Comparative assessment of the access of albendazole, fenbendazole and triclabendazole to *Fasciola hepatica*: effect of bile in the incubation medium

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SUMMARY

The work reported here describes the comparative ability of albendazole (ABZ), fenbendazole (FBZ) and triclabendazole (TCBZ) to penetrate through the tegument of mature *Fasciola hepatica*, and the influence of the physicochemical composition of the incubation medium on the drug diffusion process. The data obtained from the trans-tegumental diffusion kinetic studies were complemented with the determination of lipid-to-water partition coefficients (octanol-water) for the benzimidazole (BZD) anthelmintic drugs assayed. Sixteen-week-old *F. hepatica* were obtained from untreated artificially infected sheep. The flukes were incubated (37 °C) over 60 and 90 min in incubation media (pH 7.4) prepared with different proportions of ovine bile and Krebs’ Ringer Tris (KRT) buffer (100, 75, 50, 25 and 0% of bile) containing either ABZ, FBZ or TCBZ at a final concentration of 5 nmol/ml. After the incubation time expired, the liver fluke material was chemically processed and analysed by high performance liquid chromatography (HPLC) to measure drug concentrations within the parasite. Additionally, the octanol-water partition coefficients (PC) for each molecule were calculated (as an indicator of drug lipophilicity) using reversed phase HPLC. The 3 BZD molecules were recovered from *F. hepatica* at both incubation times in all incubation media assayed. The trans-tegumental diffusion of the most lipophilic molecules ABZ and FBZ (higher PC values) tended to be greater than that observed for TCBZ. Interestingly, the uptake of ABZ by the liver flukes was significantly greater than that measured for TCBZ, the most widely used flukicidal BZD compound. This differential uptake pattern may be a relevant issue to be considered to deal with TCBZ-resistant flukes. Drug concentrations measured within the parasite were lower in the incubations containing the highest bile proportions. The highest total availabilities of the 3 compounds were obtained in liver flukes incubated in the absence of bile. Altogether, these findings demonstrated that the entry of the drug into a target parasite may not only depend on a concentration gradient, the lipophilicity of the molecule and absorption surface, but also on the physicochemical composition of the parasite’s surrounding environment.

Key words: trans-tegumental drug diffusion, benzimidazole anthelmintics, *Fasciola hepatica*, bile acids, albendazole, fenbendazole, triclabendazole.

INTRODUCTION

The economic importance of helminth infections in livestock has long been recognized and it is probably for this reason that the most relevant advances in the chemotherapy of helminthiasis have come from the animal health area (Horton, 1990). Chemotherapy still remains the most widely used method to control parasitism in livestock (Zajac, Sangster & Geary, 2000) and human health (Quellette, 2001). The activity of most anthelmintic molecules is based on their affinity for a specific receptor, and on the kinetic properties that allow the delivery of effective drug concentrations to the receptor inside the parasite, in sufficient time to cause the therapeutic effect (Thompson et al., 1993). Anthelmintic drugs can reach target helminth parasites by either oral ingestion or by diffusion through the external surface of the parasite, or some combination of both routes (Thompson & Geary, 1995). The accumulated data show that the main route of acquisition of broad-spectrum anthelmintics by target parasites appears to be by passive diffusion through their tegument (cestodes/trematodes) (Alvarez, Sánchez & Lanusse, 1999; Alvarez et al., 2000, 2001) or cuticle (nematodes) (Ho et al., 1990; Sims et al., 1996; Cross, Renz & Trees, 1998). Consequently, the rate of penetration of a drug will mainly depend on the intrinsic lipid-to-water partition coefficient of the molecule (Mottier et al., 2003), pH/pK relationship, molecular size, concentration gradient and the surface area of contact between drug and parasite.

