ACADEMIC PRESS

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Experimental Parasitology 103 (2003) 1–7

Experimental Parasitology

www.elsevier.com/locate/yexpr

# Transtegumental diffusion of benzimidazole anthelmintics into *Moniezia benedeni*: correlation with their octanol–water partition coefficients

M.L. Mottier,[1,2] L.I. Alvarez,[1,2] M.A. Pis, and C.E. Lanusse[*,1]

*Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Pcia. de Buenos Aires, Pje. Arroyo Seco, Campus Universitario, Tandil 7000, Buenos Aries, Argentina*

Received 19 December 2002; received in revised form 8 April 2003; accepted 10 April 2003

**Abstract**

The experiments described here report on the correlation between the ex vivo diffusion of different benzimidazole (BZD) anthelmintics into the cestode parasite *Moniezia benedeni*, and their octanol–water partition coefficients (P.C.). The characterisation of the drug diffusion process into target parasites is relevant to understand the mechanism of drug penetration and the pharmacological activity of anthelmintic drugs. Specimens of the tapeworm *M. benedeni*, used as a helminth parasite model, were obtained from untreated cattle killed at the local abattoir. The collected parasites were incubated (5–210 min) with either fenbendazole (FBZ), albendazole (ABZ), ricobendazole (RBZ), oxfendazole (OFZ), mebendazole (MBZ), oxibendazole (OBZ), or thiabendazole (TBZ), in a Kreb's Ringer Tris buffer medium at a final concentration of 5 nmol/ml. After the incubation time elapsed, samples of parasite material were chemically extracted and prepared for high performance liquid chromatography (HPLC) analysis to measure drug/metabolite concentrations. Additionally, the octanol–water P.C. for each molecule was estimated as an indicator of drug lipophilicity, using reversed phase HPLC analysis. All the incubated drugs were recovered from the tapeworms as early as 5 min post incubation. There was a high correlation ($r = 0.87$) between drug lipophilicity, expressed as octanol–water P.C. (Log $P$), and drug availability within the parasite. The most lipophilic BZD compounds (FBZ, ABZ, and MBZ), with P.C. values higher than 3.7, were measured at significative higher concentrations within the tapeworm compared to those drugs with the lowest P.C. values. Considering the results from the current and previous studies, it is clear that passive diffusion is a major mechanism of BZD penetration into cestode parasites, where lipid solubility is a determinant factor influencing the diffusion of these anthelmintic molecules through the parasite tegument.
© 2003 Elsevier Science (USA). All rights reserved.

*Index Descriptors and Abbreviations*: Transtegumental diffusion; Benzimidazole anthelmintics; Cestodes; *Moniezia benedeni*; Lipophilicity; Octanol–water partition coefficient

## 1. Introduction

Helminth parasites infect a quarter of the world's total population and are a major cause of morbidity (Colley et al., 2001). Moreover, helminth infections are the most important cause of productivity losses in livestock worldwide (Parkins and Holmes, 1989). Although alternative control methods have been developed, chemically-based anthelmintic treatments are the most important tool to control parasitism in livestock (Martin, 1985). Benzimidazole (BZD) anthelmintics are effective against nematodes, cestodes and trematodes (Campbell, 1990; McKellar and Scott, 1990). Their pharmacological activity is based on the binding to β-tubulin, which produces subsequent disruption of the tubulin–microtubule dynamic equilibrium (Lacey, 1990). 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). The introduction of thiabendazole (TBZ) in the

[*] Corresponding author. Fax: +54-2293-42-6667.
*E-mail address:* clanusse@vet.unicen.edu.ar (C.E. Lanusse).
[1] Consejo Nacional de Investigaciones Científicas y Técnicas.
[2] Authors have equally contributed to the work reported here.

0014-4894/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0014-4894(03)00060-2

1960s was followed by the development of a series of BZD and BZD prodrugs (pro-BZD) as anthelmintics. Each drug represented an improvement in efficacy and spectrum of activity which firmly established the predominance of BZD anthelmintics within the chemotherapy arsenal (Lacey, 1988). BZD anthelmintics are chemically classified as BZD thiazols (TBZ and cambendazole); halogenated BZD thiols (triclabendazole); pro-BZD (febantel, netobimin, and thiophanate); and BZD methylcarbamates (albendazole [ABZ], ricobendazole [RBZ], fenbendazole [FBZ], oxfendazole [OFZ], mebendazole [MBZ], flubendazole [FLBZ], oxibendazole [OBZ], etc.). RBZ and OFZ are the active sulphoxide metabolites of ABZ and FBZ, respectively. Although they are chemically similar, their physico-chemical properties (Lacey, 1988), tubulin binding affinity (Lubega and Prichard, 1991) and pharmacological potency (Petersen et al., 1997) are different.

