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International Journal of Pharmaceutics 263 (2003) 123–132
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Intestinal elimination of albendazole sulfoxide: pharmacokinetic effects of inhibitors

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Received 14 April 2003; received in revised form 13 June 2003; accepted 25 June 2003

Abstract

Albendazole (ABZ) is an anthelmintic drug widely used in human and veterinary medicine. Intestinal and hepatic ABZ metabolism leads to albendazole sulfoxide (ABZSO), the active metabolite. This work examines the mechanism involved in intestinal elimination of ABZSO and their pharmacokinetic consequences in rat and sheep. To assess the drug intestinal elimination, an upper small intestine segment was isolated and perfused in situ with saline, after ABZSO administration (10 mg/kg i.v.). The intestinal clearance of ABZSO was 0.106 ± 0.010 ml/min, exhibiting a stereoselective intestinal elimination to (−)ABZSO form. Oxfendazole, ampicillin and cyclosporine significantly reduced the intestinal elimination of ABZSO to 0.079 ± 0.008, 0.069 ± 0.009 and 0.065 ± 0.012 ml/min, respectively. Glucose significantly induced ABZSO intestinal elimination. Pharmacokinetic results showed a clear and statistically significant interaction between ABZ metabolites and drug efflux inhibitors. In rat, an increased area under the curve (AUC) for ABZSO in the groups co-administered with ABZ plus verapamil (43%) and plus ketoconazole (29%) was obtained. In sheep, the AUC for ABZSO in the groups co-administered with the inhibitors were significantly higher 53.68% with verapamil, 78.62% with quinidine, and 50.55% with ivermectin.

Keywords: Albendazole sulfoxide; Drug-interactions; Intestinal elimination; Enantiomers

1. Introduction

Albendazole (ABZ) is a broad-spectrum anthelmintic compound (Bennett and Guyatt, 2000; Cox, 2000) also used to treat microsporidial and criptosporidial infections, emerging diseases of relevance, particularly among those treated with immunosuppressor drugs or infected with human immunodeficiency virus (Costa and Weiss, 2000; Zulu et al., 2002). In addition, its possible ability to treat hepatic carcinomas has been shown (Pourgholami et al., 2001).

After oral/intraluminal administration of ABZ, the parent compound is undetectable in plasma, since successive ABZ oxidations in the host take place. The first step is an ABZ oxidation into its pharmacologically active metabolite, albendazole sulfoxide (ABZSO), a portion of which undergoes a second and slower irreversible step to form albendazole sulfone ($ABZSO_2$), an inactive compound (Galtier et al., 1986). Hepatic and intestinal metabolism of ABZ occurs by two different microsomal enzymatic systems, cytochrome P450 (CYP450) and flavin-containing monooxygenase (FMO). Both CYP450 and FMO systems have

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doi:10.1016/S0378-5173(03)00369-7

been suggested to be responsible for the sulfoxidation of ABZ (Rawden et al., 2000), while CYP450 is the main determinant of sulfonation (Souhaili-El Amri et al., 1988). Involvement of the two pathways in ABZ metabolism has been observed in humans (Rolin et al., 1989) and rats (Moroni et al., 1995; Villaverde et al., 1995), as well as in other domestic species. Moreover, recent data suggest CYP3A4 may be the key contributor of ABZSO formation (Rawden et al., 2000). ABZSO has two antipodes, enantiomers (−) and (+), produced from ABZ, which can be separated by HPLC; (+)ABZSO is associated with the activity of FMO, whereas CYP450 participates in the production of (−)ABZSO (Delatour et al., 1991).

The poor solubility is an important handicap in ABZ therapy, since its reduced absorption from the gastrointestinal tract, due to its physicochemical properties, results in low bioavailability and reduced efficacy. Moreover, intestinal metabolism and significant secretion of ABZSO into the intestinal lumen in isolated intestinal loops have also been demonstrated (Redondo et al., 1999). After a perfusion of a 25 μM ABZ solution, these authors observed an ABZSO secretion to intestinal lumen of 0.165 ± 0.05 nmol/cm. This efflux process can be considered as causing presystemic elimination of ABZSO, influencing its bioavailability. In addition, some drugs and/or their metabolites have shown an active transport by efflux pumps such as P-glycoprotein, breast cancer resistance protein (BCRP), etc. that plays an important role in the disposition of several drugs, since its inhibition has been shown to lead to an increase in the bioavailability of those drugs (Terao et al., 1996; Wacher et al., 2001). Many prototypic inhibitors and inducers affect both CYP3A4 and P-glycoprotein. In addition, many drug interactions caused by these inhibitors and inducers involve these two systems (Lin, 2003).

