Review Article

Host pharmacokinetics and drug accumulation of anthelmintic drugs within target helminth parasites of ruminants

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Abstract

Anthelmintic drugs require effective concentrations to be attained at the site of parasite location for a certain period to assure their efficacy. The processes of absorption, distribution, metabolism and excretion (pharmacokinetic phase) directly influence drug concentrations attained at the site of action and the resultant pharmacological effect. The aim of the current review article was to provide an overview of the relationship between the pharmacokinetic features of different anthelmintic drugs, their availability in host tissues, accumulation within target helminths and resulting therapeutic efficacy. It focuses on the anthelmintics used in cattle and sheep for which published information on the overall topic is available; benzimidazoles, macrocyclic lactones and monepantel. Physicochemical properties, such as water solubility and dissolution rate, determine the ability of anthelmintic compounds to accumulate in the target parasites and consequently final clinical efficacy. The transcuticular absorption process is the main route of penetration for different drugs in nematodes and cestodes. However, oral ingestion is a main route of drug entry into adult liver flukes. Among other factors, the route of administration may substantially affect the pharmacokinetic behaviour of anthelmintic molecules and modify their efficacy. Oral administration improves drug efficacy against nematodes located in the gastroinestinal tract especially if parasites have a reduced susceptibility. Partitioning of the drug between gastrointestinal contents, mucosal tissue and the target parasite is important to enhance the drug exposure of the nematodes located in the lumen of the abomasum and/or small intestine. On the other hand, large inter-animal variability in drug exposure and subsequent high variability in efficacy was observed after topical administration of anthelmintic compounds. As it has been extensively demonstrated under experimental and field conditions, understanding pharmacokinetic behaviour and identification of different factors affecting drug activity is important for achieving optimal parasite control and avoiding selection for drug resistance. The search for novel alternatives to deliver enhanced drug concentrations within target helminth parasites may contribute to avoiding

misuse, and prolong the lifespan of existing and novel anthelmintic compounds in the veterinary pharmaceutical market.

KEY WORDS: *Anthelmintic drugs, pharmacokinetics, drug transfer, parasite exposure, resistance*

AUC	Area under the concentration *vs.* time curve
BCRP	Breast cancer resistance protein
BZD	Benzimidazoles
ML	Macrocyclic lactones

Introduction

The main helminths affecting domestic animals are located in several tissues in the body, such as gastrointestinal contents and mucosa, liver tissue, bile duct and lung tissue. Anthelmintic drugs require that effective concentrations are attained at the site of parasite location for a certain period to assure their efficacy. The processes of absorption, distribution, metabolism and excretion (pharmacokinetic phase) directly influence the concentration of drug attained in the target parasites and the resultant pharmacological effect. Lipophilicity determines the ability of the compounds to pass through cell membranes of parasites, which is related to drug accumulation in the target parasites and consequently to efficacy. The passage of the drug through the parasite structures permits it to reach a specific receptor and to produce its effect. The evaluation of efficacy for nematodes, trematodes and cestodes is based on the percentage reduction in parasite counts in dose confirmation studies. Claims for efficacy of a product should be expressed against each genus (immature stages/adults) as highly effective (>98%), effective (90–98%), moderately effective (80–89%) or insufficiently active (<80%) (Wood *et al.* 1995). In this context the physicochemical properties and pharmacokinetic disposition have a direct influence on the anthelmintic activity of different molecules (Lanusse and Prichard 1993a). The pharmacological features of anthelmintic molecules and their impact on sustained parasite control in ruminants were recently reviewed (Lanusse *et al.* 2014, 2015). The aim of the current review is to provide an overview of the relationship between the pharmacokinetic features of different anthelmintic drugs, their availability in host tissues, accumulation within target helminths and resulting therapeutic efficacy. This review mainly concerns anthelmintics used in cattle and sheep for which published information on the overall topic is available.

Activity of anthelmintics against nematodes.

Detailed knowledge on the relationship between physicochemical properties and host tissue disposition kinetics for the most widely used broad-spectrum anthelmintics in ruminants, benzimidazoles (BZD) and macrocyclic lactones (ML), has been important for generating novel research approaches for parasitic control. Understanding the processes of drug or metabolite diffusion into different target parasites, together with the available kinetic information, has been applied to elucidate the mechanism of drug penetration and the pharmacological activity of these compounds. Some complementary available pharmacological knowledge on the new drug monepantel is included in this section.

