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Review

Enantiomeric behaviour of albendazole and fenbendazole sulfoxides in domestic animals: Pharmacological implications

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Abstract

Albendazole and fenbendazole are methylcarbamate benzimidazole anthelmintics extensively used to control gastrointestinal parasites in domestic animals. These parent compounds are metabolised to albendazole sulfoxide and fenbendazole sulfoxide (oxfendazole), respectively. Both sulfoxide derivatives are anthelmintically active and are manufactured for use in animals. They metabolites have an asymmetric centre on their chemical structures and two enantiomeric forms of each sulfoxide have been identified in plasma, tissues of parasite location and within target helminths. Both the flavin-monooxygenase and cytochrome P450 systems are involved in the enantioselective biotransformation of these anthelmintic compounds in ruminant species. A relevant progress on the understanding of the relationship among enantioselective metabolism and systemic availability of each enantiomeric form has been achieved. This article reviews the current knowledge on the pharmacological implications of the enantiomeric behaviour of albendazole sulfoxide and oxfendazole in domestic animals.
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Keywords: Albendazole sulfoxide; Fenbendazole sulfoxide (oxfendazole); Enantiomeric behaviour; Enantioselective biotransformation; Domestic animals

Introduction

The introduction of benzimidazole (BZD) anthelmintic drugs was a major advance in the treatment of gastrointestinal parasites in veterinary practice. Rational use of these compounds requires knowledge of their pharmacological properties in the target animal species and may help to optimise clinical efficacy and avoid the development of resistance. The pharmacokinetic behaviour of albendazole (ABZ) and fenbendazole (FBZ) have been studied in several species including sheep, cattle, horses, donkeys, dogs, pigs, goats, buffaloes and humans (Marriner and Bogan, 1980, 1985; Ngomuo et al., 1984; Prichard et al., 1985; Marriner et al., 1986; McKellar et al., 1990, 1993, 1995, 2002; Benchaoui et al., 1993; Hennessy et al., 1993a; Sanyal, 1994, 1997, 1998; Alvarez et al., 1996; Sanchez et al., 2000; Gokbulut et al., 2006a).

The BZD sulfoxide metabolites, albendazole sulfoxide (ABZSO), fenbendazole sulfoxide (FBZSO) (also called oxfendazole, OFZ) and their respective sulfone derivatives ($ABZSO_2$ and $FBZSO_2$) are the main metabolic products found systemically after ABZ and FBZ administration. Two sequential oxidative steps, mediated by the enzymatic systems flavin-monooxygenase (FMO) and cytochrome P450, are involved in the production of the sulfoxide and sulfone metabolites in the liver. Both ABZSO and OFZ are available for anthelmintic therapy in sheep and cattle and are reduced by ruminal microflora to their parent sulfides (ABZ and FBZ, respectively) after oral or intra-ruminal administration. In sheep, no differences in the systemic availability of ABZSO were observed after administration

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of either ABZ or ABZSO. Furthermore, FBZ and its sulfoxide and sulfone metabolites were recovered in equal proportions after the administration to sheep of both FBZ and OFZ (Lanusse et al., 1995). Equivalent anthelmintic efficacy has been shown following administration of the sulfoxide derivatives, compared with that observed after treatment with the parent sulfides (Perez-Serrano et al., 1997; Williams et al., 1997).

ABZSO and OFZ have an asymmetric centre in the sulfur atom of their chemical structure (Figs. 1 and 2). Thus, two ABZSO and OFZ enantiomers have been identified in the plasma of several species, including cattle, sheep, goats, dogs, rats, mice, horses and humans following administration of the pro-chiral molecules ABZ and FBZ (Delatour et al., 1990b, 1991a,b; Benoit et al., 1992; García et al., 1999; McKellar et al., 2002; Gokbulut et al., 2007).

Since the discovery of optical isomerism by Pasteur in the nineteenth century, the study of the chiral behaviour of a pair of enantiomers has become relevant for a proper understanding of the differential pharmaco-toxicological properties of different compounds administered as a racemate. Although enantiomers have similar or identical physico-chemical properties, they may exhibit different pharmacodynamic and/or pharmacokinetic behaviour. The available knowledge on the pharmacological implications of the enantiomeric behaviour of albendazole sulfoxide and oxfendazole in domestic animals is reviewed in the current article.