The aim of this work was to study the intestinal elimination of ABZSO after its intravenous administration by using a simple in vivo model of perfused rat intestine, and the mechanism involved in this process by its competition with different compounds such as structural analogous, oxfendazole; β-lactam antibiotics, ampicillin; and agents that can be inhibitors of CYP3A and drug efflux transporters such as cyclosporin, verapamil, ketoconazole, quinidine, and ivermectin. In addition, the influence of inhibitors on the disposition of ABZSO and $ABZSO_2$, after oral ABZ administration, was studied in rat and sheep.

2. Materials and methods

2.1. Drugs and chemicals

ABZ, ABZSO and $ABZSO_2$, used to carry out the study and to prepare the standard solutions, were kindly supplied by Smithkline & Beecham (Madrid, Spain). Valbazen®, albendazole solution (2%), was purchased from Pfizer (Madrid, Spain); mebendazole, verapamil, quinidine, ivermectin, ampicillin and glucose, from Sigma Chemical Co. (St. Louis, MO, USA), and cyclosporin from Novartis (Basel, Suiza); all compounds present purities higher than 99%. The reagents for HPLC analysis were of analytical grade.

2.2. Experimental animals

Male Wistar rats (250 ± 40 g), purchased from IFFA CREDO (Barcelona, Spain), were housed three per cage in a temperature-controlled room (21–23 °C) with 40–60% humidity and a 12:12 h light/dark cycle. Rats were accustomed to these conditions for at least 1 week before any experimental study. Standard rodent diet (Panlab S.A., Barcelona, Spain) and water were available ad libitum. Before any experiments, the animals underwent an overnight fast with free access to water.

Female Merino sheep weighing 44.7 ± 8.8 kg were also used in this trial. The animals were parasite-free and for 3 weeks before administration of the drugs, the animals were fed on the same diet as that given during the experiment (a mixture of dehydrated alfalfa and dehydrated maize).

All procedures were in accordance with institutional guidelines for the care and use of laboratory animals and the Declaration of Helsinki.

2.3. Perfusion technique

For perfusion, we used a technique based on that described by Rabbaa et al. (1996). Rats were anesthesised with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and rectal temperature was monitored and kept at 37 °C. A catheter (filled with

saline containing 100 IU heparin/ml) was inserted into the carotid artery, a midline abdominal incision was made, and the bile duct was ligated.

A 30-cm small intestinal segment, caudal to the ligament of Treitz, was isolated in situ with its blood supply intact. A polyethylene catheter was inserted, through a small opening and secured by ligation with silk thread, in the proximal end of the segment to deliver the saline perfusate at a rate of 0.6 ml/min. Another catheter was fixed in a similar manner to the distal end to collect effluents. The lumen of the segment was washed with saline at 37 °C until the efflux was clear. The segment was returned to the abdominal cavity, and the abdomen was covered with a cotton pad to avoid evaporation and heat loss. After an equilibration period of 15 min, during which the segment was submitted to single pass perfusion with saline, to allow the temperature to reach 37 °C, and to determine if the animals tolerated the procedure well, the ABZSO (10 mg/kg) was administered by injection of an ethanol (10%), polyethylene glycol (40%) and saline (50%) solution over 1 min into the penis vein. In competition experiments, verapamil (5 mg/kg), cyclosporine (2.5 mg/kg), oxfendazole (5 mg/kg) and ampicillin (10 mg/kg) were administered via the carotid artery 5 min before ABZSO injection. In the experiments with glucose (6 mM), it was added to the saline perfusate. Blood samples from the carotid artery (0.35 ml) were obtained at 2, 5, 7.5, 10, 15, 30, 45 and 60 min. Intestinal effluxes were collected at 15-min intervals for 60 min. Volumes were measured, and aliquots were stored at −20 °C until drug analysis. Blood samples were centrifuged immediately at 3000 × *g* for 15 min and plasma collected and stored at −20 °C until the time of drug analysis. Six animals were used for each type of experiment.