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Benzimidazoles (BZD) are broad-spectrum anthelmintic compounds widely used in human and veterinary medicine to control nematode, cestode and trematode infections (McKellar & Scott, 1990). The BZD compounds currently marketed as anthelmintics can be grouped as BZD thiazolyls, BZD methylcarbamates, pro-BZD and halogenated BZD thiols (Lanusse & Prichard, 1993). Only a few molecules within the BZD chemical family demonstrated activity against the trematode, *Fasciola hepatica*. Albendazole (ABZ) is the only BZD methylcarbamate recommended to control fascioliasis in domestic animals, despite its activity being restricted to flukes older than 12 weeks (McKellar & Scott, 1990). Fenbendazole (FBZ), a similar BZD methylcarbamate widely used in veterinary medicine as a nematodicial drug, is not as effective as ABZ against *F. hepatica*, but a single treatment of 5 mg/kg reduced *F. gigantica* infection in sheep by up to 95% (Roberson & Courtney, 1993). Unlike other BZD compounds, the halogenated derivative triclabendazole (TCBZ) has been shown to have an efficient efficacy against the adult and juvenile stages of *F. hepatica* (Boray et al. 1983). However, TCBZ activity appears to be restricted to the liver fluke and the lung fluke, *Paragonimus* spp. (Weber, Buscher & Buttner, 1988; Calvopina et al. 1998), because the drug does not show clinical efficacy against nematodes, cestodes and other trematode (*Dicrocelium dendriticum*, *Paramphistomum* spp. and *Schistosoma mansoni*) parasites. BZD nematodicial activity is based on its binding to parasite β-tubulin (Borgers & De Nollin, 1975; Lacey, 1988; Lubega & Prichard, 1991), which inhibits polymerization into microtubules (Friedman & Platzer, 1980). Thus, all the functions ascribed to microtubules at the cellular level are altered (cell division, maintenance of cell shape, cell motility, cellular secretion, nutrient absorption and intracellular transport) (Lacey, 1988). BZD methylcarbamate molecules such as ABZ or FBZ act upon nematode microtubules at the tubulin colchicine binding site (Lacey, 1988). It is likely that a different site of action is involved on the flukicidal activity of TCBZ, which could also explain its lack of efficacy against other helminth parasites (Stitt & Fairweather, 1994). However, differences in the ability of ABZ, FBZ and TCBZ to penetrate through the *F. hepatica* external tegument may help to explain the observed differences in clinical efficacy among those chemically related drugs. Comprehension of the patterns of drug diffusion into target parasites, in conjunction with the available pharmacodynamic information on drug-receptor interactions, may substantially contribute to elucidation of the mechanisms of drug action and enhancement of their activity.

BZD anthelmintics are extensively metabolized in all mammalian species studied (Lanusse & Prichard, 1993). Albendazole sulphoxide (ABZSO), the main metabolite found in plasma after ABZ administration to sheep, has been recovered at higher concentrations compared with the parent drug in abomasal and intestinal fluids of treated sheep (Alvarez, Sánchez & Lanusse, 1999; Alvarez et al. 2000). However, in specimens of *Moniezia* spp. (Alvarez et al. 1999) and *Haemonchus contortus* (Alvarez et al. 2000) collected from the same ABZ-treated animals, the availability of ABZ parent drug was greater than that of its sulphoxide metabolite. The *in vivo* uptake studies carried out in *Moniezia* spp. and *H. contortus* demonstrated that ABZ has the capability to concentrate in the parasite. Such a pattern was not observed in liver flukes (Alvarez et al. 2000, where the ABZ ratio of area under the concentration versus time curve (AUC) between *F. hepatica* and bile was 0.33, which clearly demonstrated a lower drug accumulative process in the adult trematode parasite. These findings suggest that the drug-partitioning phenomenon between gastrointestinal fluid and parasite tissues might be different from that occurring between the surrounding bile and target liver flukes.

