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METHIMAZOLE INCREASES THE PLASMA CONCENTRATIONS OF THE ALBENDAZOLE METABOLITES OF NETOBIMIN IN SHEEP

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ABSTRACT

The influence of methimazole (MTZ) on the pharmacokinetics of netobimin (NTB) and its metabolites was investigated in adult sheep. NTB zwitterion suspension was administered at 20 mg kg^{-1} by intraruminal injection either alone or with simultaneous administration of MTZ intramuscularly at 1·5 mg kg^{-1}. Blood samples were taken serially over a 120-h period and plasma was analysed by HPLC for NTB, albendazole (ABZ), albendazole sulphoxide (ABZSO), and albendazole sulphone ($ABZSO_2$). NTB parent drug showed fast absorption, low area under the plasma concentration–time curve (AUC) and was rapidly removed from plasma after both treatments. The presence of MTZ did increase significantly the ABZ AUC (138 per cent) and mean residence time (MRT) (86 per cent). Concomitant treatment with MTZ resulted in a notably higher ABZSO plasma profile with significantly longer elimination half-life ($t_{1/2\beta}$) (390 per cent) and MRT (252 per cent) and with significantly higher AUC (95 per cent). Also, MTZ induced significant increases in $ABZSO_2$ $t_{1/2\beta}$, AUC, and MRT.

We have demonstrated a pharmacokinetic interaction between MTZ and NTB metabolites. MTZ may alter the liver biotransformation of ABZ metabolites which results in pronounced changes in the disposition kinetics of anthelmintically active metabolites.

KEY WORDS Netobimin Albendazole metabolites Methimazole Pharmacokinetic interaction

INTRODUCTION

Netobimin (NTB), N-methoxycarbonyl-N′-(2-nitro-5-propylphenylthio-N″-(2-ethyl sulphonic acid) guanidine, is a new broad spectrum anthelmintic compound. NTB exerts its anthelmintic activity after biotransformation into its corresponding 5-substituted propylthio-benzimidazole, albendazole (ABZ), and its metabolites in the host's body.[1,2] After oral and parenteral administration of NTB both in sheep and cattle, albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) are the principal metabolites found in plasma.[2-4] Experimental data have shown that NTB is reduced and cyclised into ABZ by the gastrointestinal flora.[1] Following this cyclisation, ABZ may be absorbed by the gut mucosa and oxidised into ABZSO in the liver, or

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perhaps partly oxidised (into ABZSO) in the digestive tract before absorption.[1,2] *In vitro* studies have demonstrated that ABZ sulphoxidation takes place in the liver microsomal fraction.[5,6] A flavin-containing monooxygenase (FMO) could be responsible for the ABZ oxidation into ABZSO,[5] while the cytochrome P-450 system is involved in the second, slower and irreversible oxidative step by which ABZSO is converted into $ABZSO_2$.[7]

Benzimidazole and pro-benzimidazole compounds exert their anthelmintic effects by binding to parasite tubulin.[8] The successive metabolic oxidations of benzimidazole and pro-benzimidazole thioethers in the host lead to more polar and less anthelmintically active metabolites. In terms of parasite uptake and binding to parasite tubulin, the parent sulphides (ABZ) are more potent than the sulphoxide metabolites, while the sulphone metabolites are inactive.[8,9] Therefore, the sequential metabolic steps after NTB conversion result in a considerable reduction in antiparasite activity, notably the last irreversible oxidation step that produces the $ABZSO_2$ metabolite. Additionally, the increasing polarities of the oxidised metabolites may make them less able to reach target parasites, especially tissue-dwelling parasites, in sufficient concentrations to produce a pharmacological effect.

Methimazole (MTZ) is a safe anti-thyroid drug regularly used in human and veterinary medicine, known to be a substrate for the FMO system. MTZ seems to inhibit the participation of this enzymatic pathway in the *in vitro* microsomal oxidation of different xenobiotics.[5,10] Other reports have shown that MTZ may interfere with the cytochrome P-450 system.[11]

Modulation of liver microsomal oxidation might result in changes in the pattern of biotransformation and in the resultant pharmacokinetic profile of anthelmintically-active benzimidazole metabolites. This could lead to improved anthelmintic activity against gastrointestinal and tissue-dwelling parasites both in man and animals. The purpose of this study was to determine whether or not the administration of MTZ induces changes in the pharmacokinetic behaviour of NTB parent drug and its metabolites in sheep.