2.4. Pharmacokinetic rat studies

Fifteen rats were used in the pharmacokinetic study. A 1-week washout period was allowed between the administration of each treatment studied: oral doses of ABZ alone (10.6 mg/kg), 10.6 mg/kg ABZ plus verapamil (3 mg/kg) and 10.6 mg/kg ABZ plus ketoconazole (3 mg/kg).

All treatments were administered, as a single oral dose, by gastric intubation (1 ml/animal) at 08:00 h, after a fasting period of 12 h. Blood samples (0.4 ml) were drawn by retro-orbital venous plexus puncture. To carry out the sample collection, rats were anaesthetised with ethyl ether. The samples were collected at 30 min and 1, 2, 4, 8, 10, 12, 15 and 18 h after dosing, with an average collection time of about 2 min. Three groups of five rats were set up at random and sampled with a minimum time interval of 3 h. Blood samples were transferred into heparinised Eppendorf tubes to prevent clotting and immediately centrifuged (3000 × *g* for 15 min) to obtain the plasma, which was stored at −20 °C until analysis.

2.5. Pharmacokinetic sheep studies

The experimental design consisted of 3 animals × 4 treatments, as a single dose orally, with a crossover sequence (six animals for each treatment) using the following formulations: suspension of ABZ (Valbazen®) at 7.5 mg/kg; the same formulation and dosage of ABZ plus verapamil at 3 mg/kg; the same formulation and dosage of ABZ plus quinidine at 3 mg/kg; the same formulation and dosage of ABZ plus ivermectin at 0.5 mg/kg. The inhibitors were dissolved directly in the ABZ suspension to obtain the desired dosage. To ensure full delivery of the drug each syringe was rinsed with water. A 4-week washout period was allowed before the animals participated in the second treatment. Blood samples were collected from the jugular vein into heparinised vacutainers before treatment and at 1, 2, 4, 6, 8, 10, 12, 14, 18, 24, 36, 48, 60, 72 and 96 h after treatment. Blood samples were centrifuged immediately at 3000 × *g* for 15 min and plasma was collected and stored at −20 °C until the time of drug analysis.

2.6. Analytical methodology

Aliquots of the plasma or intestinal effluxes collected (100 μl) were added in a propylene tube to 1 ml ethyl acetate containing 0.2 μg mebendazole (MBZ) as internal standard. The samples were vortexed for 30 s, centrifuged at 6000 × *g* for 6 min (Denver Instrument Co., Arvada, USA), and the organic phase was evaporated to dryness under a nitrogen stream. The dry residue was redissolved in 100 μl of methanol, shaken on a vortex and then analysed by HPLC (Redondo et al., 1998). ABZ metabolites were quantified in reverse phase on a nucleosil C-18 column with a mobile

phase composed of acetonitrile–water with acetic acid (0.5%) pumped at a rate of 1 ml/min in a gradient at the following proportions: 30–70% (5 min), 50–50% (3 min), 65–35% (4 min) and 30–70% (4 min). The absorbance detector was set at a wavelength of 292 nm.

The analytes were identified by comparison with the retention times of 97–99% pure reference standards. Under these chromatographic conditions, the retention times were 4.7 min for ABZSO, 5.5 min for $ABZSO_2$, 10.3 min for MBZ and 13.2 min for ABZ. Calibration curves for each metabolite were determined. Linear regression lines for each analyte, in the range of the tested concentrations (quadruplicate determinations), showed correlation coefficients in excess of 0.995. Unknown concentrations were calculated by comparison of each metabolite and internal standard peak using Millennium[32] software (Waters Corporation) on a Pentium III computer. The quantification limits determined were 0.012 μg/ml for ABZSO, 0.025 μg/ml for $ABZSO_2$ and 0.032 μg/ml for ABZ. There was no interference of endogenous compounds in the chromatographic determinations. Standard plasma samples were spiked with drug and metabolite concentrations of 0.5 and 2.5 mg/ml in order to ascertain the percentage recovery associated with the extraction procedure, which was performed by comparing the chromatographic peak area with that obtained by directly injecting the standard in methanol. Recovery percentages ranged from 85 to 95%.