The current experiments were designed to investigate the comparative ability of ABZ, FBZ and TCBZ to diffuse into mature *F. hepatica* and to assess the influence of the physico-chemical composition of the incubation medium on the drug diffusion process. The results obtained from the trans-regimental diffusion kinetic studies were complemented with the determination of lipid-to-water partition coefficients (octanol-water) of the anthelmintic drugs assayed, as an indicator of drug lipophilicity.

**MATERIALS AND METHODS**

**Collection of parasite material**

Eight (8) parasite-free Corriedale sheep were infected with 200 metacercariae of *F. hepatica* each, given in a gelatine capsule by the oral route. Sixteen weeks after infection the animals were killed by captive bolt plus exsanguination, following internationally accepted animal welfare guidelines. To recover adult specimens of *F. hepatica* from the liver, common bile ducts and the gall-bladder of each sheep were removed and opened. The specimens were rinsed extensively with saline solution (NaCl 0.9%) (37°C) to remove bile and/or adhering materials.

**Drug diffusion assays**

The collected flukes were maintained for 2 h before starting the incubation in a Krebs’ Ringer Tris (KRT) buffer (pH 7.4) at 37°C (McCraen & Lipkowitz, 1990). Two flukes (approximately 0.2 g) were incubated at 37°C for 60 and 90 min in 2 ml of
an incubation medium (pH 7.4) prepared with bile and KRT buffer in different proportions (100/0, 75/25, 50/50, 25/75 and 0/100), containing either ABZ, FBZ or TCBZ at a final concentration of 5 nmol/ml. This is a pharmacologically relevant concentration obtained from previously reported work where the BZD concentrations in bile were measured after conventional treatments in ruminants (Hennessy et al. 1987; Alvarez et al. 2000). Ovine bile was collected from the gall-bladder of non-infected untreated sheep killed at the local abattoir at the same time as the infected animals. There were 6 replicate incubation assays for each drug at each incubation time. Blank samples containing parasite material and incubation medium without drug, and drug-spiked medium without parasite material were incubated during the same time-intervals. Once the incubation time had elapsed, the flukes were rinsed and incubation medium without drug, and drug-incubation assays for each drug at each incubation time. Blank samples containing parasite material and incubation medium without drug, and drug-spiked medium without parasite material were incubated during the same time-intervals. Once the incubation time had elapsed, the flukes were rinsed thoroughly with saline solution, blotted on coarse-filter paper and stored at −20 °C until their preparation for high performance liquid chromatography (HPLC) to measure drug concentrations. The parasite material was processed shortly after the incubation assays.

Measurement of drug concentrations

The parasite material (0.2 g) was homogenized using an Ultraturrax® homogeniser (T 25, Ika Works Inc., Labortechnik, USA) and spiked with oxibendazole (OBZ) used as internal standard. The parasite material homogenate was mixed with 1.5 ml of methanol and shaken for 5 min to extract the drug analyte/s present in the fluke sample. The collected methanol phase was evaporated to dryness. The residue obtained was dissolved in 1 ml of a methanol/water solution (20/80) and prepared for HPLC analysis using the extraction procedure described by Alvarez et al. (1999). All the solvents and reagents used during the extraction and drug analysis processes were HPLC grade.

Experimental and spiked liver fluke samples were analysed to measure the concentrations of each drug by HPLC using a model 10 A system (Shimadzu, Kyoto, Japan). The extraction efficiency of the different analytes from parasite material samples, expressed as absolute recovery, ranged between 85 and 97.5% with a coefficient of variation (CV) of ≤15-5%. The quantification limits of the HPLC technique for all the molecules assayed was 0.27 nmol/100 mg protein. The molecules under study were identified by comparison with the retention times of pure drug standards, which were used to prepare standard solutions to construct the calibration lines for each analyte in the parasite material analysed. The linear regression lines for each analyte in the range between 0.27 and 27.2 nmol/100 mg protein (triplicate determinations) showed correlation coefficients greater than 0.995. The concentrations of each analyte were quantified by comparison of the chromatographic peak area of each analyte with that obtained for the internal standard, using the Class LC 10 Software (Shimadzu, Kyoto, Japan) on an IBM 486-AT computer. The final concentration values for the different drugs assayed are expressed as nmol/100 mg protein. The determination of parasite protein concentrations was carried out according to the methodology described by Smith et al. (1985).

