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Effects of methimazole on the kinetics of netobimin metabolites in cattle

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1. The effects of methimazole (MTZ) on the pharmacokinetic behaviour of netobimin (NTB) and its albendazole (ABZ) metabolites were studied in calves. NTB trisamine salt solution was given by subcutaneous (12·5 mg/kg) and oral (20 mg/kg) routes, either alone or co-administered with MTZ (1·5 mg/kg, intramuscularly).

2. NTB parent drug was detected only after s.c. treatments, showing rapid absorption, early C_{max} and fast disposition. ABZ was not found in plasma at any time after either s.c. or oral treatments.

3. Concomitant treatment with MTZ significantly increased the albendazole sulphoxide (ABZSO) elimination half-life ($t_{1/2\beta}$) (321%) and mean residence time (MRT) (170%) from the values obtained after s.c. treatment with NTB alone.

4. Oral treatment resulted in an ABZSO pharmacokinetic profile with an *AUC* 27% higher, a significantly longer $t_{1/2\beta}$ (151%) and MRT (124%) in the presence of MTZ.

5. We conclude that when co-administered with NTB in cattle, MTZ induces significant changes in the disposition kinetics of the anthelmintically active ABZSO metabolite.

Introduction

It has been shown that netobimin (NTB) is a pro-benzimidazole compound which exerts its anthelmintic activity by cyclization into albendazole (ABZ) metabolites (Delatour *et al.* 1986). NTB can be formulated either as an insoluble zwitterion or as a water-soluble salt for oral or parenteral administration. Recently, we have investigated the pharmacokinetic behaviour of NTB parent drug and its metabolites in sheep (Lanusse and Prichard 1990) and cattle (Lanusse *et al.* 1990, 1991). In these studies we have established that both formulation and route of administration may dramatically affect the pharmacokinetic profiles of albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$), the principal metabolites detected in plasma after NTB administration. Furthermore, our results have shown pronounced differences in the disposition kinetics of the above-mentioned metabolites between sheep and cattle.

As demonstrated by *in vitro* studies (Galtier *et al.* 1986, Souhaili-el-Amri *et al.* 1987), ABZ biotransformation takes place mainly in the liver microsomal fraction. Two distinct enzymic pathways seem to be involved in this sulphoxidation process. It was proposed that the flavin-containing monooxygenase (FMO) is responsible for the ABZ oxidation into ABZSO (Galtier *et al.* 1986), while the cytochrome P-450 system would be involved in the second and slower oxidative step, by which ABZSO is converted into $ABZSO_2$ (Souhaili-el-Amri *et al.* 1988). In terms of uptake by parasites and binding to nematode tubulin, the thioethers (like ABZ) are more potent than sulphoxide metabolites, which in turn are more potent than the sulphones (Lacey 1990, Lubega and Prichard 1991). The oxidation metabolites have a higher polarity, which makes their recycling across the gastrointestinal wall more difficult

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and facilitates their elimination (Prichard *et al.* 1985). Therefore, metabolism of ABZ into its sulphoxide, and finally into its sulphone, may result in a considerable decrease in anthelmintic activity.

Methimazole (MTZ) and other thioureas are anti-thyroid drugs, known to be substrates for the flavin-containing monooxygenase system. They seem to inhibit participation of this enzymic pathway in the *in vitro* microsomal oxidation of different xenobiotics (Tynes and Hodgson 1983). Furthermore, other reports have shown that the biotransformation of MTZ may give reactive metabolites which may bind to a cytochrome P-450 drug-binding site, temporarily decreasing its enzymic activity (Kedderis and Rickert 1985). The *in vitro* inhibition of ABZ sulphoxidation by sheep liver microsomes has been shown to be significantly greater with MTZ than with several other oxidation-impairing compounds (Galtier *et al.* 1986). In addition, MTZ drastically inhibits the ABZSO formation by cattle liver microsomes in a concentration-dependent manner (Lanusse *et al.* 1991 b).