For the enantiomeric analysis, during the HPLC analysis, the ABZSO fraction was collected in an Eppendorf tube at a retention time of 5.5 min, and evaporated under a nitrogen steam. The subsequent residue was redissolved with 20 μl of methanol and 80 μl of mobile phase (1% 2-propanol in 8 mM of Na_2HPO_4 buffer, pH 6.9). Eighty microlitres of each sample was then injected into the same HPLC system fitted with a chiral stationary column (100 mm × 4.0 mm × 5 μm, Chiral-AGP; ChromTech AB; Hägersten, Sweden) using the conditions described by Delatour et al. (1991). This step provided the relative proportions of both (−)ABZSO and (+)ABZSO (Redondo et al., 1999). Results were expressed as enantiomeric excess (e.e.), which is defined as:

$$\text{e.e.} = \frac{(-)\text{ABZSO eliminated} - (+)\text{ABZSO eliminated}}{\text{ABZSO total eliminated}} \times 100$$

2.7. Pharmacokinetic analysis

The plasma concentration (Cp) versus time curves of ABZSO and $ABZSO_2$ were determined by non-compartmental analyses based on statistical moments (Berrozpe et al., 1997). In this model, the under the curve (AUC) is the zero moment and the mean residence time (MRT) of the drug in the organism is the first moment. The AUC was calculated using the trapezoidal method ($t_0 - t_n$). These calculations were made using the PK Solutions computer program (Farrier, 1997).

In the perfusion experiments, the intestinal elimination was expressed as intestinal clearance (Cl_I). Cl_I is defined as the amount of ABZSO eliminated in 60 min by the intestinal segment divided by serum ABZSO $AUC_{0–60}$.

$$Cl_I = \frac{\text{intestinal ABZSO eliminated in 60 min}}{\text{ABZSO AUC}_{0–60}}$$

2.8. Statistical analysis

The pharmacokinetic parameters are given as mean ± S.D. The statistical significance of differences between treatments was determined in the pharmacokinetic parameters analysed by one-way analysis of variance (ANOVA). When a significant difference between treatments was observed ($P < 0.05$), a post-hoc Newman–Keuls test was carried out in order to perform pair-wise comparisons between treatment means.

3. Results

3.1. Perfusion experiments

Since ABZSO intestinal elimination over 1 h can varied between the 10 and 20% of the ABZSO formed (AUC value), the ABZSO amount eliminated at intestinal level cannot be considered modest (Table 1). Cyclosporin, oxfendazole (a benzimidazole structural analogous) and ampicillin significantly reduced the ABZSO Cl_I. The extends of inhibition were 38.7% with cyclosporin, 25.5% with oxfendazole and 34.9% with ampicillin. In addition, verapamil (a P-glycoprotein inhibitor) also reduced the ABZSO

Table 1
Plasma $AUC_{0–60}$, intestinal clearance (Cl_I), and intestinal enantiomeric excess (e.e.) of ABZSO after an i.v. dose of ABZSO (10 mg/kg) preceeded or not by the administration of different drugs in rat

Drug	$AUC_{0–60}$ (μg/min/ml)	Cl_I (ml/min)	e.e. (%)
Control (ABZSO)	292.02 ± 99.14	0.106 ± 0.010	8.04 ± 0.90
Cyclosporin	325.78 ± 47.85	0.065 ± 0.012[a]	6.04 ± 0.67
Verapamil	205.90 ± 85.51	0.081 ± 0.036	6.74 ± 0.74
Oxfendazole	361.02 ± 45.61	0.079 ± 0.008[a]	6.64 ± 0.73
Ampicillin	377.66 ± 39.07	0.069 ± 0.009[a]	6.95 ± 0.76
Glucose	338.46 ± 157.94	0.163 ± 0.039[a]	8.20 ± 0.33

[a] Significantly different from control, $P < 0.05$. Results are expressed as means ± S.D. ($n = 6$).