Octanol-water partition coefficients (PC)

The octanol-water PC (Log P) was used as an indicator of lipid solubility of the BZD molecules used in the current experiment. The methodology used to calculate this parameter was adapted from that reported by Péhourcq, Thomas & Jarry (2000). The octanol-water PC was estimated by the combination of the traditional shake-flask technique and HPLC analysis, using n-octanol (Merck, Schuchardt, Germany) and desionized ultrapure water (pH 7.4) (Simplicity® Water purification system, Millipore, Brazil) as a biphasic liquid system. Samples of 20 μl of either ABZ, FBZ or TCBZ (from 1 mM stock solutions) were added to 1800 μl of the aqueous phase were collected, evaporated to dryness and re-suspended in 150 μl of the HPLC mobile phase (27% acetonitrile, 73% water) to calculate the peak area of the analyte before partitioning (W0). In screw-capped glass tubes, the remaining 1800 μl of the aqueous phase (Vaq) were supplemented with 200 μl of an octanol phase (Voc), previously saturated with n-octanol. Under the chromatographic conditions described above, 200 μl of the aqueous phase were collected, evaporated to dryness and re-suspended in 150 μl of the HPLC mobile phase (27% acetonitrile, 73% water) to calculate the peak area of the analyte after partitioning (Wi). The partitioning of the drug between both phases (P value) was calculated using the following equation (Péhourcq, Thomas & Jarry, 2000):

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P = \frac{(W_0 - W_1)}{W_1} \frac{V_{aq}}{V_{oc}}
\]

The partition coefficient (Log P) was calculated as the logarithm of the obtained experimental P value.

Analysis of the data

The individual concentration values are reported as mean ± s.d. The statistical analysis of the data was performed as follows: (a) the comparison of the
concentrations achieved in *F. hepatica* in the different assayed incubation media, for each drug (ABZ, FBZ or TCBZ) and at each incubation time (60 and 90 min), was performed by analysis of variance (ANOVA); (b) Student’s *t*-test was used to compare drug concentrations obtained at 60 and 90 min of incubation in the different incubation media. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, San Diego, California). When ANOVA was employed and a significant *F* value was obtained, Tukey’s range test was performed to indicate order of significance. Values were considered different at *P* < 0.05.

**RESULTS**

The 3 molecules investigated were detected in *F. hepatica* after their *ex vivo* incubation. The amounts of ABZ, FBZ and TCBZ recovered from *F. hepatica* incubated in the absence of bile were significantly greater than those obtained with media containing bile (100, 75, or 50%). The comparison of the drug concentration profiles recovered in liver flukes incubated with ABZ and FBZ in media with different composition is shown in Fig. 1. The highest concentration values for ABZ (20.1 ± 8.15 nmol/100 mg protein) and FBZ (13.5 ± 4.06 nmol/100 mg protein) were measured in flukes incubated in the absence of bile. In the presence of bile in the incubation medium (100% bile), TCBZ concentrations recovered from the flukes ranged between 0.32 ± 0.07 and 0.47 ± 0.17 nmol/100 mg protein. Those TCBZ concentrations have a significant enhancement in the incubations without bile, reaching values up to 7.48 ± 2.62 (60 min) and 8.76 ± 3.16 (90 min) nmol/100 mg protein. The relative ability of ABZ and FBZ to penetrate into the liver flukes incubated in different media after 60 and 90 min is presented in Fig. 1. After 60 min of incubation, the amount of ABZ recovered from the parasite was significantly greater than that of FBZ, regardless of the composition of the incubation medium. However, the length of drug incubation for the flukes seems to play a role, as some differences in the uptake pattern between ABZ and FBZ were observed after 90 min of incubation. The slightly greater FBZ lipid solubility (compared to ABZ) may have to be taken into account to explain why the extension of the incubation time up to 90 min allowed its recovery at higher concentrations in flukes incubated in the presence of bile at 25, 50 and 75% of the total medium composition. Interestingly, the amount of ABZ recovered from *F. hepatica* incubated exclusively in ovine bile (100%) was between 32% (90 min) and 220% (60 min) higher than that measured for FBZ.