MATERIALS AND METHODS

Study design

Adult male Finn Dorset cross-bred sheep weighing 50–62 kg were used in this study. The animals were housed in parasite-free conditions in individual metabolism cages and given a pelleted sheep ration of 2000 g per day. Water was offered *ad libitum*. The health of all animals was monitored prior to and throughout the experimental periods. The treatments were given as follows.

First period. Four animals were treated with a zwitterion suspension of netobimin (150 mg ml^{-1}) (Sch-32481, Schering Plough, NJ, USA) by intraruminal (IR) administration at 20 mg kg^{-1}.

Second period. After a 4-week wash-out period, the same four animals received the zwitterion suspension of netobimin (150 mg ml^{-1}) by IR administration at 20 mg kg^{-1} together with an aqueous solution of methimazole (10 mg ml^{-1}) (2-mercapto-1-methyl-imidazole, Aldrich Chemical Co., WI, USA) given intramuscularly at 1·5 mg kg^{-1}, immediately after the NTB injection.

Due to the spurious results obtained for one animal (low NTB conversion) in the control group, three extra animals were treated with the zwitterion suspension of NTB by IR injection at 20 mg kg^{-1}. Since the results for these three extra animals were not statistically different from the original control group (three animals), they were included in the overall results of this study.

Blood samples (10 ml) were taken from the jugular vein in vacutainer tubes with sodium heparin (Becton Dickinson, Mississauga, Ont., Canada) prior to the treatments and at 0·5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 72, 96, and 120 h post-treatment. Plasma was separated by centrifugation at 3000 rev min^{-1} for 15 min, placed into plastic vials, and frozen at −20° until analysed.

Analytical methodology

After thawing, plasma samples (1 ml) were spiked with an internal standard (oxibendazole, 1 mg 10 ml^{-1} methanol) and NTB, and its metabolites extracted for further high performance liquid chromatographic (HPLC) analysis, using disposable C_{18} SepPak cartridges (part no. 51910, Waters Associates, MA, USA). Each cartridge was conditioned by washing with 5·0 ml HPLC grade methanol (Fisher Scientific, Montreal, Que., Canada) followed by 5·0 ml 0.017 M ammonium dihydrogen phosphate buffer pH 5·5. After application of spiked plasma, the cartridge was successively washed with 20 ml distilled water, 0·5 ml methanol (40 per cent), 0·4 ml methanol (100 per cent) and 2·5 ml methanol (100 per cent). NTB and ABZ metabolites eluted in the last 2·5 ml of methanol were concentrated to approximately 0·45–0·5 ml under a stream of nitrogen and refrigerated until analysed by HPLC.

Standard solutions and extracted metabolites from unknown samples were quantified on an LKB Bromma HPLC system (LKB, Bromma, Sweden) using an autosampler (LKB, model 2153), Bondex 10 μm C_{18} reverse phase column (Phenomenex, Torrance, CA, USA), LKB 2150 solvent delivery pumps, and an LKB spectral variable wavelength absorbance detector (model 2140) reading at 292 nm (ABZ metabolites) and 320 nm (NTB). The mobile phase was an acetonitrile/0·025 M ammonium acetate gradient in the following proportions: 28:72 (9 min), 42:58 (15 min), and 28:72 (9 min). The flow rate was 1 ml min^{-1}.

Identification of NTB, ABZ, ABZSO, and $ABZSO_2$ was undertaken by comparison with the retention time of the pure reference standards (supplied by Schering Plough, Kenilworth, NJ, USA). Calibration curves for each metabolite were determined. Linear regression lines for each analyte, in the range of 0·02 to 3 μg ml^{-1} (triplicate determinations), showed correlation coefficients between 0·980 and 0·992. Unknown concentrations were calculated by comparison of

each metabolite and internal standard peak area using Nelson Analytical software, model 2600, version 3·0 (Nelson Analytical Inc., Cupertino, CA, USA) on an IBM-XT computer.

The limits of detection (μg ml^{-1}) were as follows: 0·040 (NTB), 0·020 (ABZ and ABZSO), and 0·025 ($ABZSO_2$). There was no interference by MTZ or 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.[12] The equation below was used to describe the biexponential plasma concentration curves:[13]

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

where C_p = plasma concentration at time t after administration (μg ml^{-1}); B = concentration at time zero extrapolated from the elimination phase (μg · ml^{-1}); e = base of the natural logarithm; β = terminal slope obtained by linear regression analysis (h^{-1}); and k is the rapid slope obtained by feathering, which represents either the first order absorption rate constant (k_{ab}) or the first order metabolite formation rate constant (k_f) (h^{-1}). The resulting fits had determination coefficient (R^2) values of $>0·9$.