Benzimidazoles

Benzimidazole anthelmintics are widely utilised in veterinary medicine, with the methylcarbamate compounds used most commonly, such as albendazole, fenbendazole, oxfendazole and ricobendazole. The anthelmintic efficacy of BZD depends on their ability to reach high and sustained concentrations at the site of parasite location and bind to the parasite ß-tubulin (Lanusse and Prichard 1993b). These anthelmintics provide a good example of the influence of pharmaceutical formulation on pharmacokinetic disposition. The methylcarbamates are formulated as a suspension and show only limited gastrointestinal absorption due to their poor solubility in water. In ruminant species, the rumen may substantially influence the absorption pattern and the resultant pharmacokinetics and antiparasitic activity of orally delivered BZD anthelmintics. The adsorption of BZD particles to solid matter and the long residence time of digesta in the rumen delay the rate of passage of the drug down the gastrointestinal tract (Hennessy 1993). This slow passage is important as the abomasal pH facilitates the dissolution of BZD in water (McKellar and Scott 1990), which is the rate-limiting step in the systemic availability of the active drug or metabolites (Lanusse and Prichard 1993b). Only the dissolved drug is available for absorption. Gastrointestinal nematodes are exposed to the dissolved drug in gastrointestinal fluids and also to the drug recycled from plasma to those fluids. The plasma concentration profiles of BZD, including parent sulphides such as albendazole, fenbendazole and/or active sulphoxide metabolites, reflect the amount of dissolved drug at the gastrointestinal level and the overall exposure of nematodes located in the gastrointestinal tract to the drug.

The blood consuming nematode *Haemonchus contortus* is one of the most pathogenic parasites of small ruminants. *H. contortus* has been used as an *in vivo* model to understand the relationship between the routes of drug entry and efficacy. Anthelmintic drugs can reach *H. contortus* either by oral ingestion of blood or by transcuticular uptake or diffusion (Geary *et al.* 1995). Therefore, the

concentration of drug in the bloodstream and abomasal fluid or mucosa is related to the clinical efficacy against this parasite. Albendazole is not found in the bloodstream after oral administration to sheep and cattle. The active albendazole sulphoxide metabolite has been postulated to be responsible for the activity against different nematodes (Marriner and Bogan 1980). However, Alvarez *et al.* (2000) reported that, after intraruminal administration, both albendazole and albendazole sulphoxide rapidly accumulated in *H. contortus*, being detected in the parasite between 0.5 and 12 hours post-administration. As albendazole was not detected in peripheral plasma, only drug found in abomasal fluid and mucosa may reach the parasite through the nematode cuticle. It should be also pointed out that *H. contortus* may feed on portal blood as a source of albendazole. However, only low concentrations of albendazole (between 0.01–0.1 µg/mL) were recovered in portal blood (Alvarez *et al.* unpublished data), and do not explain the high concentrations of albendazole observed in *H. contortus*. Altogether, these findings confirm the importance of the transcuticular absorption process for penetration of albendazole in *H. contortus*, being the parent drug and the main molecule responsible for activity against this parasite.

Anthelmintic activity could theoretically be improved by increasing concentrations of active drug at the site of target receptor location, commonly termed the biophase. Different strategies have been assessed to increase availability of BZD and their resultant pharmacological activity. Enhanced plasma availability, measured as area under the concentration *vs.* time curve (AUC), and anthelmintic efficacy against gastrointestinal nematodes in lambs was obtained by administering three single recommended doses of oxfendazole every 12 hours (Sangster *et al.* 1991), and when the dose of albendazole was doubled from 3.8 to 7.5 mg/kg (Moreno *et al.* 2004). Other trials have evaluated the impact of different doses of albendazole on pharmacokinetic behaviour (Alvarez *et al.* 2012), and efficacy against resistant *H. contortus* (Barrere *et al.* 2012), in artificially infected lambs. Significantly higher AUC and peak concentrations in plasma for albendazole sulphoxide were observed after the administration of albendazole at both 15 and 45 mg/kg compared to the conventional treatment at 5 mg/kg. As BZD metabolites are reversibly exchanged between the bloodstream and the gastrointestinal tract (Lanusse *et al.* 1993b), the enhanced concentrations of drug in plasma associated with the increasing administered doses may account for gastrointestinal nematodes being exposed to toxic drug concentrations for extended periods. As expected, there was a strongly negative correlation between the albendazole sulphoxide AUC and peak plasma concentrations and the number of adult *H. contortus* recovered from treated lambs (Alvarez *et al.* 2012, Barrere *et al.* 2012). The impact of different albendazole doses on pharmacokinetic behaviour and efficacy against resistant *H. contortus* is summarised in Table 1.

Dietary management was also considered a useful tool to enhance the systemic availability of BZD. A temporary reduction in feed intake or a short period of fasting (12–24 hours) increased the plasma availability of BZD and improved efficacy in sheep and cattle (Hennessy *et al.* 1995; Ali and Hennessy 1995; Lifschitz *et al.* 1997; Sanchez *et al*. 1997). The reduction in feed intake and fasting delayed the gastrointestinal transit time and increased the drug dissolution time in the abomasum and consequently the amount of drug available to be absorbed. However, clinical efficacy against highly resistant gastrointestinal nematodes obtained after the oral administration of albendazole was similar in fed (48%) and fasted (49%) lambs, indicating that the 68% increase in albendazole sulphoxide systemic availability was not enough to improve anthelmintic efficacy against highly resistant nematodes (Alvarez *et al.* 2010). Therefore, the success of the strategies used to improve the BZD availability and efficacy depends on the level of drug resistance already established in the treated nematode population.