Interference *in vivo* with the liver microsomal oxidation process might affect the pharmacokinetic profiles of benzimidazole metabolites which could lead to improved clinical efficacy, as a result of higher concentrations of active metabolites being presented to the parasite for longer periods of time. The objective of this experimental work was to evaluate potential modification to the pharmacokinetic behaviour of NTB metabolites produced by the co-administration of NTB subcutaneously and orally with methimazole (intramuscularly) in calves.

Materials and methods

Animals

The study was conducted on eight Holstein Fresian bull calves, in parasite-free conditions, weighing from 110 to 130 kg. High-quality hay and water were available to them *ad libitum*. The health of all animals was closely monitored prior to, and throughout, the experimental periods. A 4-week wash-out period was allowed before the same animals participated in the second phase of the study.

Drug administration

Animals were divided into two groups of four animals each and treatments were given as follows.

Netobimin-alone treatments. Group A: animals were treated with trisamine salt solution of netobimin (250 mg/ml) (Schering Plough, NJ, USA) by subcutaneous (s.c.) injection in the shoulder area at 12·5 mg/kg. *Group B*: animals were treated with trisamine salt solution of netobimin (50 mg/ml) (Schering Plough, NJ, USA) by oral drenching at 20 mg/kg.

Netobimin-methimazole co-administrations. Group A: animals received trisamine salt solution of netobimin (250 mg/ml) by s.c. injection at 12·5 mg/kg together with a solution of methimazole (10 mg/ml in sterile physiological saline; 2-mercapto-1-methyl-imidazole, Aldrich Chemical Co., USA) given intramuscularly (i.m.) at 1·5 mg/kg, immediately after the netobimin injection. Methimazole was given i.m. in this experimental study to ensure its systemic availability. *Group B*: animals received trisamine salt solution of netobimin (50 mg/ml) by oral drench at 20 mg/kg immediately followed by an i.m. injection of methimazole (10 mg/ml) at 1·5 mg/kg. The choice of the MTZ dose rate was based on the recommended antithyroid therapeutic dose. No adverse reactions were observed at the injection sites.

Blood sample collection

In each experiment, blood samples (10 ml) were taken from the jugular vein into Vacutainer tubes (Becton-Dickinson) containing sodium heparin prior to treatment and at 0·25, 0·5, 0·75, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 32, 48, and 72 h post-administration. Plasma was separated by centrifugation at 2500 r.p.m. for 15 min, placed into plastic vials and frozen at −20°C until analysed.

Analytical methods

Immediately after thawing the plasma samples (1·0 ml), an internal standard (oxibendazole, 1 μg/10 μl methanol) was added, and NTB and its metabolites extracted using disposable C_{18} SepPak cartridges (part no. 51910, Waters Associates, MA, USA). Sample extraction procedures, h.p.l.c. equipment and analysis conditions were as previously reported (Lanusse *et al.* 1990).

Identification of NTB, ABZ, ABZSO and $ABZSO_2$ was undertaken by comparison with the retention time of pure reference standards (supplied by Schering Plough, NJ, USA). The retention times were as follows: ABZSO 5·40 min, $ABZSO_2$ 8·10 min, NTB parent compound 9·90 min, and ABZ 16·8 min. The standards were also used to prepare the calibration curve for each analyte. The linear regression lines for each analyte in the range of 0·02–2·0 μg/ml (triplicate determinations) showed correlation coefficients between 0·970 and 0·982.

Unknown concentrations were quantified by establishing the ratio between each analyte and the internal standard peak area, using Nelson Analytical Software, model 2600, version 3.0 (Nelson Analytical Inc., CA, USA) on an IBM-XT computer. The sensitivity of the assay (μg/ml) was as follows: 0·040 (NTB), 0·020 (ABZ and ABZSO) and 0·025 ($ABZSO_2$). There was no interference of methimazole or any endogenous compounds in the chromatographic determination of either NTB or its metabolites.