The elimination half-life ($t_{½\beta}$) and absorption ($t_{½ab}$) or metabolite formation half-lives ($t_{½\ for}$) 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 for each metabolite. The zero (AUC) and first moment (AUMC) area under the plasma concentration–time curves for NTB and its metabolites were calculated by trapezoidal rule[14] and further extrapolated to infinity by dividing the last experimental plasma concentration by the terminal slope (β). The mean residence time (MRT) was obtained as follows:[15]

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

Statistical analysis

Student's t-test for unpaired observations was used to test the significance of differences between mean pharmacokinetic parameters obtained after each treatment. A value of $p < 0·05$ was considered significant. The pharmacokinetic parameters are expressed as mean ±SEM.

RESULTS

Only the occasional detection of very low plasma levels of NTB parent drug and ABZ, in some of the animals that received NTB intraruminally alone, precluded pharmacokinetic analysis in these animals. Because of this, only three animals could be included in the pharmacokinetic results for these two analytes in the control group. The parent NTB compound was detected in plasma from 0·5 to 12 h after both treatments. There was no statistical difference between treatments for any of the NTB pharmacokinetic parameters calculated.

The mean plasma concentrations of ABZ, ABZSO, and $ABZSO_2$ obtained after the IR administration of NTB zwitterion suspension in the presence or absence of MTZ are plotted in Figure 1(a), (b), and (c), respectively. Pharmacokinetic parameters for ABZ after both treatments are shown in Table 1. The coadministration of MTZ with NTB significantly increased ($p < 0·05$) the MRT, AUC, and AUMC for ABZ.

The pharmacokinetic analyses for ABZSO and $ABZSO_2$ obtained after the IR administration of NTB with and without MTZ are summarised in Table 2. The presence of MTZ resulted in pronounced changes in the pharmacokinetic behaviour of ABZSO and $ABZSO_2$. Highly significant differences were obtained in terms of $t_{½\beta}$, AUC, AUMC, and MRT for both metabolites. Also $t_{½}$ (for) and t_{max} for $ABZSO_2$ were significantly longer when NTB was coadministered with MTZ.

DISCUSSION

After both treatments, the parent NTB compound showed a similar pharmacokinetic pattern with fast absorption, early C_{max} and rapid disappearance from plasma. The low NTB plasma AUCs may indicate an efficient conversion of this pro-drug into ABZ. ABZ has not previously been detected in plasma after the administration of NTB orally and subcutaneously in cattle[3,4] or after the oral administration of ABZ itself to sheep[16] and cattle.[17] Low ABZ plasma concentrations were detected in three animals of the control group of this trial that received NTB IR at 20 mg kg^{-1}, resulting in a low AUC. Both the apparent absence of ABZ in plasma or the failure to detect very low concentrations and the early appearance of ABZSO in plasma may be attributed to a first-pass oxidation in the liver by which ABZ is rapidly converted into ABZSO after its gastrointestinal absorption. It is also possible that ABZ could be partly oxidised (into ABZSO) in the digestive tract before absorption. However, the coadministration of NTB with MTZ resulted in a notably improved pharmacokinetic profile for ABZ. The significantly longer ($p < 0·05$) MRT and significantly higher AUC (138 per cent) for ABZ would indicate MTZ interference in the enzymatic pathway responsible for ABZ sulphoxidation. These pharmacokinetic differences would have been even greater if ABZ could have