In summary, the systemic availability of orally delivered BZD anthelmintics in ruminants is directly related to the amount of drug in solution in the gastrointestinal tract. Enhanced plasma concentrations correlate with increased gastrointestinal dissolution, increased drug concentrations in the gastrointestinal fluid and increased target parasite exposure. Under these circumstances, BZD clinical efficacy may be improved, but depends on the resistance status of the parasite population.

Macrocyclic lactones

Macrocyclic lactones are effective against endo- and ectoparasites in different animal species. They exhibit a broad-spectrum of antiparasitic activity against gastrointestinal and lung nematodes (McKellar and Benchaoui 1996). They are very lipophilic and relatively insoluble in water (Jackson 1989). Although all ML show a great affinity for fatty tissues, moxidectin lipophilicity is 100-fold higher than other avermectins (Hennessy 1997). From a practical point of view, the components of the formulation and the administration route substantially influence the pharmacokinetics of ML (Lo *et al.* 1985; Wicks *et al.* 1993). They are available in the veterinary pharmaceutical market to be administered by S/C, oral and topical routes (McKellar and Benchaoui 1996).

After S/C treatment, ML must be absorbed from the injection site to the bloodstream to achieve effective concentrations in the target tissues. Concentrations of ML achieved in the target tissues of cattle are greater compared to those observed in plasma, with availability in the target tissue relative to plasma varying from 1.50 to 3.44 for ivermectin, doramectin and moxidectin (Lifschitz *et al.* 1999a, 2000). The extensive distribution process of ML to the sites of parasite location, and the persistence of high concentrations in target tissues make possible their efficacy against adult and larval stages of gastrointestinal and lung nematodes, resulting in a prolonged protective efficacy

(Armour *et al.* 1985; Ranjan *et al.* 1992; Jones *et al.* 1993). A strong correlation (r=0.88–0.99) has been observed between the plasma and tissue concentration profiles of ML (Lifschitz *et al.* 1999, 2000). Therefore, plasma concentrations of ML following S/C administration predict the effect of the drugs in the tissues where nematodes are located.

The relationships between distribution to the target tissue, drug concentrations in parasites and efficacy were recently evaluated in nematode-infected lambs administered 0.2 mg/kg or 0.4 mg/kg of ivermectin and moxidectin. Exposure of *H. contortus* to both drugs was related to the dose administered and was correlated with drug concentrations in the abomasal contents (r=0.86). Whereas ivermectin completely failed to control highly resistant *H. contortus* (0% efficacy) at both doses, the efficacy of moxidectin against this ivermectin-resistant strain was 85.1 and 98.1% for 0.2 and 0.4 mg/kg doses, respectively (Lloberas *et al.* 2015). In another study, increasing the dose of ivermectin administered intraruminally from 0.2 to 2.0 mg/kg resulted in enhancement of efficacy against *H. contortus* from 48 to 98% (Alvarez *et al.* 2015). Although it is clear that increasing the dose is not a practical solution to control resistant parasites, the *in vivo* kinetic results were corroborated by *in vitro* accumulation studies of ivermectin and moxidectin in adult *H. contortus*. Accumulation of the drug in the parasites was directly related to the concentration of drug present in the environment where the nematodes were located and to the duration of parasite exposure (Lloberas *et al.* 2015).

The choice of administration route for ML are principally based on marketing and practical factors. However, with increasing resistance, selection of the administration route may be an important pharmacological tool to improve efficacy. Administration of ML by the oral route resulted in lower plasma concentrations compared to S/C treatment (Marriner *et al.* 1987; Imperiale *et al.* 2004) and supports the clinical indication to use the S/C route to control ectoparasites in ruminants. However, when efficacy was evaluated against parasite populations with a reduced susceptibility, oral administration of ML showed a better performance compared to S/C injections in lambs (Gopal *et al.* 2001; Alka *et al.* 2004). The pharmacological rationale behind the observed differences in efficacy against resistant nematodes was shown to be related to concentrations in the gastrointestinal tract. The AUC (systemic availability) for ivermectin in plasma in lambs was 129 ng.d/mL after S/C treatment and 58.4 ng.d/mL after intraruminal treatment, however concentrations in the abomasal contents were 57-fold higher after intraruminal administration. The increased concentrations in the abomasal content after intraruminal administration accounted for the greater amount of drug measured within adult *H. contortus* and the higher efficacy compared to the S/C treatment (Lloberas *et al.* 2012). In cattle, oral, S/C and pour-on administration of moxidectin resulted in high efficacy by all administration routes against a susceptible parasite such as