Pharmacokinetic considerations

Changes in the enantiomeric proportions following the administration of a racemate clearly indicate an enantioselective pharmacokinetic behaviour of a given compound. In addition, pharmacodynamic differences between enantiomers may be qualitative or quantitative (Landoni et al., 1997). After administration of a chiral drug, the observed pharmacological response may represent the combined effects of each enantiomeric form. Understanding the pharmacokinetic behaviour and metabolic fate of different BZD anthelmintics is necessary to optimise parasite control in veterinary medicine. The pharmacokinetics of ABZSO and OFZ enantiomers has been studied in several species, including cattle, goats, sheep, horses, dogs, humans and rats following FBZ, OFZ, ABZ or ABZSO administration by different routes and formulations (Delatour et al., 1990b, 1991a; Capece et al., 2000; Cristòfol et al., 2000; McKellar et al., 2002; Goudah, 2003; Sanchez Bruni et al., 2005a,b; Gokbulut et al., 2007).

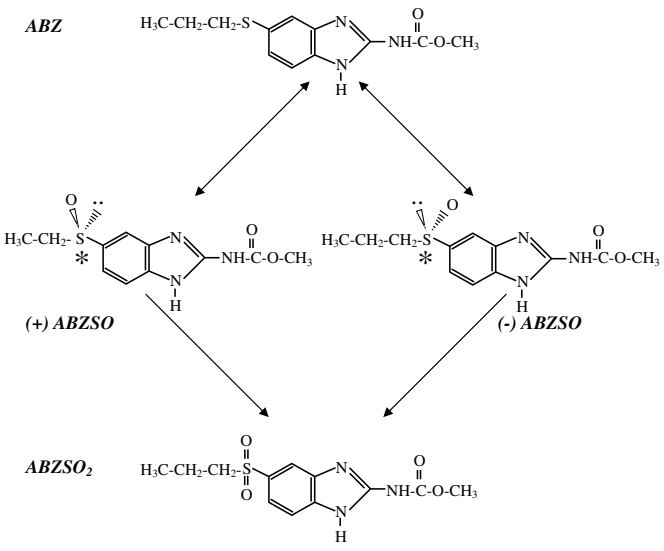

Fig. 1. Metabolic pathway of albendazole and albendazole sulfoxide.

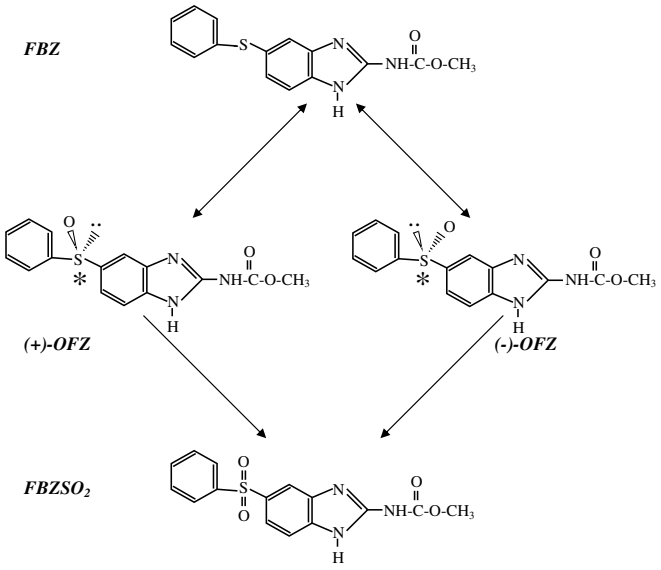

Fig. 2. Metabolic pathway of fenbendazole and oxfendazole.

Several investigations have shown species differences in the pharmacokinetic behaviour of ABZSO and OFZ enantiomers. After FBZ or ABZ administration to sheep, the (+) antipodes represented 74% and 86% of the total plasma area under the curve (AUC) of OFZ and ABZSO, respectively (Delatour et al., 1990b; Sanchez Bruni et al., 2005b). Delatour et al. (1991a) observed that the (+)-ABZSO AUC value in dogs was 70% of the total-ABZSO. Similarly, (+)-OFZ was the predominant enantiomer found in the plasma of sheep and dogs treated with FBZ (Delatour et al., 1990b; Sanchez Bruni et al., 2005b; Gokbulut et al., 2007). A predominance of the (+)-ABZSO was also seen following IV (Cristòfol et al., 2000; Goudah, 2003) and oral (Capece et al., 2000) administration of racemic ABZSO (*rac*-ABZSO) to cattle and sheep. The evolution over time of OFZ enantiomeric ratios in the plasma of horses treated with FBZ showed that (−)-OFZ predominated up to 12 h post-treatment. Afterwards, (+)-OFZ was the main enantiomeric form recovered from the bloodstream in this species (Sanchez Bruni et al., 2005a).