The anthelmintic activity of BZD compounds is dependent on two main factors: their affinity for a specific receptor (β-tubulin); and the transport properties that allow the delivery of effective concentrations of the compound at the receptor within the parasite cells, in sufficient time, to cause the therapeutic effect (Thompson et al., 1993). Anthelmintic drugs can reach target helminth parasites by either oral ingestion (from host's blood and/or intestinal contents) or by diffusion through the external surface (named cuticle in nematodes or tegument in cestodes and trematodes), or some combination of both routes (Thompson and Geary, 1995; Thompson et al., 1993). Cestode parasites do not have a gut such as the gastrodermis in trematodes or the intestine in nematodes, and each proglottis functions as an individual unit. The absence of a digestive tract facilitates the interpretation of the data obtained from drug transport studies (Thompson and Geary, 1995). Therefore, it is of interest that the only way that a given drug molecule can reach its receptor is by passing through the cestode's tegument. If passive diffusion is the main mechanism of entry of BZD anthelmintics (over active transport), restrictions imposed by tegumental lipid barriers will probably be similar to those of standard cellular membranes. Consequently, the rate of anthelmintic drug penetration will depend mainly on their lipophilicity (Thompson et al., 1993).

*Moniezia benedeni*, a sheep and cattle intestinal cestode parasite, was used as a parasite model to compare the ex vivo patterns of diffusion of different BZD anthelmintics in the current experiments. The results obtained from the transtegumental diffusion kinetic studies were correlated with the lipid-to-water partition coefficient (P.C.) of the anthelmintic drugs assayed. The logarithm of the octanol–water P.C. (Log $P$) was chosen as an indicator of drug lipophilicity, since is the most frequently used parameter for defining the lipophilic character of a given drug molecule (Péhourcq et al., 2000).

## 2. Materials and methods

### 2.1. Incubation assays

Specimens of *M. benedeni* were collected from the small intestine of untreated cattle killed at the local abattoir. The parasite material was rinsed extensively with saline solution to remove intestinal debris and adherence materials. The collected tapeworms were maintained for 2 h before starting the incubation in a Krebs Ringer Tris (KRT) buffer (pH 7.4) at 37 °C (McCracken and Lipkowitz, 1990). The tapeworm material (0.5 g) (from the middle part of the strobila) was incubated at 37 °C in 5 ml of the KRT buffer containing either ABZ, RBZ, FBZ, OFZ, MBZ, OBZ, or TBZ at a final concentration of 5 nmol/ml. This is a pharmacologically relevant concentration obtained from previously reported studies where the gastrointestinal concentrations of BZD compounds were measured after conventional treatments in ruminant species (Alvarez et al., 1999; Hennessy et al., 1993a,b; Sánchez et al., 1997). The following incubation times were used to evaluate the diffusion of the different BZD anthelmintics into *M. benedeni*: 5, 10, 15, 30, 45, 60, 90, 120, 180, and 210 min. There were four replicate assays for each incubation time. Blank samples containing parasite material and medium without drug, and, drug-spiked medium without parasite material, were incubated over the same time intervals and served as controls. Once the appropriate incubation time had elapsed, the tapeworm material was rinsed thoroughly with saline solution, blotted on coarse filter paper and prepared for high performance liquid chromatography (HPLC) analysis to determine drug concentrations. The parasite material was processed shortly after the incubation assays.

### 2.2. Measurement of cestode protein concentrations

The final concentration values for the different drugs assayed are expressed as nmol/100 mg protein. The determination of parasite protein concentrations was adapted from that described by Smith et al. (1985). The work reagent was prepared with 50 volumes of bicinchoninic acid (Sigma–Aldrich Chemical, St. Louis, USA) and 1 volume of cupric sulphate ($CuSO_4$) (Baker, Phillipsburg, USA). The bicinchoninic acid complexes with the $Cu^{2+}$ ion and with the sample proteins, giving a purple colour that can be measured by spectrophotometry. The colour obtained is directly proportional to the sample protein concentration.

### 2.3. Sample clean up, drug extraction, and HPLC analysis

The parasite material (0.5 g) was homogenised (Ultraturrax, T 25, Ika Works, Labortechnik, USA) and

spiked with the chosen internal standard (i.s.) compound. OBZ was used as i.s. to measure the concentration of ABZ, FBZ, MBZ, TBZ, RBZ, and OFZ. MBZ was the chosen i.s. to determine the concentration of OBZ. The parasite material homogenate was mixed with 1.5 ml of methanol and shaken over 5 min (twice) to extract the drug analyte(s) present in the sample. The collected methanol phase was evaporated to dryness. The residue obtained was dissolved in 1 ml 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 parasite material samples were analysed to measure the concentrations of each drug by HPLC using a model 10 A Shimadzu system (Kyoto, Japan). The efficiency extraction of the different analytes from parasite material samples expressed as absolute recovery, ranged between 69 and 93% with a coefficient of variation (C.V.) lower than 11.3%. The quantification limits for all the molecules assayed ranged between 0.32 and 3.21 nmol/100 mg protein, which can be considered as highly satisfactory. Identification of all the BZD molecules assayed was undertaken by comparison with the retention times of pure reference standards, which were also used to prepare standard solutions to construct the calibration lines for each analyte in spiked parasite material. The linear regression lines for each analyte in the range between 1.61 and 64 nmol/100 mg protein (triplicate determinations) showed correlation coefficients greater than 0.992. The concentrations of each molecule were quantified by comparison of its chromatographic peak area with that obtained for the i.s. compound, using the Class LC 10 Software (Shimadzu, Kyoto, Japan) on an IBM 486-AT computer.