Ostertagia spp. However, efficacy of the drug against resistant *Cooperia oncophora* was significantly higher after oral treatment (Leathwick and Miller 2013). Similar efficacy trends were observed after comparison of abamectin administered by oral and topical routes to cattle; efficacy against *C. oncophora* was 83 and 35% after oral and topical administration of abamectin, respectively, supporting the advantage of the oral route (Leathwick *et al.* 2016). Collectively, these results show that high efficacy against nematodes located in the gastrointestinal tract may be obtained after the administration of ML by the oral route, especially if parasites have a reduced susceptibility. The improved efficacy may be based on the enhanced drug exposure of the nematodes located in the lumen of the abomasum and/or small intestine.

Pour-on formulations of ML are internationally marketed for use in cattle. Due to self- and allo-grooming, an unpredictable portion of drug topically administered may be systemically absorbed via the oral route. This social behaviour produces highly variable kinetic profiles and also different pharmacodynamic responses in treated, and in some cases in untreated, animals (Laffont *et al.* 2003; Sallovitz *et al.* 2005; Bousquet-Melou *et al.* 2011; Toutain *et al.* 2012). A skin depot of the drug occurs following topical administration of ML and large amounts of drug are recovered from the dermal layers of skin (Sallovitz *et al.* 2003). As the skin is a barrier which limits the amount of substances reaching the bloodstream, the plasma concentrations of any given substance achieved exclusively by transdermal absorption will be lower than those are administered by oral or S/C routes. Thus, following topical administration (without licking behaviour), ML are slowly released from the skin to the systemic circulation resulting in a lower availability in plasma and in the target tissues compared to the other administration routes. If oral ingestion of the drug occurs following topical treatment with ML (licking behaviour), drug concentrations in gastrointestinal luminal fluid contents are markedly higher than those measured after the S/C injection of these compounds (Sallovitz *et al.* 2003, 2005). These factors explain the large inter-animal variability in drug exposure after topical administration of ML (Toutain *et al.* 2012) and the subsequent higher variability in the efficacy compared to other routes (Leathwick and Miller 2013).

Overall these studies demonstrate that the administration route has a direct influence on the amount of ML reaching target parasites and consequently on their efficacy against different gastrointestinal parasites, as summarised in the schematic representation shown in Figure 1.

Amino-acetonitrile derivatives: monepantel

The amino-acetonitrile derivatives represent one of the newest anthelmintic groups introduced in veterinary medicine. Monepantel, the active s-enantiomer of the molecule AAD 96, was launched into the veterinary pharmaceutical market in 2009.It is active against larval and adult stages of

gastrointestinal nematodes of sheep (Hosking *et al.* 2010), and its plasma disposition kinetics have been assessed in sheep after oral administration (Karadzovska *et al.* 2009; Lifschitz *et al.* 2014). Several metabolites of monepantel were described after *in vitro* and *in vivo* assays in sheep, but monepantel sulphone was the main metabolite detected in the bloodstream after monepantel administration (Stuchlíková *et al.* 2013, 2014). Low concentrations of monepantel were measured in plasma up to 48–72 hours post-administration, with significantly higher concentration profiles for monepantel sulphone detected in the bloodstream. The metabolite was detected in plasma up to 9–12 days after oral administration of monepantel to sheep (Lifschitz *et al.* 2014).

Although the evaluation of plasma concentration profiles contributed useful information, the characterisation of concentrations in the different tissues of the gastrointestinal tract is necessary to understand the pharmacokinetic-pharmacodynamic relationship. In the abomasal contents, monepantel concentrations were between 2000–4000 ng/g during the first 48 hours post-treatment; monepantel sulphone was also detected in the abomasal contents but concentrations were significantly lower (Lifschitz *et al.* 2014). The detection of monepantel sulphone in the abomasal contents may be explained by the exchange between the blood and the gastrointestinal tract lumen, as was demonstrated for BZD compounds in sheep (Hennessy 1993). The high efficacy of monepantel against the abomasal nematode *H. contortus* may be based on this exchange. The lipophilicity of monepantel and monepantel sulphone and the great availability of both compounds in the abomasal contents contribute to their accumulation within the parasite through a transcuticular diffusion process. The relationship between monepantel pharmacokinetics and parasite exposure to the parent drug and its active sulphone metabolite is schematically illustrated in Figure 2.