Cl_I, but not significantly. On the other hand, glucose in the perfusate increased significantly the ABZSO Cl_I, being the increase of 53.8%. Regarding to the enantiomeric analysis, the e.e. obtained from the 60-min intestinal perfusate is shown in Table 1. We found that ABZSO intestinal elimination was stereoselective, being (−)ABZSO predominant in the perfusate in all the cases since e.e. was always positive. Cl_I inhibition was related to a lower elimination of (−)ABZSO since the e.e. decreased. On the other hand, the e.e. obtained after 60 min in the control group was 3.73% in serum versus 8.04% in intestinal lumen.

3.2. Pharmacokinetic interaction studies

A comparative study between a control ABZ alone treatment and the co-administration of ABZ with inhibitors was performed in rat and sheep. Good tolerability was observed in all the cases. While the parent compound ABZ was not detected in any of the plasma samples, ABZSO and $ABZSO_2$ were the metabolites recovered in plasma after the oral administration of ABZ. The comparative mean plasma concentration versus time profiles of the major metabolite, ABZSO, obtained after the administration of each formulation are shown in Figs. 1 and 2. The plasma concentration values of ABZSO were higher at nearly all the sampling times when ABZ was co-administered with the inhibitors.

The pharmacokinetic parameters for ABZSO and $ABZSO_2$ in rat obtained following the different treatments are shown in Table 2. AUC and C_{max} for

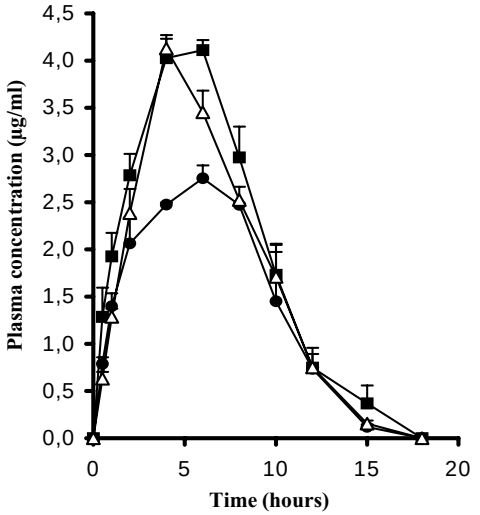

Fig. 1. Mean plasma concentrations (μg/ml) vs. time (h) curves for ABZSO obtained following the oral administration of ABZ alone (●) and after co-administration of verapamil (■) or ketoconazole (△) in rat. Each point represents means ± S.D. ($n = 5$).

ABZSO were significantly higher for the treatments with inhibitors than those obtained in the ABZ alone treatment alone treatment (34.48 ± 1.95, 31.06 ± 1.23, versus 24.2 ± 1.36). The T_{max} value only differed significantly from the control ABZ alone treatment in the case of treatment with ketoconazole. There

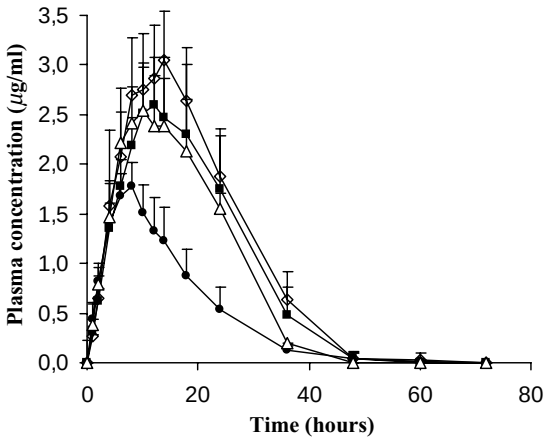

Fig. 2. Mean plasma concentrations (μg/ml) vs. time (h) curves for ABZSO obtained following the oral administration of ABZ alone (●) and after co-administration of verapamil (■), quinidine (◇), or ivermectin, (△) in sheep. Each point represents means ± S.D. ($n = 6$).