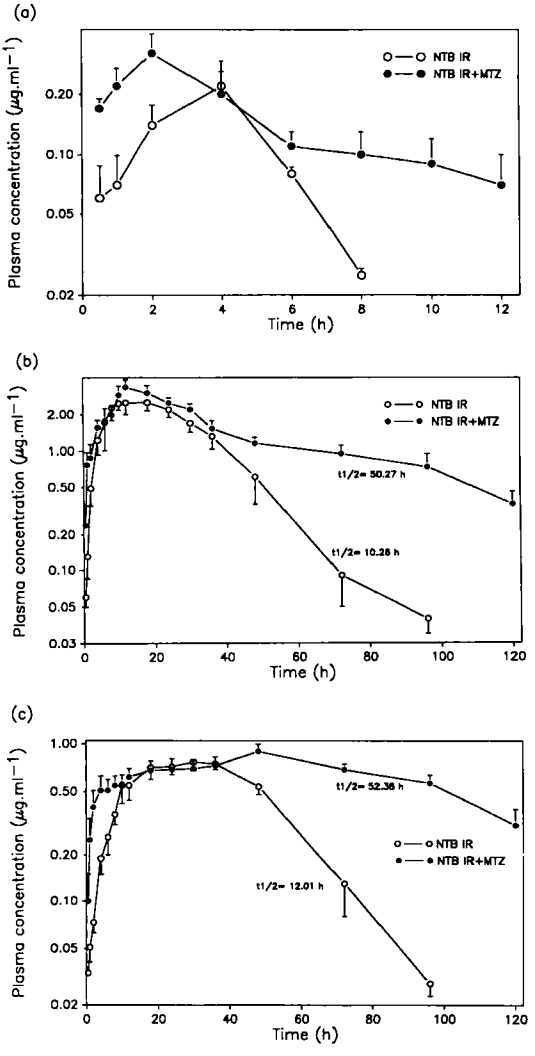

Figure 1. Mean plasma concentrations ±SEM of albendazole (a), albendazole sulphoxide (b), and albendazole sulphone (c) following the intraruminal administration of netobimin (NTB) zwitterion suspension (20 mg kg^{-1}) either alone or with methimazole (MTZ) (IM, 1·5 mg kg^{-1}) to sheep

been measured in all the animals of the control group (three animals were not included in the pharmacokinetic analysis of ABZ because this analyte was not detected in plasma).

As is apparent in the plot of ABZSO levels (Figure 1(b)) and from the pharmacokinetic results in Table 2, the concomitant treatment of NTB with MTZ caused dramatic changes in the disposition kinetics of ABZSO. ABZSO is, perhaps, the most relevant metabolite in terms of the clinical efficacy of NTB.

Table 1. Pharmacokinetic parameters for albendazole obtained after intraruminal (IR) administration of netobimin (20 mg kg^{-1}) either alone or with methimazole (MTZ) (IM, 1.5 mg kg^{-1}) to sheep

Parameter	Albendazole	
	IR alone	IR + MTZ
$AUC_{0\to\infty}$ ($\mu g.h\ ml^{-1}$) †	0·87 ± 0·17	2·07 ± 0·61*
AUMC ($\mu g.h^2\ ml^{-1}$)	3·51 ± 0·51	7·58 ± 1·64*
MRT (h)	2·76 ± 0·10	5·14 ± 1·05*
C_{max} ($\mu g\ ml^{-1}$)	0·21 ± 0·05	0·33 ± 0·09
t_{max} (h)	3·33 ± 0·67	2·50 ± 0·50

Values are expressed as means ±SEM with $n = 3$ for the IR alone treatment, and $n = 4$ for the IR + MTZ treatment.
*Statistically different from the IR alone treatment at $p < 0·05$.
† AUC extrapolated to infinity.

Table 2. Pharmacokinetic parameters for albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) obtained after IR administration of netobimin (20 mg kg^{-1}) either alone or with methimazole (MTZ) (IM, 1·5 mg kg^{-1}) to sheep

Parameter	ABZSO		$ABZSO_2$	
	IR alone ($n = 6$)	IR + MTZ ($n = 4$)	IR alone ($n = 6$)	IR + MTZ ($n = 4$)
$t_{½}$ for (h)	6·03 ± 0·81	4·83 ± 1·00	8·17 ± 0·62	14·21 ± 3·08*
$t_{½\beta}$ (h)	10·26 ± 1·02	50·27 ± 10·67***	12·01 ± 0·95	52·38 ± 12·60***
$AUC_{0\to lev}$ ($\mu g.h\ ml^{-1}$)†	98·69 ± 15·43	157·83 ± 18·87*	37·38 ± 3·29	76·94 ± 4·85***
$AUC_{0\to\infty}$ ($\mu g.h\ ml^{-1}$)‡	99·45 ± 15·67	193·92 ± 32·50*	37·89 ± 3·29	110·18 ± 8·79***
AUMC ($\mu g.h^2\ ml^{-1}$)	2749 ± 652	14328 ± 4407*	1352 ± 184	11961 ± 2804**
MRT (h)	17·63 ± 1·95	62·10 ± 11·58**	23·66 ± 1·66	84·37 ± 19·08**
t_{max} (h)	3·26 ± 0·52	3·91 ± 0·45	0·86 ± 0·08	0·95 ± 0·06
C_{max} ($\mu g.ml^{-1}$)	16·33 ± 3·12	17·50 ± 4·50	25·00 ± 2·86	39·00 ± 5·74*

Values are expressed as means ± SEM
*Significantly different from the IR alone treatment at $p < 0·05$.
**Significantly different from the IR alone treatment at $p < 0·01$.
***Significantly different from the IR alone treatment at $p < 0·001$.
† AUC from time of administration to the last experimental value (lev).
‡ AUC extrapolated to infinity.