Although all drugs penetrated the trematode’s tegument, the rates of penetration were different. In all cases, the concentrations of the most lipophilic BZD methylcarbamates (FBZ, ABZ) recovered in *F. hepatica* were higher than those of TCBZ. The partition coefficients (*Log P*) obtained for FBZ, ABZ and TCBZ were 3.99, 3.82 and 3.48, respectively. The relative ability of ABZ and FBZ to penetrate into the liver flukes incubated in different media after 60 and 90 min is presented in Fig. 1. After 60 min of incubation, the amount of ABZ recovered from the parasite was significantly greater than that of FBZ, regardless of the composition of the incubation medium. However, the length of drug incubation for the flukes seems to play a role, as some differences in the uptake pattern between ABZ and FBZ were observed after 90 min of incubation. The
The relative diffusion of ABZ and TCBZ into *F. hepatica* after 60 and 90 min of incubation with (100%) or without (0%) bile is shown in Fig. 2. The diffusion of ABZ was significantly greater than that observed for TCBZ in all the incubation conditions under investigation. The diffusion of ABZ into *F. hepatica* incubated in ovine bile was between 281% (60 min) and 434% (90 min) higher than that measured for TCBZ. In the absence of bile, ABZ diffusion was between 129% (90 min) and 151% (60 min) higher than that of TCBZ.

The concentrations of FBZ and TCBZ (mean ± S.D.) measured in *F. hepatica* incubated without bile during 60 and 90 min, and the octanol-water partition coefficients (PC) for both molecules are compared in Fig. 3. The diffusion of FBZ into the trematode parasite was between 50 and 80% higher than that of TCBZ.

**DISCUSSION**

The relationship between *F. hepatica* and its surrounding environment occurs both across its external (tegument) and internal (gastrodermal cavity) surfaces (Thompson & Geary, 1995). The relative importance of these 2 available routes for drug uptake in *F. hepatica* is still unclear. However, the higher absorption surface of the tegument probably determines its major relevance in drug diffusion from the surrounding medium. This statement is supported by the fact that *F. hepatica* can survive long periods under *in vitro* conditions, in the absence of detectable nutrient absorption across the intestine (Smith & Clegg, 1981). Additionally, the higher concentrations of the lipophilic ABZ parent drug recovered in *F. hepatica*, compared to the more polar sulphoxide metabolite under *ex vivo* conditions (Alvarez et al. 2000) may also contribute to demonstrate the relevance of the trans-tegumental drug passage. A large number of experiments have shown that different chemical substances, as well as anthelmintic drugs, are mainly taken up through the external surface, as opposed to oral ingestion, in *H. contortus* (Rothwell & Sangster, 1997; Alvarez et al. 2000), *Ascaris suum* (Ho et al. 1990; Alvarez
The accumulated data show that anthelmintic drugs move across the external surface of helminth parasites by passive diffusion. In this process, the membrane behaves as an inert lipid-pore boundary, and drug molecules traverse this barrier either by diffusion through the lipoprotein region or, alternatively, filtering through aqueous pores (channels) without the cellular expenditure of energy if they are of sufficiently small size (Baggot, 1982). The rate of diffusion is proportional to the area of diffusion surface, the concentration gradient across the membrane and to the lipid-to-water partitioning of the drug (Baggot, 1982), and it is inverse to the medium viscosity of the drug-containing medium (Hörtér & Dressman, 2001). Lipid solubility is a major factor determining drug penetration across nematode cuticle (Alvarez et al. 2000, 2001) as well as through the tegument of cestodes (Alvarez et al. 1999; Mottier et al. 2003) and trematodes (Fetterer & Rew, 1984; Alvarez et al. 2000, 2001). Although, there are relevant structural differences between cuticle and tegument, the mechanism of drug entry to both type of structures seems to be equally dependent on lipophilicity as a major physicochemical determinant of drug capability to reach therapeutic concentrations within the target parasite. The logarithm of the octanol-water PC (Log P) was chosen as an indicator of drug lipophilicity since it is the most frequently used parameter for defining the lipophilic character of a given drug molecule (Péhourcq et al. 2000). This coefficient represents the fraction of molecules that distribute in an organic phase (octanol) versus an aqueous phase (water), and provides an estimate of how readily a molecule will penetrate a lipoidal membrane such as the trematode tegument. In all cases, the most lipophilic BZD methylcarbamates (FBZ, ABZ) were recovered at higher concentrations, as compared to TCBZ, in the incubated liver flukes.