### 2.4. Octanol-water partition coefficients

The octanol–water P.C. (Log $P$) was used as an indicator of lipid solubility of the BZD molecules used in the current transtegumental diffusion experiments. The methodology used to calculate this parameter was adapted from Péhourcq et al. (2000). The octanol–water P.C. was estimated by the combination of the traditional shake-flask technique and HPLC analysis, using *n*-octanol (Merck, Schuchardt, Germany) and deionized ultrapure water (pH 7.4) (Simplicity, Water purification system, Millipore, Brazil) as a biphasic liquid system. ABZ, RBZ, FBZ, OFZ, MBZ, OBZ, or TBZ (20 μl of either, from 1 mM stock solutions) were added to 1980 μl of deionized ultrapure water 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 300 μl of the HPLC mobile phase (37% acetonitrile and 73% water) to calculate the peak area of the analyte before partitioning ($W_0$). In screw-capped glass tubes, the remaining 1800 μl of the aqueous phase ($V_{aq}$) were supplemented with 200 μl of an octanol phase ($V_{oc}$), previously saturated with deionized water (pH 7.4). The mixture was shaken during 90 min in a mechanical shaker (Cole Parmer, Vernon Hills, IL, USA) at 15 °C. The mixture was then centrifuged (1500*g*, 5 min) and 1 ml of the lower aqueous phase was recovered, evaporated to dryness, resuspended in 150 μl of mobile phase, and injected into the HPLC system to determine drug concentration in the aqueous phase after partitioning ($W_1$). The partitioning of the drug between both phases ($P$ value) was calculated using the following equation (Péhourcq et al., 2000):

$$P = \frac{(W_0 - W_1)}{W_1} \cdot \frac{V_{aq}}{V_{oc}}.$$

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

### 2.5. Analysis of the correlation data

Once the concentration values (expressed as nmol/100 mg protein) for each compound were determined for each individual incubation assay, the area under the concentration vs time curve (AUC) was calculated by the trapezoidal rule method (Gibaldi and Perrier, 1982), using the PkSolution 2.0 program (Summit Research Services, Ashland, OH, USA). The AUC value (expressed as nmol min/100 mg protein) was considered as an indicator of the total drug availability within the cestode parasite. The individual concentrations and AUC values are presented as means ± SD (four replicates). The AUC and Log $P$ values obtained for each molecule assayed were compared by analysis of variance (ANOVA), using the Instat 3.0 Software (Graph Pad Software, San Diego, California, USA). The Tuckey's range test was used to indicate the order of significance when a significant $F$ value was obtained. A value of $P < 0.05$ was considered statistically significant.

## 3. Results

The concentrations profiles (X ± SD) of FBZ, OFZ, ABZ, RBZ, MBZ, OBZ, and TBZ measured in *M. benedeni* at different times post-incubation in a KRT medium are shown in Table 1. All the molecules were detected in the cestode parasite as early as 5 min post-incubation, demonstrating fast drug diffusion from the medium to the parasite tissues. The amount of drug recovered over time increased during the incubation period, with the maximum concentrations ranging between 14.0 and 89.1 nmol/100 mg protein. Although all the BZD compounds investigated penetrated the

Table 1
Concentrations (nmol/100 mg protein, $X \pm SD$) of fenbendazole (FBZ), oxfendazole (OFZ), albendazole (ABZ), ricobendazole (RBZ), mebendazole (MBZ), oxibendazole (OBZ), and thiabendazole (TBZ) measured in *Moniezia benedeni* at different times post-incubation