These studies on the plasma enantiomeric behaviour of ABZSO shows that (+)-ABZSO predominates in the

plasma of most species studied including man (Delatour et al., 1991a; Marques et al., 1999; Capece et al., 2000; Cristòfol et al., 2000; Goudah, 2003), whilst its (−) antipode is recovered in higher proportions in the plasma of rats (Delatour et al., 1990a, 1991a; Capece et al., 2003) and mice (García et al., 1999; García et al., 2003).

Tissue distribution

The lack of water solubility is an important limitation for the formulation of the most potent BZD methylcarbamate anthelmintics, allowing their formulation only as suspensions for oral/intra-ruminal administration in ruminants. The oxidised sulfur atom present in ABZSO and OFZ chemical structures enhanced their hydrosolubility and this physico-chemical property was exploited to develop aqueous solutions introduced for subcutaneous administration to cattle in South America. Independent of the route of administration, the pharmacological effect of the active BZD compounds depends on the presence of sustained concentrations of active drug/metabolites at the site of parasite location. Most of the BZDs, including the asymmetric derivatives reach the receptors within the parasites throughout passive drug transfer (Takayanagui et al., 2002; Mottier et al., 2006).

ABZSO is well distributed in the body after IV administration. The volume of distribution (Vd) values for this molecule ranged between 0.67–1.2 L/kg for cattle and sheep, respectively (Cristòfol et al., 2000; Formentini et al., 2005). As consequence of the lack of differences in their physico-chemical properties, ABZSO enantiomers have similar pattern of distribution reaching parasite location tissues, such as the digestive mucosa, ileal and abomasal fluids, liver and lung (Cristòfol et al., 2001). The Vd values for the enantiomeric forms after *rac*-ABZSO intravenous administration ranged from 0.60–0.79 L/kg for (+)-ABZSO and from 0.55–0.85 L/kg for (−)-ABZSO (Cristòfol et al., 2000, 2001).

Regardless of the route of administration, (−)-ABZSO and (−)-OFZ are depleted faster from the bloodstream than their respective (+) antipodes in domestic animals. Following *rac*-ABZSO IV administration to sheep and cattle, (−)-ABZSO was depleted faster from the bloodstream than its (+)-ABZSO antipode (Cristòfol et al., 2000, 2001; Goudah, 2003). A faster (−)-ABZSO depletion was also observed after the oral administration of NTB, ABZ or ABZSO to sheep, cattle, dogs and goats (Delatour et al., 1990b; Benoit et al., 1992; Capece et al., 2000, in press; Gokbulut et al., 2006b, 2007). Similarly, a more rapid elimination of (−)-OFZ, compared to (+)-OFZ, was seen when either FBZ or *rac*-OFZ were orally administered to dogs, sheep and horses (Delatour et al., 1990b; McKellar et al., 2002; Sanchez Bruni et al., 2005b; Gokbulut et al., 2007). In consequence, a major contribution to the antiparasitic effect of (+) enantiomers may be expected since their (−) antipodes are rapidly eliminated from the body. Conversely, in rats and mice, (−)-ABZSO was the main enantiomeric form recovered in the systemic circulation (Delatour et al., 1991a; Capece et al., 2003; García et al., 2003). Species differences on the disposition of ABZSO enantiomers are attributed to the relative participation of the hepatic enzymatic systems involved in the biotransformation of ABZ and ABZSO.

The BZD anthelmintics are distributed or re-distributed from the plasma into the gastrointestinal (GI) tract. Following intra-ruminal administration of netobimin (NTB) to cattle, the plasma profiles of ABZSO and $ABZSO_2$ metabolites reflected their GI disposition (Lanusse et al., 1993a). Peak concentrations of both metabolites were followed by a well-defined elimination phase in both plasma and GI compartments (rumen, abomasum and ileum). However, whereas plasma concentrations fell to undetectable levels (at 30–36 h post-treatment), the profiles of ABZSO and $ABZSO_2$ in the rumen, abomasum and ileum showed an 'extra slow' elimination phase up to 72 h post-treatment. A plasma-gastrointestinal pH gradient may facilitate the secretion of these molecules to the lumen of the GI tract (Lanusse and Prichard, 1993).