Regardless of the time of incubation, the availability of ABZ in liver flukes incubated in the absence and presence of bile was significantly higher than those measured for FBZ. It has been suggested that the sulphoxide metabolites of both ABZ and FBZ may contribute substantially to the nematocidal (Lanusse & Prichard, 1993) and flukicidal (Fetterer, Rew & Knight, 1982) activities of the parent compounds. A series of free and conjugated ABZ and FBZ metabolites have been recovered in the bile of treated sheep (Hennessy et al. 1989; Hennessy, Prichard & Steel, 1993). However, the concentration profiles of the anthelmintically active unconjugated ABZ sulphoxide metabolite measured in bile were higher than those of FBZ sulphoxide (oxfendazole). Altogether, the greater trans-tegumental rate of ABZ diffusion into F. hepatica reported in the current work, and the higher availability of its active metabolite in bile may account for the advantageous flukicidal activity of ABZ compared to FBZ. However, other factors such as a differential portal blood concentration profile and differences in affinity for fluke β-tubulin should be considered in order to understand the low flukicidal activity of FBZ, a compound that is chemically closely related to ABZ and shows an equivalent spectrum of activity against nematode parasites.

ABZ and TCBZ are the only BZD compounds used as flukicidal drugs in domestic animals. While ABZ is recommended for flukes older than 12 weeks, TCBZ is active against both mature and immature stages of F. hepatica (Boray et al. 1983), being the most extensively used flukicidal drug in veterinary medicine (Coles & Stafford, 2001). The intensive use of TCBZ in endemic areas of fascioliasis has resulted in the development of liver flukes resistant to this compound (Overend & Bowen, 1995; Mitchell, Maris & Bonniwell, 1998; Moll et al. 2000; Thomas, Coles & Duffus, 2000), which is considered a major problem for veterinary therapeutics. A recent study has shown that ABZ is active against TCBZ-resistant isolates of F. hepatica (Coles & Stafford, 2001). If it is assumed that TCBZ and ABZ may act on tubulin in F. hepatica, then differences in uptake or metabolism of these 2 drugs could explain their differential efficacy against TCBZ-resistant flukes (Robinson et al. 2002). The drug biotransformation capacity of the liver fluke, recently characterized by Solana, Rodríguez & Lanusse (2001), could have potential involvement in the development of resistance to BZD compounds. It is possible that TCBZ may target a molecule other than β-tubulin, which would explain why ABZ continues to act against TCBZ-resistant flukes. However, the comparative ability of ABZ and TCBZ to penetrate through the tegument of susceptible liver flukes shown here provides some useful information. The diffusion of ABZ was significantly greater than that observed for TCBZ in all the incubation conditions under investigation similar results were observed for FBZ regardless of its lower flukicidal activity. This would suggest that the lower PC value obtained for TCBZ could play against its trans-tegumental diffusion ability. Finally, the greater trans-tegumental diffusion capability of ABZ compared to TCBZ may account for its efficacy pattern against TCBZ-resistant flukes, which is a relevant finding to be considered in fascioliasis control.