Pharmacokinetic analysis of data

The plasma concentration versus time curves for NTB and/or its metabolites after each treatment were fitted with the PKCALC computer program (Shumaker 1986) coupled to an augmented copy of the stripping program ESTRIP (Brown and Manno 1978). The following equation was used to describe biexponential plasma concentration curves (Notari 1987):

$$Cp = Be^{-\beta t} - Be^{-kt}$$

where: Cp = plasma concentration at the time t after administration (μg/ml); B = concentration at time zero extrapolated from the elimination phase (μg/ml); e = base of the natural logarithm; β = terminal slope (h^{-1}); and k is the rapid slope obtained by feathering which represents either the first-order absorption rate constant (K_{ab}) or first-order metabolite formation rate constant (K_f) (h^{-1}). Appropriate lag times were used where necessary.

The elimination half-life ($t_{1/2\beta}$) and absorption ($t_{1/2\,ab}$) or metabolite formation half-lives ($t_{1/2\,for}$) were calculated as $\ln 2/\beta$ and $\ln 2/k$, respectively. The peak plasma concentration (C_{max}) and time to peak concentration (t_{max}) were read from the plotted concentration–time curve of each metabolite. The area under the plasma concentration–time curve (AUC) was calculated by trapezoidal rule (Gibaldi and Perrier 1982) and further extrapolated to infinity by dividing the last experimental plasma concentration by the terminal slope (β).

Since the i.v. route was not used, the total body clearance (Cl_b) and volume of distribution (method of area) (Vd_{area}) for NTB parent drug represent their true values divided by the bioavailability (F) (Gibaldi and Perrier 1982). Therefore, these parameters are reported and calculated as follows:

$$Cl_b/F = \frac{\text{dose}}{AUC} \qquad Vd_{area}/F = \frac{\text{dose}}{AUC \cdot \beta}$$

Statistical moment theory was applied to calculate the mean residence time (MRT) for NTB and its metabolites as follows (Perrier and Mayersohn 1982):

$$\text{MRT} = \frac{AUMC}{AUC} - \frac{1}{k}$$

where AUC and k are as defined previously and $AUMC$ is the area under the curve of the product of time and the plasma drug concentration versus time from zero to infinity (Gibaldi and Perrier 1982).

The rate of metabolic conversion (RMC) of NTB into ABZ metabolites was calculated as follows:

$$\text{RMC} = \frac{AUC_{ABZSO} + AUC_{ABZSO_2}}{AUC_{NTB} + AUC_{ABZSO} + AUC_{ABZSO_2}}$$

Statistical analysis

Statistical comparison of mean pharmacokinetic parameters for NTB and its metabolites in the absence and presence of MTZ was performed using Student's t-test for paired observations. A value of $P < 0.05$ was considered significant. The pharmacokinetic parameters are reported as mean ± SEM.

Results

While NTB parent drug was detected only after subcutaneous treatment, ABZ was not found in plasma at any time after either subcutaneous or oral administrations. The comparative mean plasma concentrations of NTB, ABZSO and $ABZSO_2$ after subcutaneous and oral treatments with NTB trisamine solution (without MTZ) at recommended doses are plotted in figure 1. The appearance of

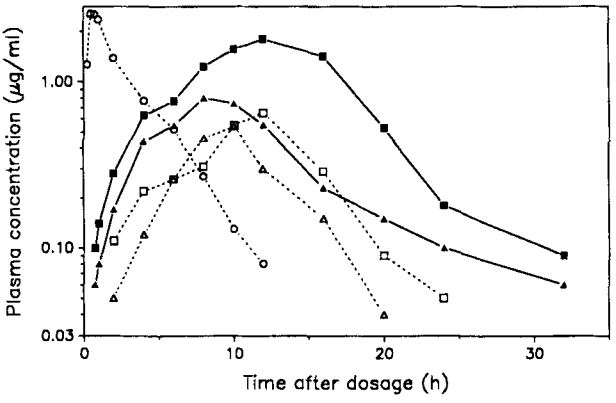

Figure 1. Mean plasma concentration of netobimin (parent compound), albendazole sulphoxide and albendazole sulphone after administration of netobimin trisamine by subcutaneous and oral routes to calves.