| Time (min) | FBZ | OFZ | ABZ | RBZ | MBZ | OBZ | TBZ |
|---|---|---|---|---|---|---|---|
| 5 | $20.5 \pm 3.0^{a}$ | $5.88 \pm 0.6^{c}$ | $11.0 \pm 4.3^{bcf}$ | $4.68 \pm 0.9^{c}$ | $15.9 \pm 2.1^{ae}$ | $11.9 \pm 0.5b^{ef}$ | $10.2 \pm 3.0^{bce}$ |
| 10 | $20.7 \pm 2.6^{a}$ | $7.31 \pm 1.1^{cd}$ | $11.8 \pm 3.8^{bc}$ | $6.38 \pm 0.6^{d}$ | $9.09 \pm 0.6^{bcd}$ | $12.9 \pm 1.7^{b}$ | $10.3 \pm 1.9^{bcd}$ |
| 15 | $28.2 \pm 5.8^{a}$ | $10.8 \pm 1.5^{bc}$ | $16.0 \pm 4.3^{b}$ | $6.84 \pm 1.4^{c}$ | $13.1 \pm 1.2^{bc}$ | $15.2 \pm 1.1^{b}$ | $11.7 \pm 1.4^{bc}$ |
| 30 | $34.3 \pm 2.3^{a}$ | $9.97 \pm 2.3^{cd}$ | $24.7 \pm 6.7^{b}$ | $7.86 \pm 1.2^{d}$ | $15.9 \pm 3.8^{c}$ | $17.2 \pm 2.9^{bc}$ | $11.2 \pm 2.1^{cd}$ |
| 45 | $22.1 \pm 2.2^{a}$ | $11.2 \pm 3.1^{b}$ | $21.5 \pm 2.4^{a}$ | $9.52 \pm 1.1^{b}$ | $18.6 \pm 3.1^{a}$ | $13.0 \pm 2.0^{b}$ | $12.9 \pm 1.4^{b}$ |
| 60 | $29.4 \pm 8.4^{a}$ | $9.81 \pm 0.9^{c}$ | $25.3 \pm 7.4^{ab}$ | $10.2 \pm 3.3^{c}$ | $19.9 \pm 4.6^{abc}$ | $15.2 \pm 2.7^{bc}$ | $12.2 \pm 0.8^{c}$ |
| 90 | $36.0 \pm 7.0^{a}$ | $14.6 \pm 3.3^{bc}$ | $21.0 \pm 4.1^{b}$ | $8.54 \pm 1.0^{c}$ | $22.4 \pm 6.9^{b}$ | $17.8 \pm 0.9^{bc}$ | $12.8 \pm 1.5^{bc}$ |
| 120 | $44.5 \pm 5.5^{a}$ | $12.9 \pm 1.9^{d}$ | $24.9 \pm 7.0^{b}$ | $9.50 \pm 0.4^{d}$ | $23.8 \pm 8.3^{bc}$ | $16.1 \pm 3.6^{bcd}$ | $13.9 \pm 1.2^{cd}$ |
| 180 | $51.8 \pm 13^{a}$ | $12.5 \pm 2.1^{d}$ | $78.1 \pm 6.1^{b}$ | $14.0 \pm 0.8^{cd}$ | $28.0 \pm 8.2^{c}$ | $15.4 \pm 2.7^{cd}$ | $13.9 \pm 1.0^{cd}$ |
| 210 | $53.0 \pm 5.7^{a}$ | $12.1 \pm 1.7^{cd}$ | $89.1 \pm 13.0^{b}$ | $11.1 \pm 1.2^{d}$ | $26.7 \pm 7.0^{c}$ | $15.8 \pm 2.0^{cd}$ | $16.2 \pm 2.7^{cd}$ |

[a,b,c,d,e,f] Mean values not having a common superscript within a row are significantly different at $P < 0.05$.

cestode's tegument, the rates of penetration were different. Diffusion of the sulphides FBZ and ABZ was significantly greater ($P < 0.05$) compared to their respective sulphoxide metabolites (OFZ and RBZ) and to other BZD molecules at almost all the incubation times assayed.

The total drug availabilities expressed as $AUC_{0-210\,min}$, obtained in *M. benedeni* after the ex vivo incubations, are compared in Fig. 1. Higher $AUC_{0-210\,min}$ values were obtained for the most lipophilic molecules, FBZ (8205 nmol min/100 mg protein) and ABZ (8133 nmol min/100 mg protein), compared to MBZ (4607 nmol min/100 mg protein), OBZ (3254 nmol min/100 mg protein), TBZ (2731 nmol min/100 mg protein), OFZ (2471 nmol min/100 mg protein), and RBZ (2094 nmol min/100 mg protein). There were no statistically significant differences between the amount of ABZ and FBZ recovered in the tapeworm. Diffusion of MBZ was greater ($P < 0.05$) than that of OBZ. Besides, there were no significant differences between OBZ vs TBZ vs OFZ, or between TBZ vs OFZ vs RBZ. However, the diffusion of OBZ was significantly greater ($P < 0.05$) than that of RBZ.

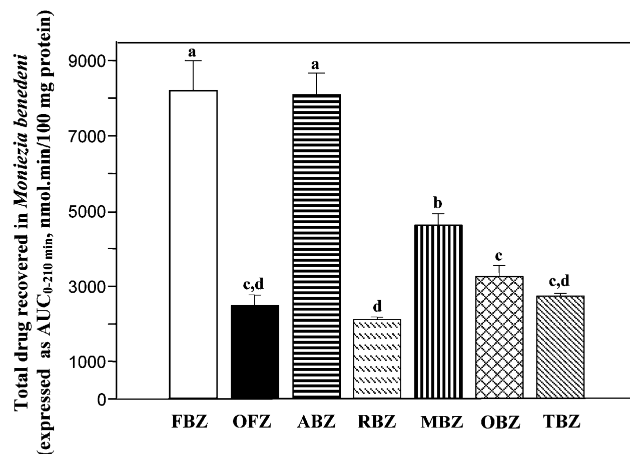


Fig. 1. Comparative total drug recovered in *Moniezia benedeni*, expressed as area under the concentration vs time curves (AUC) after its ex vivo incubation (210 min) with different benzimidazole anthelmintics. Data shown are the means ± SD ($n = 4$). Mean AUC values not having a common superscript are significantly different at $P < 0.05$. FBZ, fenbendazole; OFZ, oxfendazole; ABZ, albendazole; RBZ, ricobendazole; MBZ, mebendazole; OBZ, oxibendazole; and TBZ, thiabendazole.