The partitioning of monepantel and monepantel sulphone between the intestinal content and mucosal tissue differs; the accumulation of monepantel in the fluid content of the intestine is mainly related to the non-absorbed orally administered drug. In contrast, the high concentrations of monepantel sulphone recovered from mucosal tissues comes from the blood-mucosa transfer in different intestinal segments (Lifschitz *et al.* 2014). Active intestinal secretion, mediated by the P-glycoprotein and breast cancer resistance protein (BCRP), has been observed for other anthelmintic compounds such as ivermectin (Lifschitz *et al.* 2012). However, the active transport of monepantel and monepantel sulphone along the intestine has not been fully elucidated. The interaction of monepantel and monepantel sulphone with ruminant BCRP cloned in cultured cells was recently demonstrated (Halwachs *et al.* 2014), and may explain the high concentrations of monepantel sulphone measured in the milk of dairy cows (Mahnke *et al.* 2016). Furthermore, *in vitro* exposure of *H. contortus* larvae to extremely high concentrations of monepantel increased the expression of different transporter-proteins (Raza *et al.* 2016). The involvement of transporter-proteins in the

pharmacokinetics and resistance mechanisms of monepantel should be evaluated, as they may affect the effective drug concentrations reached at the target tissue and in the parasites. This evaluation would be also useful to detect potential drug-drug interactions when this compound is combined with other drugs, as is recommended for the optimisation of parasite control in livestock.

The required concentrations of anthelmintic drugs at the site of parasite location to inhibit parasite establishment have not been determined. This information is very important to predict the efficacy and the persistence of action of these compounds against the different parasites. Monepantel and monepantel sulphone affect the motility of free-living nematodes and adult *H. contortus* at 50–100 ng/mL but full lethality occurs at drug concentrations above 1,000 ng/mL (Kaminsky *et al.* 2008). The drug concentrations of monepantel measured in the gastrointestinal tract of lambs (Lifschitz *et al.* 2014) are in agreement with the minimal effective concentrations determined in *in vitro* experiments. Although monepantel sulphone was detected in plasma between 9–12 days post-administration, the low drug concentrations measured may not be sufficient to obtain a good persistence of antiparasitic activity against different gastrointestinal nematodes (Lifschitz *et al.* 2014).

The characterisation of pharmacokinetic behaviour is useful for comprehension of the varying efficacy of monepantel against nematodes located in different systemic tissues. Monepantel is not recommended to control lung nematodes at the dose used for gastrointestinal nematodes (Hosking 2010). The low drug concentrations obtained in plasma after oral administration of monepantel to sheep at 2.5 mg/kg may be below the minimal concentrations necessary to obtain optimal efficacy against lung nematode parasites (Lifschitz *et al.* 2014). Other examples of dose-limiting parasites for monepantel are the nematodes located in the large intestine, such as *Oesophagostomum venulosum*. The dose of 2.5 mg/kg was established as a suitable minimum dose rate due to the difficulties in killing fourth-stage larvae of *O. venulosum*. However, variable efficacy was observed in different studies (Hosking *et al.* 2009). The low efficacy against this parasite may be based on a pharmacodynamic limitation, but also the pharmacokinetics features of monepantel. The mucosal tissue of the large intestine is the preferred location of fourth-stage larvae of *O. venulosum*. The maximal concentration of monepantel achieved in the large intestine mucosa (225 ng/g) is lower than in the small intestine (762 ng/g in duodenum and 562 ng/g in ileum) (Lifschitz *et al.* 2014), and may explain the variable efficacy against *O. venulosum* .

In summary, the high concentrations of monepantel and monepantel sulphone obtained in the gastrointestinal target tissues (fluids and mucosal tissue) during the first 2–4 days post-administration are important for the efficacy of this drug against gastrointestinal nematodes. The

low concentrations of the parent drug and metabolites in plasma observed after oral administration of monepantel are not sufficient to obtain a good effectiveness against nematodes located in other tissues.

Cestodicidal and flukicidal activity of anthelmintics

Cestode nematodes, commonly known as tapeworms, are an important class of endoparasitic organisms. Losses produced by cestodes in ruminants are due to deaths from gross parasitism, lowered vitality, and poor growth and performance. The absence of a digestive system in cestodes simplifies the interpretation of the functional properties of the external surface (tegument) and the mechanisms of drug accumulation. The tegument of cestodes is structurally adapted to interact with the surrounding environment and to perform all functions normally associated with intestinal tissue (Thompson and Geary 2003). Therefore the only way that a given drug molecule can reach its receptor is by passing through the tegument.

Fasciolosis, caused by the trematode liver fluke *Fasciola hepatica,* is the cause of considerable loss in sheep and cattle production systems all over the world (Courtney and Roberson 1995). The use of flukicidal compounds is the main tool to control liver fluke. The activity of most anthelmintic molecules is based on their affinity for a specific receptor, and consequently it is important to understand the main route of drug entry into *F. hepatica*. There are two potential routes of drug entry; oral ingestion from blood and transtegumental diffusion of the drug present in bile. The relative importance of these routes for different compounds is examined in this section.