No. of calves = 4; s.c. dose was 12·5 mg/kg and oral dose was 20 mg/kg. NTB, s.c. (○---○); ABZSO, s.c. (△---△); $ABZSO_2$, s.c. (□---□); ABZSO, oral (▲——▲); $ABZSO_2$, oral (■——■).

detectable plasma levels of ABZSO and $ABZSO_2$ was delayed after the subcutaneous treatment (2–4 h) in comparison with the oral administration (0·5–0·75 h). Using the doses recommended for cattle, the administration of NTB salt solution by oral drenching resulted in both ABZSO and $ABZSO_2$ *AUC* being significantly higher ($P < 0·01$) than for the subcutaneous treatment. The $t_{1/2\beta}$ and MRT values for both metabolites were also significantly longer ($P < 0·05$) after the oral treatment.

The pharmacokinetic parameters for NTB parent drug obtained after the subcutaneous injection of NTB either alone or co-administered with MTZ are presented in table 1. MTZ did not significantly affect the pharmacokinetic parameters for the NTB parent compound except that the C_{max} was significantly lower following the co-administration.

The pharmacokinetic analyses for ABZSO and $ABZSO_2$ obtained after the subcutaneous administration of NTB in absence or presence of MTZ are shown in

Table 1. Pharmacokinetic parameters for netobimin parent compound (NTB) obtained after its subcutaneous administration (12·5 mg/kg) to calves either alone or with methimazole.

Parameter	NTB *alone* ($n = 4$)	NTB + *methimazole* ($n = 4$)
k_{ab} (h^{-1})	5·20 ± 1·71	5·83 ± 1·21
$t_{1/2\,ab}$ (h)	0·18 ± 0·05	0·14 ± 0·03
β (h^{-1})	0·325 ± 0·026	0·229 ± 0·015
$t_{1/2\beta}$ (h)	2·17 ± 0·19	3·07 ± 0·21
AUC (ug · h/ml)	8·70 ± 2·14	4·81 ± 0·57
AUMC ($\mu g \cdot h^2$/ml)	28·9 ± 7·7	22·1 ± 2·9
MRT (h)	3·04 ± 0·31	4·38 ± 0·27
C_{max} (μg/ml)	2·87 ± 0·19	1·02 ± 0·16*
t_{max} (h)	0·75 ± 0·10	0·75 ± 0·10
Vd_{area}/F (l/kg)	5·25 ± 1·09	11·9 ± 1·3
Cl_b/F (ml/h per kg)	1650 ± 295	2735 ± 384
RMC (%)	56·8 ± 7·1	71·3 ± 4·9

Values are expressed as means ± SEM.
*Statistically different from the NTB-alone treatment at $P < 0·0$.

Table 2. Pharmacokinetic parameters for ABZSO and $ABZSO_2$ after subcutaneous administration of netobimin (12·5 mg/kg) to calves either alone or with methimazole (i.m., 1·5 mg/kg).