Table 2
Correlation between drug diffusion (measured as total drug amount recovered in *Moniezia benedeni* expressed as AUC values) and drug lipophilicity (measured as the octanol–water partition coefficient)

| Anthelmintic molecule | Octanol–water partition coefficient | Total drug recovered in *Moniezia benedeni* (expressed as $AUC_{0-210\,min}$)* |
|---|---|---|
| FBZ | $3.93^{a}$ | $8205^{a}$ |
| ABZ | $3.83^{a,b}$ | $8133^{a}$ |
| MBZ | $3.73^{b}$ | $4607^{b}$ |
| OBZ | $2.60^{c}$ | $3254^{c}$ |
| TBZ | $2.55^{c}$ | $2731^{c,d}$ |
| OFZ | $2.03^{d}$ | $2471^{c,d}$ |
| RBZ | $1.24^{e}$ | $2094^{d}$ |
| Correlation coefficient ($r$) | 0.87 | |

*Note.* FBZ, fenbendazole; ABZ, albendazole; MBZ, mebendazole; OBZ, oxibendazole; TBZ, thiabendazole; OFZ, oxfendazole; and RBZ, ricobendazole.

[a,b,c,d,e] Mean values not having a common superscript within a column are significantly different at $P < 0.05$.

* AUC, area under the concentration vs time curve from 0 to 210 min (expressed as nmol min/100 mg protein).

The correlation between drug diffusion over 210 min of incubation (measured as total drug amount recovered in *M. benedeni* expressed as AUC values) and drug lipophilicity (measured as the octanol–water P.C.) is shown in Table 2 and Fig. 2. There was a high correlation ($r = 0.87$) between drug lipophilicity and drug availability within the parasite.

## 4. Discussion

The absence of a digestive system in cestode parasites simplifies the interpretation of the functional properties of the external surface (tegument). The cestode surface

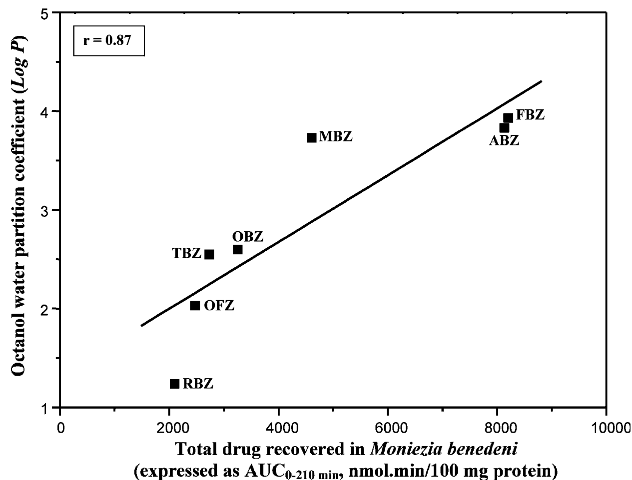


Fig. 2. Correlation between the total drug recovered in *Moniezia benedeni*, expressed as area under the concentration vs time curves (AUC) after 210 min of incubation, and the octanol-water partition coefficients (Log *P*) of different benzimidazole anthelmintic compounds. FBZ, fenbendazole; ABZ, albendazole; MBZ, mebendazole; OBZ, oxibendazole; TBZ, thiabendazole; OFZ, oxfendazole; and RBZ, ricobendazole.

must be structurally adapted to perform all functions normally associated with intestinal tissue. Consequently, all the interactions between the cestode and its surrounding environment occur across the tegument (Thompson and Geary, 1995). Drug molecules move across cell membranes either by passive diffusion or specialised transport processes. In the passive diffusion 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. The rate of diffusion of a drug will depend mainly on the diffusion surface, the concentration gradient across the membrane, the pH/pK relationship and the lipid-to-water partitioning of the molecule. Specialised transport is the other potential mechanism of drug entry to target parasites. This type of transport process is relatively selective toward the chemical nature of the substance and requires direct expenditure of energy (Baggot, 1982).

