents collected from different segments of the sheep intestine.

High concentrations of MNP and $MNPSO_2$ were measured along the intestine. The partitioning of both compounds between the intestinal content and mucosal tissue was different for parent MNP and the sulphone metabolite. Whereas the significantly high accumulation of MNP observed along the intestine (fluid content) may be mainly related to the non-absorbed orally administered drug, the high $MNPSO_2$ sulphone concentrations recovered from the mucosal tissues may be due to its blood-mucosa transfer in the different intestinal segments.

The active intestinal secretion of lipophilic anthelmintics such as ivermectin was previously demonstrated. The involvement of *P*-glycoprotein on the intestinal secretion of ivermectin was corroborated by *in vivo* and *ex vivo* trials (Laffont et al., 2002; Ballent et al., 2006). While parent drugs such as albendazole and fenbendazole did not show interaction with the transporter BCRP, their sulphoxide metabolites showed a highly efficient transport by BCRP in culture cells (Merino et al., 2005). Therefore, the involvement of ABC transporters in the intestinal secretion of MNP and $MNPSO_2$ should be examined. This may be relevant to understand its pharmacological behavior but also to detect potential drug–drug interactions if MNP is co-administered with other drugs, which appears to be a need for the future of parasite control in livestock.

There is not published information that correlates the pharmacokinetics of MNP and its clinical efficacy. The required concentrations of MNP and $MNPSO_2$ at the site of parasite location to inhibit parasite establishment and/or development have not been determined. However, the characterization of drug accumulation in target tissues may provide information to predict the drug concentration below which the effectiveness against larval and adult parasites begins to decrease. The AADs act on a nematode specific acetylcholine receptor and produce marked effects on the movement, growth and viability of nematodes (Kaminsky et al., 2008). *In vitro* experiments have shown that the phenotypic effects of these compounds on free-living nematodes and adult *H. contortus* are observed at 50–100 ng/ml but full lethality occurs at drug concentrations above 1000 ng/ml. This latter level of drug concentration is in the range of those measured in the sheep GI tract in the current study.

The data reported by Sager et al. (2010) may also supply useful information on the pharmacokinetic-pharmacodynamic relationship for this novel drug. These authors studied the speed at which a reduction of nematode eggs in the faeces of sheep occurs after MNP treatment. A significant reduction of eggs was obtained at 36 h post-treatment and the faecal egg counts were reduced to 0 at 72 h post-treatment. This time-course of MNP pharmacological activity correlates to the highest MNP concentrations in the abomasum during the first 48 h post-treatment (see Fig. 2).

The low to moderate action of MNP against nematodes located in different systemic tissues may be explained by using the kinetic data described here. It seems unlikely that MNP will adequately control lung nematodes at the dose used for GI nematodes (Hosking, 2010). The current work corroborated that MNP and $MNPSO_2$ plasma concentrations were significantly lower than those measured in the GI tract. As the exposure of lung nematodes to the parent drug and its sulphone metabolite is directly related to the drug systemic availability, the concentration profiles in the bloodstream after oral treatment may not be sufficient to obtain a good efficacy level against parasites located in the lungs. Another interesting issue is related to the efficacy of MNP against nematodes located in the large intestine such as *Oesophagostomum venulosum*. A low efficacy (52%) against the fourth-stage larvae (L_4) of *O. venulosum* was obtained after the oral administration of MNP at 1.25 mg/kg. Therefore, the dose of 2.5 mg/kg was established as a suitable minimum dose rate (Kaminsky et al., 2009). However variable efficacy (between 15% and 98%) was observed against L_4 of *O. venulosum* in different efficacy studies (Hosking et al., 2009). A similar situation was observed for *Trichuris ovis* as MNP did not reduce worm burdens in naturally infected sheep (Sager et al., 2009). Although a reduced sensitivity of these parasites (a pharmacodynamic-based limitation) to MNP may explain its low efficacy, a pharmacokinetic issue should not be ruled out. The L_4 of *O. venulosum* and adult *T. ovis* bury into the mucosal tissue of the large intestine. The lower maximal concentration of MNP achieved in the current study in the large intestine mucosa (225 ng/g) compared to that measured in small intestine (562 ng/g in ileum and 762 ng/g in duodenum) may explain the efficacy levels obtained against *O. venulosum* and *T. ovis*.

The evaluation of persistent antiparasitic activity of MNP and $MNPSO_2$ underlines the relevance of the drug concentrations achieved in the GI tissues. Although $MNPSO_2$ plasma concentrations were detected until 9–12

days post-administration, efficacy studies confirmed that MNP is a short-acting anthelmintic (Hosking, 2010). Thus, MNP anthelmintic activity may be based on the great drug/metabolite accumulation in the GI tissues and fluid contents during the first 2 to 3 days post-treatment. It is also likely that the level of drug concentration below 0.1 μg/ml measured in plasma between 4 and 9 days post-treatment may not be sufficient to obtain a good activity against the different species of GI located nematodes. This feature of MNP would be an advantage compared to anthelmintics with long persistence of activity. It seems unavoidable that declining concentrations of long persistent drug permit resistant genotype larvae to establish, whereas susceptible genotypes cannot survive (Sutherland and Leathwick, 2011).

The development of resistance to all the older anthelmintic groups in sheep is a seriously increasing problem in many countries (Kaplan and Vidyashankar, 2012; Cezar et al., 2010). There is a real need to include new pharmacological tools in nematode control programs but it is also necessary to further optimize the use of anthelmintic drugs (Kaminsky et al., 2013). The risk of abusing these novel anthelmintics may rapidly lead to the development of resistance, as it was recently reported for MNP in goats (Scott et al., 2013). The emergence of new anthelmintic compounds into the veterinary pharmaceutical market reinforces the need for deeper understanding of their pharmacological properties to avoid their misuse and therefore, delay the appearance of resistance. The current work characterized the accumulation and disposition of MNP and its main metabolite $MNPSO_2$ at the sites where the main target nematodes reside. The pharmacological data provided here are considered an important contribution to improving our understanding of the relationship between the plasma and tissue pharmacokinetics and the nematodicidal activity of MNP in sheep.