Benzimidazoles

Cestocide activity

Cestode parasites, such as *Moniezia* spp., reside in the intestinal lumen immersed in the fluid. The anthelmintically active drug or metabolites dissolved in the intestinal fluid must diffuse from the fluid into the parasite through the external tegument. After only 0.5 hour post-treatment, concentrations of albendazole were detected in cestode parasites that were higher than those recovered in the intestinal fluid of the same infected lamb. This pattern was observed at all the sampling times during the first 6 hours post-treatment (Alvarez *et al.* 1999). These findings confirm the rapid and efficient uptake of albendazole by cestode parasites. Furthermore, albendazole sulphoxide was the analyte recovered at the highest concentrations in the intestinal fluid of treated animals. However, in the tapeworms collected from those animals, the availability of albendazole was significantly greater than that of its sulphoxide metabolite (Alvarez *et al.* 1999). The greater *in vivo* uptake of albendazole compared to albendazole sulphoxide by tapeworms can be explained by the higher lipophilicity of the parent molecule. This finding was considered an indirect indication

that passive diffusion is the main mechanism of BZD entry into tapeworms. In fact, there is a close correlation between molecular lipophilicity (expressed as the octanol-water partition coefficient) and the amount of drug recovered within *M. benedeni*, under *in vitro* conditions (Mottier *et al.* 2003).

Flukicide activity

The BZD compounds currently marketed as flukicidal include the methylcarbamate BZD compound albendazole, and the halogenated BZD compound triclabendazole (Lanusse and Prichard 1993b). Triclabendazole shows excellent efficacy against both the mature and immature stages of the liver fluke in sheep and cattle, which is a differential feature compared to other available trematodicidal drugs (Boray *et al.* 1983). Triclabendazole is rapidly oxidised to form triclabendazole sulphoxide and triclabendazole sulphone. The parent compound is short lived, and both triclabendazole sulphoxide and triclabendazole sulphone are the main unconjugated compounds recovered in the bloodstream and bile of treated sheep (Hennessy *et al.* 1987). It has been demonstrated that both metabolites can induce severe disruption to *F. hepatica in vitro* (Halferty *et al.* 2009).

The route of drug entry of triclabendazole into *F. hepatica* was initially assessed i*n vitro*. Equivalent triclabendazole sulphoxide concentrations were recovered from mouth-ligated (unable to orally ingest) and non-ligated adult *F. hepatica* (Mottier *et al.* 2004, 2006), which demonstrates the importance of drug entry through the external surface. The time-course and pattern of *in vivo* accumulation of triclabendazole and its metabolites into adult *F. hepatica* were evaluated in specimens recovered from infected sheep (Moreno *et al.* 2014). Triclabendazole sulphoxide and triclabendazole sulphone were the only molecules recovered in the bloodstream, with peak plasma concentrations of 10.8 and 12.6 µg/mL, respectively. The same metabolites were also the main analytes accumulated within adult flukes, reaching similar peak concentrations at 24 hours post-treatment (Moreno *et al.* 2014). In the same experiment, high concentrations of triclabendazole were quantified in bile, that certainly provides the potential for substantial chemical contact with the liver-dwelling *F. hepatica*. However, low concentrations of triclabendazole (0.14 µg/g at 24 hours post-treatment) were measured within collected flukes. Contrary to the observations in the *in vitro* trials, these data confirm that oral ingestion is a main route of drug entry into adult liver flukes *in vivo* exposed to triclabendazole and its metabolites. The presence of low concentrations of triclabendazole within the adult fluke may be related to some degree of transtegumental diffusion from bile or by a direct oral ingestion from portal blood.

The physicochemical characteristics of the fluids surrounding the parasite may play a role in drug accumulation (Alvarez *et al.* 2004). The partitioning of the active drug or metabolites between an aqueous fluid (buffer or incubation medium in the *in vitro* assays) and the lipoid-tissue of the parasite may facilitate the accumulation of the drug within the parasite. This drug partitioning phenomenon may be different *in vivo* for sites of parasite location such as the biliary tract, where the bile-induced micelle formation may affect the diffusion of the active drug or metabolite into the target parasite. The amounts of albendazole, fenbendazole and triclabendazole recovered from *F. hepatica in vitro* incubated in the absence of bile were significantly greater than those obtained after incubation in bile (Alvarez *et al.* 2004). Consequently, the physicochemical features of the environment where the target parasite is immersed play a pivotal role in the process of drug access, indicating that some helminths may be protected from the deleterious effect of a drug when living in their preferred location site. This phenomenon may also explain many therapeutic failures observed in parasite control in both human and veterinary medicine which, in some cases, have contributed to exposing the target parasites to sub-therapeutic drug concentrations. As an example, high efficacy against susceptible gastrointestinal nematodes has been demonstrated for albendazole at 5 mg/kg in sheep, but a higher dose (7.5 mg/kg) is recommended to kill adult fluke (McKellar and Scott 1990). The location of *F. hepatica* in the bile ducts and the low diffusion pattern of drug or metabolite from bile may partly explain the need for a higher dose for this parasite (Alvarez *et al.* 2004).