A markedly different terminal slope (β) resulted in an elimination half-life four-fold longer ($p < 0·001$) for this active metabolite following the coadministration of MTZ. While there was no difference in terms of C_{max} and t_{max}, the overall ABZSO MRT was 252 per cent longer ($p < 0·01$) and the AUMC and AUC were significantly higher ($p < 0·05$) when MTZ was coadministered with NTB.

MTZ also induced significant modifications in the pharmacokinetic behav-

iour of the $ABZSO_2$ metabolite, resulting in significantly longer $t_{½\beta}$ and MRT and significantly higher AUC and AUMC in comparison with the NTB treatment alone.

The FMO system, in an NADPH-dependent reaction, is of primary importance in the conversion of ABZ into ABZSO by sheep[5,18] and cattle[18] liver microsomes. Thermal inactivation of the FMO system has been shown to significantly reduce the NADPH consumption of MTZ and thiourea, two well-characterised FMO substrates, as well as that of ABZ.[18] *In vitro*, MTZ drastically inhibits oxidation of ABZ into ABZSO by sheep and cattle liver microsomes.[18] Thus, it is likely that the observed modifications in the pharmacokinetics of these metabolites are due to substrate competition between MTZ and ABZ for the FMO pathway. Thus, a reduced rate of oxidation could attenuate the liver first-pass phenomenon, resulting in an increased plasma pharmacokinetic profile for ABZ. Delayed ABZ oxidation could result in an extended time of ABZSO formation which would explain the substantial modifications observed in the disposition kinetics of this metabolite. Furthermore, ABZSO has been shown to be efficiently reduced back to ABZ in the gastrointestinal tract of sheep.[19] This may also contribute to extending the residence time of these metabolites in the presence of MTZ.

It has been shown that the biotransformation of MTZ might produce some reactive metabolites which may bind to a cytochrome P-450 drug-binding site, temporarily decreasing its enzymatic activity.[11] This pathway has been reported as being responsible for the transformation of ABZSO into $ABZSO_2$.[7] Therefore, the substantial changes in the disposition and plasma concentrations of ABZSO could also be related to the effect of MTZ on the P-450 system. Such an effect would explain the significant increase in ABZSO AUC, $t_{½\beta}$ and MRT, and is consistent with the significantly longer ($p < 0{\cdot}05$) $t_{½}$ for and delayed t_{max} ($p < 0{\cdot}05$) obtained for $ABZSO_2$ in presence of MTZ. However, the disposition of the $ABZSO_2$ metabolite is formation rate-dependent and the kinetic modifications more likely reflect the changes in ABZSO disposition induced by MTZ-impairment of the FMO system.

Additionally, (+) and (−) ABZSO enantiomers have been recently identified in plasma of different species treated with ABZ.[20,21] While the cytochrome P-450 system has been related to the formation of the (−) enantiomer, the FMO system would be involved in the production of the (+) ABZSO enantiomer, which accounts for approximately 86 per cent of the total ABZSO plasma AUC in sheep.[21] While it is more likely that MTZ reduced the relative proportion of the (+) enantiomer by competition for the FMO system, changes in the enantiomer ratio produced by an MTZ-mediated P-450 impairment might also be expected. The relative biological activity of both enantiomers and their rate of metabolic reduction into the more potent ABZ thioether in the gastrointestinal tract remain to be determined.

The improved pharmacokinetic profile and residence time for the anthelmintically-active ABZ and ABZSO metabolites, obtained after the coadministration

of MTZ with NTB, may be highly important in terms of clinical efficacy. It could be expected to enhance anthelmintic efficacy. This could be an interesting approach to the design of new strategies for the control of filarial nematodes in man and animals, an unsolved problem in antiparasite therapy.

In conclusion, we have demonstrated that the pharmacokinetic interaction between MTZ and NTB in sheep results in substantial changes in the plasma profiles and disposition kinetics of NTB metabolites. However, the implications of this interaction on human and veterinary parasite control need further evaluation.

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