Benzimidazole sulfoxide metabolites are weak bases (pKa values around 7.8) and at plasma pH there will be a higher proportion of these molecules in the lipophilic non-ionic form, which would facilitate its passive diffusion from plasma to different tissues including the digestive tract. A greater plasma/abomasum pH gradient, compared to that of the rumen and ileum, would produce a strong ionic trapping effect which may have accounted for the significantly higher concentrations of ABZSO and $ABZSO_2$ found in the abomasal content in comparison with plasma and other GI contents (Lanusse and Prichard, 1993).

ABZ sulfa-metabolites may also reach the intestinal lumen through intestinal secretion (Redondo et al., 1999) and biliary excretion (Hennessy et al., 1989). Moreover, the extensive ionic trapping in the abomasum has been shown following intra-ruminal administration of ABZ to sheep (Alvarez et al., 1999) and cattle (Sanchez et al., 1997) and also following the SC and IV administration of ABZSO to cattle (Lanusse et al., 1998; Cristòfol et al., 2001). Enhanced AUC values in abomasal and intestinal fluids (compared to plasma) were observed for both ABZSO enantiomeric forms in these GI compartments (Cristòfol et al., 2001). These increased concentration profiles were found in abomasal and intestinal fluids compared to their respective mucosal tissues, which may reflect the higher affinity of ABZSO for the most polar medium represented by the GI fluids. This observation is consistent with the higher hydrophilicity of this metabolite compared to its parent compound ABZ.

The transfer of ABZSO enantiomers across membranes of parasite location tissues, and also their uptake/accumulation within the parasite, are not enantioselective processes and depend on the passive diffusion of both molecules. In general, the tissue distribution pattern of (+) and (−)-ABZSO reflect the enantioselective disposition of both antipodes in the plasma of steers treated with

ABZSO (Cristòfol et al., 2001). Thus, a higher availability of the (+) enantiomeric form was observed in several target tissues/fluids following *rac*-ABZSO intravenous administration to cattle. Moreover, (+)-ABZSO was the main enantiomer recovered from *Fasciola hepatica* specimens collected from the bile ducts of ABZ-treated sheep (Alvarez et al., 2000).

In humans ABZSO crosses the blood–brain barrier (Takayanagui et al., 2002) and also the placenta, reaching the fetus and embryos in higher and sustained concentrations in both sheep and rats (Cristòfol et al., 1995, 1997; Capece et al., 2002, 2003). In patients with active brain parenchymal neurocysticercosis, plasma concentrations of ABZSO enantiomers in humans were around two-fold higher than those observed in cerebrospinal fluid (Takayanagui et al., 2002). In sheep, the AUC ratio between (+)-ABZSO and (−)-ABZSO in the maternal plasma was 2.8, while in the fetal plasma this ratio decreased to 1.6 (Capece et al., 2002). Such difference may indicate an enantioselective transport process through the placental membranes (i.e. the transference of (−)-ABZSO is more efficient) and also metabolic differences between mother and fetus. In fact, a lower cytochrome P450-mediated oxidation of (−)-ABZSO in the fetus liver may also have accounted for a higher proportion of this enantiomer in the fetal plasma. Interestingly, (−)-ABZSO is thought as the main substrate for the production of $ABZSO_2$ in a cytochrome P450-mediated reaction (Benoit et al., 1992).

Metabolism

Benzimidazole and pro-BZD anthelmintics are extensively metabolised in domestic animals and man. Their metabolic pattern and the resultant pharmacokinetic behaviour are relevant in the attainment of high and sustained concentrations of pharmacologically active drug/metabolites at the target parasite (Lanusse and Prichard, 1993). The metabolism of BZD compounds occurs in both hepatic and extra-hepatic tissues (Villaverde et al., 1995; Virkel et al., 2004) as well as in GI fluids (Lanusse et al., 1992; Capece et al., 2001; Virkel et al., 2002) and involves oxidation of the parent sulfides, reduction of their respective sulfoxide derivatives as well as acetylation, hydroxylation and conjugation reactions.