Among BZD there are differences in the flukicidal activity of two similar compounds, albendazole and fenbendazole. Fenbendazole differs from albendazole in the aromatic group substitution at the 5-BZD ring system. The rate of microsomal sulphoxidation of albendazole to albendazole sulphoxide in sheep and cattle were higher than those observed for fenbendazole to oxfendazole (Virkel *et al.* 2004). For this reason, low concentrations of fenbendazole are recovered in plasma following oral or intraruminal administration to sheep and cattle, whilst albendazole is not detected in the bloodstream after oral administration to both species. Albendazole and fenbendazole share broad spectrum activity against gastrointestinal and lung nematodes and against cestodes, but fenbendazole and oxfendazole show low efficacy against *F. hepatica* (McKellar and Scott 1990). This is an important difference for similar compounds, which could be based on a pharmacodynamic or pharmacokinetic restriction. Although both BZD methylcarbamates have very low water solubility, the water solubility of albendazole is much higher than fenbendazole (McKellar and Scott 1990), with the metabolite albendazole sulphoxide being the most water soluble compound (Ceballos *et al.* 2012). These differences account for a significantly greater drug availability in plasma of albendazole metabolites compared to fenbendazole metabolites after oral administration of the parent drug to sheep. In fact the AUC of albendazole sulphoxide was 2.2-fold

higher than oxfendazole (Lanusse *et al.* 1995). The usual dose of oxfendazole (5 mg/kg) used against nematodes in sheep has low flukicidal efficacy (Furmaga *et al.* 1982). However, at a dose of 30 mg/kg oxfendazole demonstrated a high clinical efficacy against liver fluke (Gomez-Puerta *et al.* 2012). The increased dose of oxfendazole in sheep was also associated with enhanced plasma availability of oxfendazole, as shown in Table 2 (Alvarez *et al.* unpublished data). We can conclude that the low activity of oxfendazole against *F. hepatica* is based on a pharmacokinetic issue. When concentrations of oxfendazole in plasma were similar or higher than those observed for albendazole, this compound had flukicidal activity.

Closantel

Closantel is an ecto-endo parasiticide compound, that is highly effective for the treatment of adult fluke. In addition it shows good activity against immature specimens aged 6–8 weeks, but is not effective against earlier stages (Ali and Bogan 1987). It is formulated for oral or parenteral administration in ruminants. Closantel is an extremely lipophilic molecule, extensively bound (>99%) to plasma proteins, mainly albumin (Ali and Bogan 1987). As a consequence of its high plasma protein binding and low metabolism, an extended therapeutic concentration of closantel is observed in treated animals. Recently, the *in vivo* pattern of closantel accumulation in adult *F. hepatica* was investigated after oral or S/C treatment of sheep (Ceballos *et al.* 2015). Closantel was recovered in plasma, *F. hepatica*, bile and liver in all animals sacrificed at 12, 24 and 36 hours post-treatment. Concentrations of closantel in plasma increased after oral administration from 36 µg/mL (12 hours post-treatment) to 38.8 µg/mL (24 hours post-treatment) and 57 µg/mL (36 hours post-treatment). A similar trend was observed in adult liver flukes, in which concentrations were 16.1, 22.6 and 33.8 µg/g at 12, 24 and 36 hours post-treatment, respectively (Ceballos *et al.* 2015). The highest concentrations of closantel were measured in plasma and *F. hepatica*, either after oral or S/C administration, whereas low concentrations were measured in bile and liver tissue samples, ranging from 2.17–6.40 µg/mL after oral treatment, and from 0.66–3.27 µg/mL after S/C treatment (Ceballos *et al.* 2015). This is consistent with its limited tissue distribution due to high protein binding (Michiels *et al.* 1897). The pattern of drug accumulation in *F. hepatica* matched the plasma availability of closantel. In fact a significant positive correlation ($p<0.05$) was observed between individual concentrations of closantel in plasma and *F. hepatica* after oral and S/C administration. This high correlation can only be explained by the oral ingestion of closantel due to the haematophagous behaviour of *F. hepatica*.

In summary, the results obtained under *in vivo* conditions shows that the presence of flukicidal drugs in bile may facilitate some degree of drug accumulation by a transtegumental diffusion

process. However, the available information clearly indicates that oral ingestion is the main route of drug entry into the liver fluke exposed to flukicidal drugs *in vivo*.