Lipid solubility is a relevant factor to determine drug diffusion into a target parasite. However, although lipophilicity is an important condition to define drug diffusivity through lipoidal tissues, it does not account for all factors that control this process. The results presented here demonstrated...
that the presence of bile in the incubation medium modified the diffusion of ABZ, FBZ and TCBZ into *F. hepatica*. The higher the proportion of the KRT buffer in the incubation medium, the greater the concentrations of the 3 molecules recovered within the flukes. Why did bile modify drug diffusion into the parasite? Bile is an hepatic aqueous secretion composed of biliary acids and pigments, lipids, amino acids and glucose, amongst others. Biliary secretion has different functions, such as providing an excretory route for metabolic detoxification products, including metabolites and drugs; neutralize the H+ in the duodenum; and providing a source of bile acids that are necessary for fat digestion and absorption. Bile acids are surfactants and they reduce the surface tension of water. This enables water to wet surfaces that are normally water-repellent, dissolving substances that are normally insoluble in water and emulsifying substances that do not normally mix with water. Consequently, bile acids act as detergents and bring water-insoluble material into solution by forming a negatively charged aggregate called a micelle. This increases the surface area enormously and facilitates the diffusion across the lipid membrane into the cell. Solubilization into simple bile salt micelles has been reported for many poorly water-soluble drugs, which has been correlated with higher intestinal drug absorption (Del Estal et al. 1993; Virkel et al. 2003). Drug solutions in micellar media consist of 2 separate phases: an aqueous phase with a fraction of the drug free in solution, and a micellar phase with the remaining fraction of the drug solubilized in micelles (Poelma et al. 1991). The inclusion of lipophilic drugs into micelles increases their solubility in an aqueous environment (Hörter & Dressman, 2001). However, the concentration of the free drug in solution is considered as the only driving force for diffusion. Under our experimental conditions, the presence of amphiphilic bile components in the incubation medium may have induced the micellar solubilization of ABZ, FBZ and TCBZ reducing the proportion of free drug in solution and thus decreasing drug diffusibility through the parasite tegument, which would explain the reduced drug penetration observed in the medium containing the higher bile proportions.

The knowledge of anthelmintic drug concentrations achieved in tissues/fluids of parasite location contributes to an understanding of differences in clinical efficacy. Furthermore, the comparative *ex vivo* diffusion studies provide relevant information on drug capability to reach its specific receptors inside the target parasite. Understanding the mechanisms involved in drug access to the target parasite, together with drug pharmacodynamics, will enhance overall comprehension of anthelmintic drug activity. However, results reported here demonstrate that drug concentrations at the site of parasite location and the lipid solubility of the anthelmintic molecule are not the only parameters to consider when drug kinetics is evaluated. The physicochemical characteristics of the tissues and fluids surrounding the parasite may play a relevant role in drug diffusion into the parasite. The findings described here, together with those previously reported from *in vivo* drug uptake studies, indicate that availability is dependent upon features of the environment where the parasite is located. For instance, a given ABZ concentration (e.g. 5 nmol/ml or g) may not represent the same in the gastrointestinal fluid content, in a mucosal tissue or in the bile. The partitioning of the active drug/metabolites between an aqueous gastrointestinal fluid and the lipidoidal tissue of the target parasite may facilitate the accumulation of the drug within the parasite, as has been shown for *H. contortus* (Alvarez et al. 2000). This drug partitioning phenomenon may be different for other sites of parasite location such as the biliary tract, where the bile-induced micelle formation may affect the diffusion of the active drug/metabolite into the target parasite (e.g. liver fluke). These findings seem to indicate that 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 an anthelmintic drug when living in their pre-dilective location tissues. 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 exposure of target parasites to subtherapeutic drug concentrations.

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