The results reported in the present work clearly show a high correlation between molecular lipid solubility (expressed as Log *P*) and the availability (expressed as AUC) of BZD compounds within the helminth parasite used as a model. Considering the obtained P.C. values and the observed diffusion patterns, the drugs assayed could be grouped into three "classes": (A) high P.C. values—high diffusion rates (FBZ and ABZ); (B) medium P.C. values—medium diffusion rates (MBZ); and (C) low P.C. values—low diffusion rates (OBZ, TBZ, OFZ, and RBZ). The sulphides FBZ and ABZ are more lipophilic (higher octanol–water P.C.) and thus more soluble in the lipoidal surface membranes of the helminth parasites (Fetterer and Rew, 1984), than are their respective sulphoxide metabolites (OFZ and RBZ). Moreover, FBZ and ABZ showed higher Log *P* values in comparison with the others BZD compounds assayed in the current work (TBZ, OBZ, and MBZ). These physico-chemical differences may explain the significantly higher concentrations that these molecules reached inside the parasite material at almost all the incubation times. The octanol–water P.C. for FBZ was slightly higher than that obtained for ABZ, without being statistical significant. This greater FBZ lipophilicity was well correlated with the higher concentration this molecule reached inside the parasite at almost all the incubation times during the first 120 min compared with ABZ. However, higher concentrations of ABZ were measured at the last two points of sampling (180 and 210 min). Although it is difficult to explain this phenomenon, it could be related to an increased permeability produced by tegument damage induced by the presence of the drug. If this hypothesis is valid, then the parasite tegument would offer a weak barrier to drug penetration. Since the results obtained here demonstrate that the higher the lipophilicity, the greater the ability of the BZD compounds to cross through the helminth tegument, it can be concluded that passive diffusion, over active transport, is the main route of entry of BZD anthelmintics to cestode parasites, which has been also postulated to occur for TBZ in *Trichostrongylus colubriformis* (Sangster and Prichard, 1984).

In spite of the fact that TBZ has been postulated as the most hydrosoluble BZD compound (Ngomuo et al., 1984), under our experimental conditions the TBZ Log *P* value was 2.55 (higher than that of OFZ and RBZ), which was well correlated with a medium-range drug availability in the parasite. These ex vivo results could not be directly related to the in vivo behaviour, since TBZ is rapidly metabolised in the liver to 5-hydroxythiabendazole (a more polar and inactive metabolite), thus limiting the duration of exposure of parasites to TBZ (Weir and Bogan, 1985). Additionally, in terms of binding to parasite tubulin, TBZ is the less potent BZD molecule (Lacey et al., 1987; Lubega and Prichard, 1991). These two factors may explain the lack of activity of TBZ against tapeworms (McKellar and Scott, 1990). Interestingly, in contrast to the high Log *P* value obtained for MBZ, diffusion of this drug into *M. benedeni* was lower than that measured for a molecule with a similar octanol–water P.C. such as ABZ (ABZ tended to show higher Log *P* but these differences were not statistically significant). However, MBZ is active against human and sheep tapeworms after its administration at the recommended dose level. It is clear that in vivo efficacy is determined by various primary factors such as systemic availability of an adequate BZD concentration in the host and consequently at the site of parasite

location, diffusion into the parasite and tubulin binding. It is not surprising that the molecules with the highest diffusion rates, namely FBZ, ABZ, and MBZ, also show greater ability to bind the high affinity receptors (β-tubulin) (Lubega and Prichard, 1991), greater in vivo efficacy (Campbell and Rew, 1986; Marriner and Armour, 1986) and ex vivo activity (Bartlett et al., 1992; Petersen et al., 1997).

Measurements of drug levels in tissues of *M. benedeni* demonstrated that the drug added to the incubation medium was the predominant analyte recovered inside the parasite. Nevertheless, cestodes incubated with ABZ or FBZ contained RBZ (5–30%) or OFZ (5%), respectively, as metabolic products. On the other hand, when cestode parasites were incubated with RBZ or OFZ, 3.9% of ABZ or FBZ, respectively, were detected in the parasite tissues. These results are not surprising since the oxidative and reductive metabolic activities of different helminth parasites has been recently shown in our laboratory (Solana et al., 2001).

It has been difficult to establish a correlation between the ex vivo diffusion of RBZ and OFZ, their in vitro activity against parasite tubulin and their in vivo anthelmintic potency. OFZ and RBZ, are very highly potent anthelmintics in terms of recommended therapeutic doses. However, they showed lower diffusion rates and lower affinity binding for tubulin compared to other drugs with similar anthelmintic potency. The in vivo anthelmintic activity of the sulphoxides OFZ and RBZ, may be facilitated by their gastrointestinal microbial sulphoreduction (Lanusse et al., 1992; Virkel et al., 2002) into more active and more lipophilic sulphide forms (FBZ and ABZ, respectively). Higher concentration profiles of the sulphoxide metabolite (RBZ) compared to ABZ parent drug, have been reported in the gastrointestinal fluids of ABZ-treated sheep (Alvarez et al., 2000) and cattle (Sánchez et al., 1997). However, the higher lipophilicity of ABZ measured in the current experiments, may explain its greater penetration through the external parasite surface, compared to its sulphoxide derivative, observed in tapeworms (Alvarez et al., 1999) and *Haemonchus contortus* (Alvarez et al., 2000) collected from infected sheep after ABZ treatment. Since ABZ has not been detected in peripheral plasma, only drug from the pool found in abomasal fluid and mucosa could be able to reach the parasite through the nematode cuticle. *H. contortus* may feed on portal blood and the relevance of the portal circulation as a source of ABZ should be considered. However, the low ABZ concentrations recovered in portal blood (Alvarez et al., unpublished observations) from catheterised ABZ-treated sheep, do not explain the high ABZ availability observed in *H. contortus*. Altogether, these findings confirm the relevance of the drug diffusion process through the external parasite surface even in a blood-sucking parasite (Alvarez et al., 2000).