After ABZ administration, the parent drug was not detected in the plasma of treated animal and this has been attributed to a first-pass oxidation in the liver. In fact, the oxidation of ABZ to ABZSO has been shown to be catalysed by the liver microsomal mixed function oxidases (cytochrome P450 and flavin-monooxygenase [FMO] systems) in rats (Moroni et al., 1995), pigs (Souhaili El-Amri et al., 1987), sheep (Galtier et al., 1986; Lanusse et al., 1993b), calves (Lanusse et al., 1993b), some wild animals (Velík et al., 2005a) and humans (Rawden et al., 2000). Cytochrome P450 is primarily involved in ABZ hepatic sulfoxidation in rats (Moroni et al., 1995) and humans (Rawden et al., 2000). This enzymatic system is also the major contributor to FBZ hepatic sulfoxidation in rats (Murray et al., 1992).

Inhibition of the cytochrome P450-mediated sulfoxidation by piperonyl butoxide showed the participation of this enzymatic system in the hepatic metabolism of FBZ in horses (McKellar et al., 2002). Conversely, it has been demonstrated that FMO is primarily involved in ABZ hepatic sulfoxidation in sheep (Galtier et al., 1986; Lanusse et al., 1993b; Virkel et al., 2004) and cattle (Lanusse et al., 1993b; Virkel et al., 2004). For example, the FMO-mediated sulfoxidation of ABZ and FBZ accounted for the major production of total-ABZSO (60%) and total-OFZ (80%) in both species (Virkel et al., 2004).

On the other hand, the parent drug FBZ and its sulfoxide metabolite are found in the bloodstream after the administration of both FBZ and OFZ to sheep (Lanusse et al., 1995). The hepatic sulfoxidation of FBZ to form OFZ has been shown in rats (Murray et al., 1992), horses (Montesissa et al., 1989; McKellar et al., 2002), pigs, sheep and cattle (Montesissa et al., 1989). Furthermore, both anthelmintically active sulfoxide derivatives undergo a second, slower and irreversible oxidative step, yielding the inactive sulfone ($ABZSO_2$ and $FBZSO_2$) metabolites, which are also found in the bloodstream after administration of their respective parent sulfides.

Enantioselectivity of metabolic products occurs when chiral metabolites are generated differentially (in qualitative or quantitative terms) from a single achiral substrate (Testa and Mayer, 1988). Two different Km values for the production of each ABZSO enantiomer have been reported after ABZ (pro-chiral molecule) incubation with liver microsomes obtained from rats (Moroni et al., 1995) and calves (Virkel et al., 2000). These observations are consistent with the involvement of two different enantioselective enzymatic pathways on the liver sulfoxidation of ABZ. Indeed, FMO and cytochrome P450 are known to be oppositely enantioselective (Cashman, 1998). In rat liver, the FMO system produces ~63–69% of (+)-ABZSO, whereas the cytochrome P450 isoenzymes 2C6 and 2A1 are mainly involved in the production of (−)-ABZSO (Moroni et al., 1995). The FMO-mediated liver sulfoxidation of ABZ was enantioselective (100%) towards the (+)-ABZSO production in both sheep and cattle (Virkel et al., 2004). On the other hand, cytochrome P450 was found to be mainly involved in the production of (−)-ABZSO in the liver of these species.

Both enzymatic systems are also involved in the hepatic sulfoxidation of FBZ in sheep and cattle. The FMO-mediated sulfoxidation of FBZ generated both (+)-OFZ and (−)-OFZ enantiomers in these species, however the percentage enantioselectivity towards (+)-OFZ production was 65% in sheep and 79% in cattle (Virkel et al., 2004). Thus, both enantiomeric forms of ABZSO and OFZ were produced by the liver microsomal cytochrome P450 in both species. Inhibition of the hepatic cytochrome P450-mediated sulfoxidation of FBZ by piperonyl butoxide

reduced the production of (−)-OFZ in horses (McKellar et al., 2002).

Clearly, the relative involvement of both FMO and cytochrome P450 enzymatic systems on the production of ABZ and FBZ sulfoxides accounts for the observed enantioselective plasma disposition of these metabolites in the different species studied. For example, (+)-ABZSO and (+)-OFZ prevailed in the plasma of sheep, cattle and horses, which is consistent with a higher FMO-mediated production of these enantiomers in the liver. A minor contribution of the cytochrome P450 system correlates well with lower plasma AUC values observed for (−)-ABZSO and (−)-OFZ. Also, (−) enantiomers are thought to be the primary substrates for the cytochrome P450-mediated production of their inactive sulfone metabolites in sheep and goats (Delatour et al., 1990b), which may accounted for their faster depletion from plasma at least in these species. Conversely, after the individual administration of each ABZSO enantiomer to rats, both enantiomeric forms are converted into $ABZSO_2$ to the same extent, which may indicate a lower cytochrome P450 substrate enantioselectivity for the sulfonation reaction in this species.