Table 2
Pharmacokinetic parameters for ABZSO and $ABZSO_2$ obtained after the oral administration of ABZ co-administered with verapamil or ketoconazole in rat

	ABZ (control)	ABZ + verapamil	ABZ + ketoconazole
ABZSO			
C_{max} (μg/ml)	2.70 ± 0.17	4.19 ± 0.08[a]	4.10 ± 0.12[a]
T_{max} (h)	6.40 ± 0.89	5.60 ± 0.89	4.00 ± 0.00[a]
AUC_{0-t} (μg h/ml)	24.2 ± 1.36	34.48 ± 1.95[a]	31.06 ± 1.23[a]
MRT (h)	6.12 ± 0.26	6.04 ± 0.21	6.22 ± 0.15
$ABZSO_2$			
C_{max} (μg/ml)	0.71 ± 0.06	1.04 ± 0.08[a]	0.89 ± 0.08[a]
T_{max} (h)	7.60 ± 2.19	6.00 ± 1.41	9.20 ± 1.09
AUC_{0-t} (μg h/ml)	6.30 ± 0.70	11.00 ± 1.01[a]	8.06 ± 0.19[a]
MRT (h)	8.18 ± 0.19	7.92 ± 0.33	8.86 ± 0.17[a]

[a] Significantly different from control, $P < 0.05$. Results are expressed as means ± S.D. ($n = 5$).

Table 3
Pharmacokinetic parameters for ABZSO obtained after the oral administration of ABZ either alone or co-administered with verapamil, quinidine or ivermectin in sheep

	ABZ	ABZ + verapamil	ABZ + quinidine	ABZ + ivermectin
ABZSO				
C_{max} (μg/ml)	2.38 ± 0.22	2.69 ± 0.48	2.91 ± 0.62	2.75 ± 0.29
T_{max} (h)	7.00 ± 1.2	12.7 ± 2.4[a]	13.5 ± 1.0[a]	10.5 ± 2.5
AUC_{0-t} (μg h/ml)	40.8 ± 2.6	62.8 ± 14.5[a]	72.9 ± 16.9[a]	61.5 ± 10.8[a]
MRT (h)	15.2 ± 1.7	17.4 ± 1.3	17.2 ± 2.2	15.7 ± 1.3
$ABZSO_2$				
C_{max} (μg/ml)	0.72 ± 0.04	1.22 ± 0.31[a]	1.33 ± 0.18[a]	1.07 ± 0.04[a]
T_{max} (h)	20.0 ± 3.5	22.9 ± 6.9	17.0 ± 5.3	22.5 ± 5.7
AUC_{0-t} (μg h/ml)	18.6 ± 1.7	35.2 ± 12.2[a]	37.4 ± 6.4[a]	28.0 ± 4.0[a]
MRT (h)	21.6 ± 0.1	24.9 ± 3.0	22.5 ± 3.7	21.5 ± 1.4

[a] Significantly different from control, $P < 0.05$. Results are expressed as means ± S.D. ($n = 6$).

were no differences in terms of MRT for any of the treatments compared with control. Pharmacokinetic results for $ABZSO_2$ showed AUC and C_{max} values for $ABZSO_2$ significantly higher than those obtained in the control ABZ alone treatment. The MRT value only differed significantly from the control ABZ alone treatment in the case of treatment with ketoconazole. The T_{max} values for the different treatments were not statistically different.

The pharmacokinetic parameters for ABZSO and $ABZSO_2$ obtained following the different treatments are shown in Table 3. The ABZSO AUC values obtained in all the treatments with inhibitors were significantly higher than those obtained in the control ABZ-alone treatment: 53.68% with verapamil, 78.62% with quinidine, and 50.55% with ivermectin.

In addition, all the treatments with the inhibitors resulted in longer T_{max}. There were no differences in terms of MRT for any of the treatments as compared with the control. The results of the pharmacokinetic analysis for $ABZSO_2$ showed AUC and C_{max} values for $ABZSO_2$ significantly higher for the treatments with inhibitors than those obtained in the ABZ-alone treatment. There were no differences for $ABZSO_2$ in terms of MRT, or T_{max} for any of the treatments as compared with the ABZ-alone control.