Parameter	ABZSO		$ABZSO_2$	
	NTB alone ($n=4$)	NTB + methimazole ($n=4$)	NTB alone ($n=4$)	NTB + methimazole ($n=4$)
K_f (h^{-1})	0·362 ± 0·022	0·230 ± 0·036	0·292 ± 0·017	0·275 ± 0·001
$t_{1/2for}$ (h)	1·93 ± 0·10	3·23 ± 0·49	2·39 ± 0·29	2·52 ± 0·09
β (h^{-1})	0·267 ± 0·009	0·083 ± 0·002**	0·234 ± 0·027	0·207 ± 0·018
$t_{1/2\beta}$ (h)	2·62 ± 0·10	8·43 ± 0·24**	3·09 ± 0·37	3·44 ± 0·34
AUC ($\mu g \cdot h/ml$)	4·59 ± 0·34	2·96 ± 0·74	6·44 ± 0·83	7·59 ± 0·90
AUMC ($\mu g \cdot h^2/ml$)	48·2 ± 1·9	51·5 ± 14·5	76·0 ± 9·7	100 ± 17
MRT (h)	7·84 ± 0·44	13·3 ± 0·9*	8·37 ± 0·35	9·36 ± 0·67
C_{max} ($\mu g/ml$)	0·60 ± 0·05	0·19 ± 0·04*	0·69 ± 0·09	0·70 ± 0·05
t_{max} (h)	10·0 ± 0·8	9·50 ± 0·50	11·5 ± 0·5	11·5 ± 0·5

Values are expressed as means ± SEM.
* Significantly different from the control treatment (NTB alone) at $P<0·01$.
** Significantly different from the control treatment (NTB alone) at $P<0·001$.

table 2. ABZSO was detected in plasma between 4 and 20 h (NTB alone) and between 2 and 32 h (NTB + MTZ) post-administration. A significantly higher ($P<0·01$) ABZSO C_{max} was obtained after treatment with NTB alone. However, co-administration of NTB + MTZ resulted in a longer metabolite formation half-life than the subcutaneous treatment with NTB alone. The terminal slope (β) and the $t_{1/2\beta}$ for ABZSO were significantly longer ($P<0·001$) after the co-administration of NTB + MTZ (see table 2). Also ABZSO MRT was significantly longer ($P<0·01$) after NTB + MTZ than after NTB alone. $ABZSO_2$ showed a similar pharmacokinetic profile after both subcutaneous treatments.

Neither NTB parent drug nor ABZ was detected in plasma after both oral treatments with NTB. The mean plasma concentrations of ABZSO obtained after oral administration of NTB alone or in combination with MTZ in calves are shown in figure 2. In table 3 the results of the pharmacokinetic analyses for ABZSO and $ABZSO_2$ metabolites after the above-mentioned oral NTB treatments are summarized. The $t_{1/2\beta}$ and MRT values were significantly longer ($P<0·05$) for the

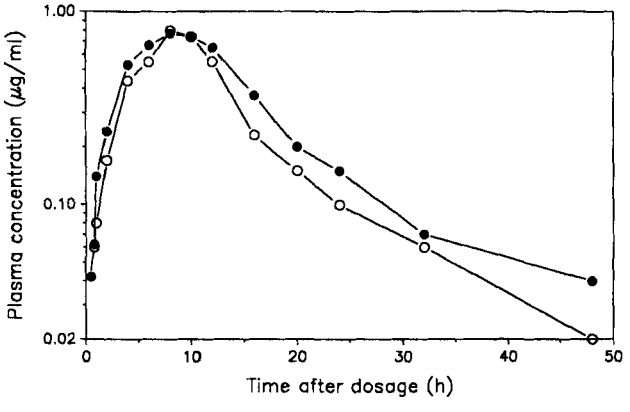

Figure 2. Mean plasma concentration of albendazole sulphoxide after oral administration of netobimin trisamine to calves either alone or with methimazole.

No of calves = 4; netobimin trisamine dose was 20 mg/kg and methimazole dose i.m. was 1·5 mg/kg. NTB alone (○——○); NTB + MTZ (●——●).

Table 3. Pharmacokinetic parameters for ABZSO and $ABZSO_2$ after oral administration of netobimin (20 mg/kg) to calves either alone or with methimazole (i.m., 1·5 mg/kg).