Induction of a cytochrome P450 isoenzyme, possibly belonging from CYP 1A subfamily, was observed following ABZ administration in multiple doses to goats (Benoit et al., 1992) and mouflon (Velík et al., 2005b). Repeated administration of ABZ led to decreased AUC values for ABZSO and increased AUC values for $ABZSO_2$ in the plasma of goats. It has been shown that this auto-inductive effect of ABZ is due to a substantial increase in the activity and the expression of a CYP1A isoenzyme in rats, humans (Arteinza et al., 2000) and mouflon (Velík et al., 2005b). This enhanced metabolism was shown to be related to an increased (−)-ABZSO consumption after repeated administration of ABZ to goats (Benoit et al., 1992), which may confirm the involvement of cytochrome P450 in the production of $ABZSO_2$. Overall, the inductive effect of ABZ may give rise to a considerable decrease in the therapeutic efficacy, which in addition may contribute to the development of parasitic resistance.

In comparison to the liver, where oxidative metabolism predominates, the GI microflora is very active in reductive reactions of foreign compounds, particularly those containing nitro (Acosta de Pérez et al., 1992) and sulfoxide (Renwick et al., 1986; Rowland, 1986) groups. Drug metabolic processes taking place in the rumen are particularly important in ruminant therapeutics. Once ABZSO and OFZ have been distributed from the plasma to different GI compartments both molecules could be reduced back by the GI microflora, providing a source of ABZ in the GI tract. The in vitro ruminal sulforeduction of both ABZSO (Lanusse et al., 1992; Virkel et al., 1999) and OFZ (Beretta et al., 1987) has been demonstrated in sheep and cattle.

The comparative sulforeduction of ABZSO and OFZ enantiomers was described in ruminal fluid obtained from sheep and cattle under in vitro conditions (Capece et al., 2001; Virkel et al., 2002). A higher rate of depletion was observed for the (+) enantiomeric form when ABZSO was incubated with ruminal fluid from both species. The concentrations of ABZ formed were between 55% and 158% greater after incubation of cattle ruminal fluid with (+)-ABZSO, compared to that produced when (−)-ABZSO was the incubated substrate. Similarly, a higher production of ABZ was obtained when (+)-ABZSO was incubated with sheep ruminal fluid. The metabolic profile of both OFZ enantiomers followed a similar pattern to that observed for ABZSO enantiomers. These results showed that (+) enantiomers may be the main substrates for the ruminal sulforeduction to form ABZ. Interestingly, a bidirectional chiral inversion of one enantiomer into its antipode was also observed for ABZSO (Virkel et al., 2002). Thus, the (+) enantiomer appeared in the incubation medium when (−)-ABZSO was the incubated substrate, and also the (−) antipode was detected after (+)-ABZSO incubation with ruminal fluid obtained from both sheep and cattle.

FBZ and ABZ were recovered in high concentrations from the faeces of donkeys orally treated with either ABZ, FBZ or OFZ (Gokbulut et al., 2006a). The presence of FBZ in OFZ treated donkeys suggests the GI reduction of the administered compound in this animal species. The microflora present in the caecum (the largest forestomach cavity in horses and donkeys) may be involved in such metabolic reaction. The extra-hepatic sulforeduction of ABZSO into ABZ was also suggested in rats (Capece et al., 2008). These authors recovered small amounts of ABZ in rat urine after oral administration of ABZSO. Conversely, the sulforeduction of OFZ into FBZ was not observed in dogs (Gokbulut et al., 2007).