4. Discussion

4.1. Perfusion experiments

These experiments have shown ABZSO eliminated at intestinal level from the blood systemic stream (Table 1). Several systemic administered drugs have shown significant intestinal clearances, such as

antihistaminic agents (diphenhydramine), antibiotic drugs (salicilamide and gentisamide) or anthelmintics (ivermectin) (Kumar et al., 1999; Lin et al., 1999; Laffont et al., 2002).

Since we observed a clear enantioselectivity in the intestinal elimination of ABZSO, it can thus be considered that passive diffusion is not the sole mechanism for intestinal excretion of ABZSO, because e.e. would otherwise be identical in the serum and in the intestinal efflux. Since the enantiomer physicochemical properties are very similar, the stereoselective transport is probably due to an active transport system involvement. An enantioselective transport by P-glycoprotein has been shown in the case of intestinal elimination of ofloxacin and talinolol (Rabbaa et al., 1996; Hanafy et al., 2001).

Clear inhibitor effects of the ABZSO Cl_I with concomitant cyclosporin, oxfendazole and ampicillin were shown; and clear inductor effects with glucose in the perfusate (Table 1). In general, inhibitions and inductions were directly related to a change in the (−)ABZSO elimination as it is shown by the e.e. (Table 1).

The effect observed with concomitant cyclosporin (a 38.7% of inhibition) of the ABZSO intestinal elimination, could be considered unspecific, since this drug interacts with a great variety of transporters and enzymes, including P-glycoprotein. However, one of the main metabolites of cyclosporin, AM1, is actively transported by P-glycoprotein (Gan et al., 1995); therefore, a P-glycoprotein competition with ABZSO could be taken place. However, after intravenous ABZSO administration and concomitant verapamil, this P-glycoprotein inhibitor did not have a clear effect on the ABZSO intestinal elimination (Table 1). However, the main interactions between P-glycoprotein inhibitors and several drugs have been shown after the oral co-administration of the inhibitors. In addition, P-glycoprotein interaction occurs in the luminal surface of the enterocyte and very closely related to CYP3A metabolism. Moreover, the P-glycoprotein inhibition by verapamil is strongly dependent of its intraluminal availability (Gramatte and Oertel, 1999). Also it is important to notice that verapamil is quickly metabolised at hepatic and intestinal levels (Fromm et al., 1996). Controversial results about the effect of verapamil on intestinal ivermectin secretion in the different segments of the small intestine have been recently observed (Laffont et al., 2002).

In addition, Spahn-Langguth et al. (1998) observed that the enantioselective transport disappeared with inhibition of active efflux transporters such as P-glycoprotein. In our case, a reduction of the e.e. in the group treated with verapamil was observed (Table 1).

Regarding the inhibition of Cl_I observed with concomitant oxfendazole (25.5%), it is clearly related with the structural analogy between this drug and ABZSO, resulting in a transport competition. This transport inhibition due to structural analogy has been shown with ofloxacin, ciprofloxacin and pefloxacin, using quinolone derived drugs (Rabbaa et al., 1996; Dautrey et al., 1999).

In the case of the competition between ABZSO and ampicillin, with an inhibition of the intestinal elimination (Cl_I) of 24.9%, it could be the result of a common secretory pathway of ABZSO and β-lactam antibiotic drugs. A secretory efflux of some penicillin drugs (benzilpenicillin, amoxicillin and ampicillin itself) that was glucose-dependent and disappeared without ATP, has been shown (Saitoh et al., 1996). In addition, the pharmacokinetic of amoxicillin, an analog to ampicillin, is affected by ivermectin, a P-glycoprotein substrate (Tsai, 2001). However, this antibiotic is a well-known substrate of the organic anion transporter (Hill et al., 2002).

Intestinal ABZSO elimination seems to occur via complex mechanism combining passive diffusion and active transport. The transporter(s) involved are ATP/glucose dependent since the ABZSO elimination (Cl_I) is inhibited by cyclosporin and induced by glucose in the perfusate. The involvement of more than one transporter system could occur, being the main candidates P-glycoprotein, and also MRP2 (multidrug resistance protein 2) and BCRP. Ampicillin (Saitoh et al., 1996), fluoroquinolones (Rabbaa et al., 1996) and several other drugs have been shown to be transported to the intestinal lumen by more than one transporter system.