Parameter	ABZSO		$ABZSO_2$	
	NTB alone ($n=4$)	NTB + methimazole ($n=4$)	NTB alone ($n=4$)	NTB + methimazole ($n=4$)
K_f (h^{-1})	0·270 ± 0·034	0·318 ± 0·048	0·256 ± 0·020	0·291 ± 0·033
$t_{1/2for}$ (h)	2·71 ± 0·41	2·40 ± 0·50	2·75 ± 0·21	2·47 ± 0·25
β (h^{-1})	0·126 ± 0·016	0·085 ± 0·008	0·152 ± 0·021	0·269 ± 0·037
$t_{1/2\beta}$ (h)	5·71 ± 0·69	8·64 ± 0·49*	4·94 ± 0·95	2·70 ± 0·30
AUC (ug·h/ml)	9·83 ± 1·16	12·5 ± 1·7	24·4 ± 1·3	28·9 ± 3·0
AUMC ($\mu g \cdot h^2$/ml)	124 ± 8	185 ± 17	335 ± 29	418 ± 59
MRT (h)	9·36 ± 0·70	11·6 ± 0·2*	9·74 ± 0·68	11·0 ± 0·4
C_{max} (μg/ml)	0·87 ± 0·19	0·82 ± 0·09	1·90 ± 0·08	2·27 ± 0·13
t_{max} (h)	8·50 ± 0·50	8·50 ± 0·50	12·5 ± 1·3	16·0 ± 0·0

Values are expressed as means ± SEM.
* Significantly different from the control treatment (NTB alone) at $P<0·05$.

NTB + MTZ treatment than for the oral administration of NTB alone. The higher mean *AUC* value for ABZSO in the combined treatment was not significantly different from the treatment with NTB alone. However, the *AUMC* was significantly higher ($P<0·05$) for the co-administration of NTB + MTZ compared with the oral NTB alone treatment. All parameters obtained from the $ABZSO_2$ kinetic analyses showed no statistically significant difference between the treatments.

Discussion

Pharmacokinetics of NTB and its metabolites

As previously suggested (Delatour *et al.* 1986, Lanusse and Prichard 1990), the gastrointestinal tract seems to be the principal site of NTB reduction and cyclization into ABZ, both after oral (or intra-ruminal) and parenteral administration. Either oxidation in the gut mucosa before absorption, or a first-pass oxidation in the liver microsomal fraction, may account for ABZ not being detected in plasma after both oral and subcutaneous treatments with NTB in calves. In the present study, using recommended doses of NTB, oral administration resulted in a significantly different pharmacokinetic profile and disposition kinetics for both ABZSO and $ABZSO_2$ metabolites than did the subcutaneous treatment (figure 1). These differences are due to a less efficient NTB cyclization into ABZ metabolites following subcutaneous administration, as previously reported in sheep (Lanusse and Prichard 1990). The parenterally administered NTB must be absorbed, distributed and, by secretion or bile elimination, reach the gastrointestinal tract. The rapid elimination of the parent compound after subcutaneous administration may account for less efficient conversion into its cyclized metabolites compared with oral treatment, after which the absence of NTB and the high profile of its metabolites in plasma indicate a more successful conversion.

Influence of methimazole on the disposition of NTB metabolites

Co-administration with MTZ had little effect on the pharmacokinetic behaviour of NTB parent drug after its subcutaneous administration. The difference observed in C_{max} was consistent with a slightly higher rate of NTB metabolic conversion for the combined NTB + MTZ treatment (table 1).

Important modifications in the disposition kinetics of ABZSO were obtained after the co-administration of NTB (subcutaneous) and MTZ, compared with the treatment with NTB alone. While there was no difference in terms of *AUC*, the overall elimination half-life of ABZSO was three times as long ($P<0{\cdot}001$) and the MRT was almost twice that ($P<0{\cdot}01$) in the presence of MTZ (table 2).