It has been shown that transcuticular diffusion is a common means of entry for non-nutrient and non-electrolyte substances in nematode parasites (Geary et al., 1995). Much of the research on broad-spectrum anthelmintics has been focused on elucidating the main route of entry of these drugs into helminth parasites. It has been shown under in vivo/ex vivo studies that transcuticular diffusion is the predominant pathway for the entry of anthelmintic drugs into nematodes (Alvarez et al., 1999–2001; Cross et al., 1998; Ho et al., 1994). Thus, lipid solubility is a major factor determining drug penetration across nematode cuticle, and it may be directly related to clinical efficacy, which may also agree with the data reported here for a cestode parasite. Although, there are relevant structural differences between the external surface of nematodes (cuticle) and cestodes (tegument), the mechanism of drug entry to both type of helminths seems to be equally dependent on lipophilicity, as a major physico-chemical determinant of drug capability to reach therapeutic concentrations within the target parasite.

Although follow-up studies on surface parasite biochemistry and drug transport mechanisms are required, the results reported here are a further contribution to the understanding of the processes involved in drug penetration to cestode parasites. The correlation between lipid solubility (measured as octanol–water P.C.) and drug availability in a target parasite has been shown for the first time The information reported here may be useful to optimise anthelmintic therapy.

## Acknowledgments

Lourdes Mottier is a recipient of a fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. This research was partially supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 08-07277) (Argentina), Universidad Nacional del Centro de la Pcia. de Buenos Aires (Argentina) and Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

## References

Alvarez, L., Sánchez, S., Lanusse, C., 1999. In vivo and ex vivo uptake of albendazole and its sulphoxide metabolite by cestode parasites: relationship with their kinetics behaviour in sheep. Journal of Veterinary Pharmacology and Therapeutics 22, 77–86.

Alvarez, L., Imperiale, F., Sánchez, S., Murno, G., Lanusse, C., 2000. Uptake of albendazole and albendazole sulphoxide by *Haemonchus contortus* and *Fasciola hepatica* in sheep. Veterinary Parasitology 94, 75–89.

Alvarez, L., Mottier, L., Sánchez, S., Lanusse, C., 2001. Ex vivo diffusion of albendazole and its sulphoxide metabolite into *Ascaris suum* and *Fasciola hepatica*. Parasitology Research 87, 929–934.

Baggot, D., 1982. Disposition and fate of drugs in the body. In: Booth, N., McDonald, L. (Eds.), Veterinary Pharmacology and Therapeutics. Iowa State University Press, Iowa, p. 37.

Bartlett, M., Edlind, T., Durkin, M., Shaw, M., Queener, S., Smith, J., 1992. Antimicrotubule benzimidazoles inhibit in vitro growth of *Pneumocystis carinii*. Antimicrobial Agents and Chemotheraphy 36, 779–782.

Campbell, W., Rew, R., 1986. Chemotherapy of parasitic diseases. Plenum Press, New York.

Campbell, W., 1990. Benzimidazoles: veterinary uses. Parasitology Today 6, 130–133.

Colley, D., LoVerde, P., Savioli, L., 2001. Medical helminthology in the 21st century. Science 293, 1437–1438.

Cross, H., Renz, A., Trees, A., 1998. In vitro uptake of ivermectin by adult male *Onchocerca ochengi*. Annals of tropical Medicine and Parasitology 92, 711–720.

Fetterer, R., Rew, R., 1984. Interaction of *Fasciola hepatica* with albendazole and its metabolites. Journal of Veterinary Pharmacology and Therapeutics 7, 113–118.

Geary, T., Blair, K., Ho, N., Sims, S., Thompson, D., 1995. Biological functions of nematode surfaces. In: Bothroyd, J., Komuniecki, R. (Eds.), Molecular Approaches to Parasitology. New York, p. 57–76.

Gibaldi, M., Perrier, D., 1982. In: Pharmacokinetics. M. Dekker, Inc., New York, pp. 45–109.

Hennessy, D., Prichard, R., Steel, J., 1993a. Biliary secretion and enterohepatic recycling of fenbendazole metabolites in sheep. Journal of Veterinary Pharmacology and Therapeutics 16, 132–140.