4.2. Pharmacokinetic interaction studies

The pharmacokinetic interaction of verapamil and ketoconazole with ABZSO is clear, since the results showed an increase of the ABZSO and $ABZSO_2$

bioavailability following the co-administration of ABZ with these drugs. In rats, ABZSO AUC, with verapamil became 34.48 ± 1.95 μg h/ml and with ketoconazole 31.06 ± 1.23 μg h/ml, versus 24.2 ± 1.36 23 μg h/ml. Increased ABZSO plasma concentrations resulted in significantly higher AUC values in sheep with the different treatments: 53.68% in the case of co-administration with verapamil; 78.62% in the case of quinidine, and 50.55% with ivermectin. Although the differences in ABZSO C_{max} between the combination and control treatments did not reach statistical significance, they tended to be higher in the presence of inhibitors. Moreover, it seems that drug absorption may have been delayed, because the ABZSO T_{max} was increased in all treatments. In addition, to the best of our knowledge this is the first time that this effect has been described in a ruminant species.

In rats, the AUC and C_{max} values of two P-glycoprotein substrates, talinolol and the ivermectin, were significantly increased after the co-administration of verapamil (Alvinerie et al., 1999; Laffont et al., 2002).

Although it is know that the parent compound ABZ does not interact with P-glycoprotein (Merino et al., 2002), Zhu (1999) has proposed that P-glycoprotein substrates could be metabolites formed intracellularly from lipophilic drugs and effluxed from the cell. This metabolite transport mediated by intestinal P-glycoprotein is consistent with the disposition of CYP3A and P-glycoprotein as an intestinal barrier to drug absorption (Wacher et al., 2001).

Since verapamil, quinidine and ivermectin are P-glycoprotein and CYP3A susbstrates and kenotoconazole is an inhibitor of both proteins, a decreased elimination of ABZSO and some kind of interaction with ABZ metabolism is to be expected. The exposure of ABZSO can be increased through metabolic interactions and the decreased in intestinal drug clearance. Regarding this fact, CYP3A and P-glycoprotein are co-expressed in the intestine and the liver and their expression is co-ordinately regulated by the pregnane (steroid) xenobiotic receptor (Synold et al., 2001). These two proteins are believed to work in concert for the elimination of xenobiotics, thus therapeutics that are inhibitors or substrates of both would be expected to lead to drug–drug interactions, resulting in increased levels of the co-administered drug circulating in the plasma (Dantzig et al., 2003). The relative contribution of CYP3A and P-glycoprotein to overall interaction is still not completely clarified because the complex interplay involved between intestinal and hepatic CYP3A4 and P-glycoprotein (Lin, 2003).

In addition, drug efflux transporters could exist in locations other than the intestine (liver, kidney) and hence the observed increase in the ABZSO systemic concentrations could be the result of a modification of the elimination processes at several sites.

The interaction of these inhibitors with antiparasitic drugs is quite important, with respect to parasite resistance to the anthelmintic drugs mediated by parasite P-glycoprotein efflux proteins. This can be seen in the increase in the efficacy of ivermectin and moxidectin against *Haemonchus contortus* resistant to moxidectin after secondary infection in gerbils, following co-administration with verapamil (Molento and Prichard, 1999).

Regarding the possibility to inhibit ABZSO intestinal elimination for therapeutic purposes, this could be a good chance to increase ABZSO oral bioavailability for systemic parasitosis. In intestinal parasitosis, such as microsporidial and critosporidial infections, the therapeutic aim is to achieve high concentration of the active drug, ABZSO, in the intestinal lumen and this work showed an inductor effect of glucose of the ABZSO intestinal elimination.

These results supported an increase of exposure of ABZSO by a possible decrease of clearance and an increase of bioavailability by probable mixed effects resulting from metabolic interactions and the effect of intestinal CYP3A and drug efflux transporters.

Acknowledgements

This work was supported by Ministry of Science and Technology (Spain) with the research project AGF98-1036CO2-02 and a grant (to G.M.) from Plan Nacional de Formacion de Personal Investigador (PN98 9789011G).

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