The results of the present oral NTB experiments also demonstrated that concomitant MTZ treatment affects the pharmacokinetic profile of ABZSO, perhaps the most relevant metabolite in terms of NTB anthelmintic activity. The mean *AUC* value for ABZSO was 27% higher after the co-administration of NTB + MTZ, but this difference was not significant. This was probably due to the spurious results obtained in one animal of the group receiving NTB alone. Using the *Q* test for extraneous values (Dean and Dixon 1951), the *AUC* value for ABZSO for that animal in the control group could be rejected with 90% confidence. The elimination of this spurious observation resulted in an *AUC* value for ABZSO significantly higher ($P<0{\cdot}05$) for the oral NTB + MTZ treatment than for the oral NTB alone treatment, when analysed by unpaired *t* test. Furthermore, the *AUMC* value, a parameter that reflects the area under the first moment in the plasma concentration versus time curve, was significantly higher ($P<0{\cdot}05$) for the oral NTB + MTZ treatment than for the oral administration of NTB alone. This confirms the modification of the pharmacokinetic behaviour of ABZSO in the presence of MTZ. In addition, the concomitant administration of MTZ with oral NTB significantly increased the $t_{1/2\beta}$ (151%) and MRT (124%) for ABZSO.

In a model-independent pharmacokinetic description, the smaller slope represents the rate-limiting step. Although the ratio k/β was outside of the range proposed for the existence of the 'flip-flop' phenomenon (Notari 1987), in our data the β value could represent either the elimination rate or the metabolite formation rate. On the other hand, MRT is a non-compartmentally derived parameter, which represents an averaged time of permanence of all drug or metabolite molecules in the body, characterizing the distribution and elimination process. Thus, the significant differences obtained for $t_{1/2\beta}$ and MRT are strong evidence of a MTZ-mediated alteration in the pharmacokinetic behaviour of ABZSO. MTZ may impair the hepatic microsomal oxidation of the ABZ metabolites of NTB.

In view of the fact that MTZ is known to be a substrate for the flavin-containing monooxygenase (FMO) system (Tynes and Hodgson 1983) and the fact that this system would primarily be involved in the sulphoxidation of ABZ to ABZSO (Galtier *et al.* 1986, Lanusse *et al.* 1991 b), it is highly likely that the observed modifications in the ABZSO pharmacokinetics are due to competition between MTZ and ABZ in the FMO pathway. Thus, an inhibitory effect of MTZ at this microsomal enzymic level may not dramatically affect the plasma levels (*AUC*, C_{max}), but may dramatically affect the disposition kinetics and residence time of ABZSO after both subcutaneous and oral administration of NTB in cattle.

The efficacy of an antiparasite drug depends on a toxic concentration being presented to the parasite for sufficient time to lead to irreversible damage. The intrinsic action of the benzimidazole molecule on the parasite is based on the disruption of cell functions that requires a lag time until the parasite's survival is threatened. The parasite may be able to survive in the short term, but if the impairment of essential functions is maintained for a sufficiently long time, the ability of the parasite to survive will be affected. Thus, slow decline of the active drug/metabolites elimination phases and long residence times in the bloodstream,

leading to an extended time of parasite exposure, are more important than high peak concentrations followed by fast elimination. Even with low plasma concentrations, the prolonged duration of the anthelmintically active ABZSO, induced by the co-administration of MTZ and NTB, is highly important in terms of clinical efficacy. Conversely, the administration of a larger dose of the anthelmintic compound may result in higher peak plasma concentrations, but not in a prolonged time of parasite exposure to active drug concentrations.

MTZ is an inexpensive and safe compound, regularly used in human and veterinary medicine. In addition, high bioavailability, and a relatively long half-life (Jansson *et al.* 1985) may ensure that a single dose of MTZ will result in a prolonged residence time which would facilitate successful inhibition of ABZ sulphoxidation.

The results reported here demonstrated that the co-administration of MTZ with parenterally and orally administered NTB in cattle results in significant changes in the pharmacokinetic behaviour of the ABZSO metabolite. However, more research is necessary in order to adjust dosage levels, route of administration and to determine whether MTZ will enhance the clinical efficacy of NTB and other benzimidazole compounds.

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