Species differences in the stereoselective metabolism of ABZ and FBZ

There are species differences on the enantioselective biotransformation process of ABZ and FBZ. The enantiomeric ratio of (+)-ABZSO/(−)-ABZSO ranged between 2.8 and 3.8 after ABZ incubation with mouflon hepatocytes (Velík et al., 2003). Conversely, rat hepatocytes produced both enantiomeric forms in equal proportions (enantiomeric ratio = 1.0–1.1). The mean ABZSO enantiomeric ratio (±) found after ABZ incubation with sheep liver microsomes was 4.11, higher than in cattle (2.63) (Virkel et al., 2004). Stereoselective metabolism of drugs is most commonly the major contributing factor to stereoselectivity in pharmacokinetics, and differences in the amount or activity of the involved enzymes may be the source of the species differences in the kinetics of the enantiomers. For example, $FBZSO_2$ was not detected in dogs (Gokbulut et al., 2007), while it was detected in donkeys (Gokbulut et al., 2006a), horses (McKellar et al., 2002; Sanchez Bruni et al., 2005a), sheep (Marriner and Bogan, 1981a,b; Sanchez Bruni et al., 2005b), pigs (Petersen and Friis, 2000), cattle (Short et al., 1987a; Knox and Steel, 1997), goats (Short et al., 1987b) and rabbits (Short et al., 1987c). These find-

ings may indicate that different metabolic routes are involved in OFZ elimination among species (Short et al., 1987a,b, 1988a).

The oxidative metabolism of FBZ was studied in hepatic subcellular fractions prepared from livers of cattle, sheep, goats, chickens, ducks, turkeys, rats, rabbits and catfish. All these species produced the sulfoxide metabolite OFZ, and p-hydroxyfenbendazole (FBZ-OH) was produced by all species except sheep (Short et al., 1988b). Horses metabolise FBZ and OFZ more quickly than ruminants, with low and short bioavailability and residence time (Marriner and Bogan, 1985; McKellar et al., 2002; Gokbulut et al., 2006b). These metabolic differences among species may influence the disposition of the enantiomers.

The stereospecific ABZ sulfoxidation to (−)-ABZSO was lower in young male goats and deer than in castrated male goats, non castrated male mouflon and non castrated male sheep (Velík et al., 2005a). In the same study, these authors observed that (−)-ABZSO production from ABZ was higher in roe deer stag, fallow buck and red deer stag. In different studies carried out in rats, the proportion of (+)-ABZSO/(−)-ABZSO was 1.0–1.1 (Velík et al., 2003). Overall, a higher proportion of (−)-ABZSO was observed in rats, whilst in man and other domestic and wild animal species predominated the production of (+) ABZSO.

Excretion

ABZ, FBZ and their respective metabolites are excreted in urine and faeces (Gyurik et al., 1981; Short et al., 1988a; Hennessy et al., 1989; Capece et al., 2008). The magnitude of the metabolite excretion depends on the animal species and on the component formed in the metabolic pathways. For example, Short et al. (1988a) observed that after FBZ administration, $FBZSO_2$ was the major metabolite excreted in turkey, whereas in chickens and ducks it was hydroxyfenbendazole (FBZ-OH). In sheep, 47% of a total dose of FBZ was excreted in bile (Hennessy et al., 1993b), and 34% as conjugated metabolites. In goats, the main metabolite excreted was FBZ-OH (Short et al., 1987b). Small amounts were recovered as OFZ, indicating that the oxidative reaction is not the main pathway for FBZ elimination, compared to ABZ, because the sulfoxide (ABZSO) is the main metabolite recovered in urine (Hennessy et al., 1989; Capece et al., 2008).

A stereoselective intestinal elimination of (−)-ABZSO was observed in rats and sheep (Merino et al., 2003). However, similar plasma and urine ABZSO enantiomeric proportions in rats may indicate that the urinary elimination of ABZSO enantiomers was not enantioselective in this species (Capece et al., 2008). On the other hand, a higher renal clearance was observed for (−)-ABZSO compared to (+)-ABZSO in humans (Lanchote et al., 2004), which may indicate stereoselective urinary excretion in this species. There are species differences in the renal excretion of sulfoxide enantiomers and these may help to explain differences in their plasma disposition kinetics. The intestinal excretion of ABZSO seems to occur though a complex mechanism, combining passive diffusion and active transport with involvement of ATP/glucose dependent or other transporters which help to explain why this process could be enantioselective (Merino et al., 2003).

In ewes treated with the pro-drug NTB, (+)-ABZSO was the main enantiomeric form in plasma, whilst the ratio of the faecal AUC of (−)-ABZSO (172.22 μg h/g) and (+)-ABZSO (187.19 μg h/g) was 0.92 (Gokbulut et al., 2006b); this observation may indicate an enantioselective biliary/faecal elimination of this sulfoxide metabolite.