Hennessy, D., Sangster, N., Steel, J., Collins, G., 1993b. Comparative pharmacokinetics behaviour of albendazole in sheep and goats. International Journal for Parasitology 23, 321–325.

Ho, N., Sims, S., Vidmar, T., Day, J., Barshum, C., Thomas, E., Geary, T., Thompson, D., 1994. Theoretical perspectives on anthelmintic drug discovery: interplay of transport kinetics, physicochemical properties, and in vitro activity of anthelmintic drugs. Journal of Pharmacological Sciences 83, 1052–1059.

Lacey, E., 1988. The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. International Journal for Parasitology 18, 885–936.

Lacey, E., 1990. Mode of action of benzimidazoles. Parasitology Today 6, 112–115.

Lacey, E., Brady, R., Prichard, R., Watson, T., 1987. Comparison of inhibition of polymerisation of mammalian tubulin and helminth ovicidal activity by benzimidazole carbamates. Veterinary Parasitology 23, 105–119.

Lanusse, C., Gascon, L., Prichard, R., 1992. Metabolism of albendazole and albendazole sulphoxide by ruminal and ileal fluids of sheep and cattle. Xenobiotica 22, 419–426.

Lubega, G., Prichard, R., 1991. Interaction of benzimidazole anthelmintics with *Haemonchus contortus* tubulin: binding affinity and anthelmintic efficacy. Experimental Parasitology 73, 203–209.

Marriner, S., Armour, J., 1986. Nematode infections of domestic animals: gastrointestinal infections. In: Campbell, W., Rew, R. (Eds.), Chemotherapy of parasitic diseases. Plenum Press, New York, pp. 287–305.

Martin, P., 1985. Nematode control schemes and anthelmintic resistance. In: Anderson, N., Waller, P. (Eds.), Resistance in nematodes to anthelmintic drugs. CSIRO Division of Animal Health, Glebe, pp. 29–40.

McCracken, R., Lipkowitz, K., 1990. Structure–activity relationship of benzimidazole anthelmintics: a molecular modelling approach to in vivo drug efficacy. Journal of Parasitology 76, 853–864.

McKellar, Q., Scott, E., 1990. The benzimidazole anthelmintic agents—a review. Journal of Veterinary Pharmacology and Therapeutics 13, 223–247.

Ngomuo, A., Marriner, S., Bogan, J., 1984. The pharmacokinetics of fenbendazole and oxfendazole in cattle. Veterinary Research Communications 8, 187–193.

Parkins, J., Holmes, P., 1989. Effects of gastrointestinal helmith parasites on ruminant nutrition. Nutrition Research Reviews 2, 227–246.

Péhourcq, F., Thomas, J., Jarry, C., 2000. A microscale HPLC method for the evaluation of octanol–water partition coefficients in a series of new 2-amino-2-oxazolines. Journal of Liquid Chromatography and Research Technology 23, 443–453.

Petersen, M., Friis, C., Bjørn, H., 1997. A new in vitro assay of benzimidazole activity against adult *Oesophagostomum dentatum*. International Journal for Parasitology 27, 1333–1339.

Sánchez, S., Alvarez, L., Lanusse, C., 1997. Fasting induced changes on the pharmacokinetic behaviour of albendazole and its metabolites in cattle. Journal of Veterinary Pharmacology and Therapeutics 20, 38–47.

Sangster, N., Prichard, R., 1984. Uptake of thiabendazole and its effects on glucose uptake and carbohydrate levels in the thiabendazole-resistant and susceptible *Trichostrongylus colubriformis*. Journal of Parasitology 72, 798–800.

Smith, P., Krohn, R., Hermanson, G., Mallia, A., Gartner, F., Provenzano, M., Fujimoto, E., Goeke, N., Olson, B., Klenk, D., 1985. Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150, 76–85.

Solana, H., Rodríguez, J., Lanusse, C., 2001. Comparative metabolism of albendazole and albendazole sulphoxide by different helminth parasites. Parasitology Research 87, 275–280.

Thompson, D., Geary, T., 1995. The structure and function of helminth surfaces. In: Marr, J., Muller, M. (Eds.), Biochemistry and Molecular Biology of Parasites. Academic Press, London, pp. 203–232.

Thompson, D., Ho, N., Sims, S., Geary, T., 1993. Mechanistic approaches to quantitate anthelmintic absorption by gastrointestinal nematodes. Parasitology Today 9, 31–35.

Virkel, G., Lifschitz, A., Pis, A., Lanusse, C., 2002. In vitro ruminal biotransformation of benzimidazole sulphoxide anthelmintics: enantioselective sulphoreduction in sheep and cattle. Journal of Veterinary Pharmacology and Therapeutics 25, 15–23.

Weir, A., Bogan, J., 1985. Thiabendazole and 5-hydroxythiabendazole in the plasma of sheep. Journal of Veterinary Pharmacology and Therapeutics 8, 413–414.