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References

Ballent, M., Lifschitz, A., Virkel, G., Sallovitz, J., Lanusse, C., 2006. Modulation of the *p*-glycoprotein-mediated intestinal secretion of ivermectin: in vitro and in vivo assessments. Drug Metab. Dispos. 34, 457–463.

Baker, K.E., George, S.D., Stein, P.A., Seewald, W., Rolfe, P.F., Hosking, B.C., 2012. Efficacy of monepantel and anthelmintic combinations against multiple-resistant *Haemonchus contortus* in sheep, including characterisation of the nematode isolate. Vet. Parasitol. 186, 513–517.

Cezar, A.S., Toscan, G., Camillo, G., Sangioni, L.A., Ribas, H.O., Vogel, F.S.F., 2010. Multiple resistance of gastrointestinal nematodes to nine different drugs in a sheep flock in southern Brazil. Vet. Parasitol. 173, 157–160.

Epe, C., Kaminsky, R., 2013. New advancement in anthelmintic drugs in veterinary medicine. Trends Parasitol. 29, 129–134.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, second ed. (revised and expanded). Marcel Dekker, Inc, New York, NY, USA.

Hennessy, D.R., 1993. Pharmacokinetic disposition of benzimidazole drugs in the ruminant gastrointestinal tract. Parasitol. Today 9, 329–333.

Hosking, B.C., Dobson, D.P., Stein, P.A., Kaminsky, R., Bapst, B., Mosimann, D., Mason, P.C., Seewald, W., Strehlau, G., Sager, H., 2009. Dose confirmation studies for monepantel, an amino-acetonitrile derivative, against fourth stage gastro-intestinal nematode larvae infecting sheep. Vet. Parasitol. 160, 251–257.

Hosking, B.C., Kaminsky, R., Sager, H., Rolfe, P.F., Seewald, W., 2010. A pooled analysis of the efficacy of monepantel, an amino-acetonitrile derivative against gastrointestinal nematodes of sheep. Parasitol. Res. 106, 529–532.

Hosking, B.C., 2010. The control of gastro-intestinal nematodes in sheep with the amino-acetonitrile derivative, monepantel with a particular focus on Australia and New Zealand. In: PhD Thesis. University of Ghent, Ghent, Belgium, pp. 115 (Chapter 3).

Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Schorderet Weber, S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, F., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A., Maser, P., 2008. A new class of anthelmintics effective against drug-resistant nematodes. Nature 452, 176–180.

Kaminsky, R., Mosimann, D., Sager, H., Stein, P., Hosking, B., 2009. Determination of the effective dose rate for monepantel (AAD 1566) against adult gastro-intestinal nematodes in sheep. Int. J. Parasitol. 39, 443–446.

Kaminsky, R., Rufener, L., Bouvier, J., Lizundia, R., Schorderet Weber, S., Sager, H., 2013. Worms—a license to kill. Vet. Parasitol. 195, 286–291.

Kaplan, R.M., Vidyashankar, A.N., 2012. An inconvenient truth: global warming and anthelmintic resistance. Vet. Parasitol. 186, 70–78.

Karadzovska, D., Seewald, W., Browning, A., Smal, M., Bouvier, J., Giraudel, J.M., 2009. Pharmacokinetics of monepantel and its suphone metabolite, monepantel suphone, after intravenous and oral administrations in sheep. J. Vet. Pharmacol. Ther. 32, 359–367.

Laffont, C.M., Toutain, P.L., Alvinerie, M., Bousquet-Melou, A., 2002. Intestinal secretion is a major route for parent ivermectin elimination in the rat. Drug Metab. Dispos. 30, 626–630.

Merino., G., Jonker, J.W., Wagenaar, E., Pulido, M.M., Molina, A.J., Alvarez, A.I., Schinkel, A.H., 2005. Transport of anthelmintic benzimidazole drugs by breast cancer resistance protein (BCRP/ABCG2). Drug Metab. Dispos. 33, 614–618.

Rufener, L., Baur, R., Kaminsky, R., Mäser, P., Sigel, E., 2010. Monepantel allosterically activates DEG-3/DES-2 channels of the gastrointestinal nematode *Haemonchus contortus*. Mol. Pharmacol. 78, 895–902.

Sager, H., Hosking, B., Bapst, B., Stein, P., Vanhoff, K., Kaminsky, R., 2009. Efficacy of the amino-acetonitrile derivative, monepantel, against experimental and natural adult stage gastro-intestinal nematode infections in sheep. Vet. Parasitol. 159, 49–54.

Sager, H., Rolfe, P., Strehlau, G., Allan, B., Kaminsky, R., Hosking, B., 2010. Quarantine treatment of sheep with monepantel—rapidity of fecal egg count reduction. Vet. Parasitol. 170, 336–339.

Scott, I., Pomroy, W.E., Kenyon, P.R., Smith, G., Adlington, B., Moss, A., 2013. Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Vet. Parasitol. 198, 166–171.

Stuchlíková, L., Jirásko, R., Vokřál, I., Lamka, J., Spulák, M., Holčapek, M., Szotáková, B., Bártíková, H., Pour, M., Skálová, L., 2013. Investigation of the metabolism of monepantel in ovine hepatocytes by UHPLC/MS/MS. Anal. Bioanal. Chem. 405, 1705–1712.

Sutherland, I.A., Leathwick, D.M., 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? Trends Parasitol. 27, 176–181.