Pharmacodynamic aspects and side effects

An effective antiparasitic treatment depends on the ability of drugs to reach high, effective and sustained concentrations within the parasites. The relevance of drug uptake by helminth parasites was observed by Mottier et al. (2006), when living cestode and nematode specimens were found to contain significantly low concentrations of FBZ compared to dead parasites.

The uptake/accumulation of BZD compounds within the parasite depends mainly on their passive drug transfer. After ABZ administration to sheep, similar concentrations of (+)-ABZSO were measured in plasma, bile and *Fasciola hepatica* specimens recovered from bile ducts (Alvarez et al., 2000) and this enantiomer may play a major part in ABZ's trematodicidal action.

The anthelmintic activity of BZD compounds also depends on the affinity for the cytosolic proteins of different helminth parasites. For example, ABZSO showed high affinity for cestode cytosolic proteins compared to those of nematodes and trematodes (Solana et al., 2002). It has been shown that this binding is enantioselective; the enantiomeric binding ratios (−)-ABZSO/(+)-ABZSO were 43/57, 36/64 and 91/9, for *Ascaris* spp., *Moniezia* spp. and *Fasciola hepatica*, respectively (Solana et al., 2002).

The relative potency of ABZSO enantiomers have been evaluated under in vitro conditions (Bolás-Fernández et al., 2004). These authors observed that (+)-ABZSO has higher activity against *Trichinella spiralis* than its antipode or the racemic form. At 0.5 μg/mL in the incubation medium, all three compounds were highly effective in reducing the viability of the larvae, whereas at lower concentrations (0.1 μg/mL) only (+)-ABZSO induced a significant reduction in larval viability compared to controls. Using *Haemonchus contortus* larvae as model, the efficacy of *rac*-ABZSO and (+)-ABZSO were 94%, whereas the efficacy of (−)-ABZSO was 72% (Mottier et al., 2004). These observations suggested a low pharmacological activity of (−)-ABZSO. On the other hand, (−) ABZSO is the main substrate for the production of $ABZSO_2$, as explained above. Altogether these observations support the idea that the contribution of (−)-ABZSO is relatively low compared to (+)-ABZSO under in vivo conditions.

OFZ and ABZSO exhibit high potency against susceptible parasites. However, they show low diffusion rates and

low affinity for parasite tubulin compared to their parent compounds (Lacey et al., 1987; Lubega and Prichard, 1991). For this reason, the in vivo anthelmintic efficacy of the sulfoxide enantiomers may be facilitated by the gastrointestinal sulforeduction into their more active and lipophilic sulfide forms.

In general, BZD anthelmintics compounds are safe. Data on their teratogenic effects after NTB, ABZ and ABZSO administration to rats (Delatour et al., 1981; Cristòfol et al., 1997; Navarro et al., 1999; Capece et al., 2003; Teruel et al., 2003), sheep (Fabre et al., 1989; Navarro et al., 1998) and also in cell cultures of rat embryos (Whittaker and Faustman, 1991) are available. These authors observed that these compounds cause weight loss, skeletal malformations and embryotoxicity. In fact, the detection of ABZSO in rats embryos (Cristòfol et al., 1997; Capece et al., 2003) and sheep fetuses (Cristòfol et al., 1995; Capece et al., 2002) may explain the malformations attributed to these molecules (Cristòfol et al., 1997; Navarro et al., 1998, 1999; Capece et al., 2003). However, whether these effects have been produced by one or both enantiomeric forms is unknown. OFZ also induced mutagenic effects and embryotoxicity, including teratogenicity in mice (El-Makawy et al., 2006). Studies concerning the teratogenic effect of each enantiomeric form separately may improve the understanding of the toxicological effect of these compounds.

Conclusions

Pharmacokinetic differences of OFZ and ABZSO enantiomers were observed in all studied animal species after administration of the racemic form and/or after administration of their respective pro-chiral sulfide compounds. However, there is a lack of information on the kinetic behaviour after separate administration of each enantiomeric form to domestic animals, particularly in ruminants. Since the sulfoxide compounds are more hydrophilic than the sulfide compounds, the preparation of the enantiomers for parenteral administration may be feasible. However, the available data on the pharmacological behaviour of the BZD sulfoxide enantiomers could be only exploited after the specific activity of each enantiomer against different target parasites has been established. Limited information is available on individual anthelmintic activity of the enantiomers in domestic animals and further work is required to assess the possible manufacture and use of the different BZD sulfoxide enantiomers in livestock animals.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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