Concluding remarks

The pharmacokinetics of an anthelmintic drug involves the time course of drug absorption, distribution, metabolism and elimination from the host, which, in turn, determines the concentration of the active drug reaching the site of parasite location. The understanding of pharmacokinetic behaviour and identification of different factors affecting drug activity is important for achieving optimal parasite control and avoiding selection for resistance. *In vivo* minimal drug concentrations required to affect gastrointestinal helminths have not been determined, so the characterisation of drug concentration profiles in tissues where parasites are located, and within parasites, provides the understanding of the amount of drug reaching the target receptor site. From a pharmacokinetic point of view, increasing drug availability is a well established pharmacological tool for treatment optimisation and to delay the development of anthelmintic resistance. Any pharmacological strategy which facilitates the attainment of high drug concentrations at the sites of parasite location, for sufficient time, is important to induce a successful antiparasitic effect. In the case of BZD compounds, the dissolution of the orally administered dose is the limiting step in the systemic availability of the active drug or metabolites. Advances in pharmaceutical technology using new drug delivery systems, which solve the dissolution difficulties, constitute an attractive alternative to improve BZD formulations.

The complex interactions between route of administration, formulation, drug physicochemical properties and the resultant kinetic behaviour need to be understood to optimise drug efficacy. ML are administered by oral, S/C or topical routes, formulated as solutions. The administration route of ML is an important factor that influences the exposure of nematodes located in the lumen of the abomasum and/or small intestine. Administration of ML by the oral route facilitates the achievement of higher drug concentrations and enhanced efficacy against nematodes located in the gastrointestinal tract compared to S/C treatment. Some novel pharmacological information for the new compound monepantel is now available. It shows that the very high concentrations of the parent drug and its metabolite achieved in the gastrointestinal tract during the first 2–3 days after oral treatment play an important role in its nematodicidal activity, being critical to induce the pharmacological action at the monepantel biophase.

Several obstacles confront pharmaceutical companies in their search to bring innovative new anthelmintic drugs to the market. Only two new compounds, monepantel and derquantel, were launched against gastrointestinal nematodes during the last 30 years. Understanding the

pharmacological properties of the traditional and novel anthelmintic chemical entities and the development of new "intelligent" formulations that increase the dose reaching the target parasites may help to avoid misuse and prolong their lifespan in the veterinary pharmaceutical market.

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Table 1. Results of studies [a] examining the effect of dose of albendazole (ABZ) in artificially infected lambs on concentrations of ABZ and the active metabolite, albendazole sulphoxide (ABZSO), in plasma, abomasal fluid and the target nematode (*Haemonchus contortus*), host systemic availability of ABZSO and efficacy.

	Dose		
	5 mg/kg	**15 mg/kg**	**45 mg/kg**
Concentration of ABZSO in plasma (µg/mL) [b]	2.24	3.3	8.06
Concentration of ABZ in abomasal fluid (µg/mL) [b]	1.01	6.33	10.3
Concentration of ABZSO in abomasal fluid (µg/mL) [b]	7.96	14.5	35.5
Concentration of ABZ in *H. contortus* (µg/g) [b]	1.08	1.38	4.62
Concentration of ABZSO in *H. contortus* (µg/g) [b]	2.55	3.71	4.31
Systemic availability of ABZSO (AUC)	21	159	390
Efficacy (%)[c]	16	59	94

[a] Data from Alvarez *et al.* 2011, 2012 and Barrere *et al.* 2012.
[b] Measured 12 hours post-treatment.
[c] Based on ??.
AUC=Area under the plasma concentration *vs.* time curve (µg.h/mL)

Table 2. Mean (± SD) area under the plasma concentration *vs.* time curve (AUC), peak plasma concentration (C_{max}) and flukicidal efficacy of oxfendazole in sheep following treatment at two different doses [a].

	Dose	
	5 mg/kg	**30 mg/kg**
Plasma AUC (µg.h/mL)	18.0 ± 3.10	88.1 ± 21.3
Plasma C_{max} (µg/mL)	0.60 ± 0.10	2.50 ± 0.60
Flukicidal activity (%)[b]	14	100

[a] Data from Furmaga *et al.* (1982); Gomez-Puerta *et al.* (2012) and Alvarez *et al.* unpublished data
[b] Based on ??.

Figure 1. Summary of differences between the route administration of macrocyclic lactones on the attainment of effective drug concentrations at the sites of target nematode location and therapeutic outcomes against gastrointestinal nematodes in ruminants

Figure 2. Representation of the relationship between the pharmacokinetic behaviour of monepantel (MNP ○) and its metabolite, monepantel sulphone (●) and parasite exposure to both molecules in the abomasum, illustrating the accumulation of both anthelmintically active molecules within adult *Haemonchus contortus*. Data from Lifschitz *et al.* (2014). AUC=area under the concentration *vs.* time curve.