

## ORIGINAL PAPER

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## Pharmacokinetics of netobimin and microsomal metabolism of albendazole in infected gerbils with *Echinococcus granulosus*

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**Abstract** A comparison of the pharmacokinetic profiles of netobimin (NTB), albendazole sulfoxide (ABZSO) and albendazole sulfone (ABZSO<sub>2</sub>) was performed in gerbils (*Meriones unguiculatus*) with intra-abdominal hydatidosis and in healthy gerbils. The infection was developed after peritoneal inoculation of protoscolices of *Echinococcus granulosus* from sheep. Plasma concentrations of NTB, ABZSO and ABZSO<sub>2</sub> were measured by HPLC after oral administration of 50 mg NTB kg<sup>-1</sup>. The results showed an incomplete biotransformation of NTB over the experimental time; and this increased in infected animals. ABZSO and ABZSO<sub>2</sub> pharmacokinetic profiles were unaffected and were similar in both non-infected and infected animals. Both hepatic and intestinal microsomal sulfoxidase activities were measured. Since infected gerbils induced hepatic activity and decreased intestinal activity, the total activity was not different in infected and non-infected animals. In summary, intra-abdominal hydatid disease affected the pharmacokinetic profile of NTB, but ABZSO and ABZSO<sub>2</sub> plasma concentrations were not different in infected and non-infected gerbils.

### Introduction

Benzimidazoles are broad-spectrum drugs used against liver flukes, tapeworms and both lung and gastrointestinal roundworms. The benzimidazoles show limited water solubility and small differences in solubility may have a major influence on their absorption and clinical efficacy.

A way to overcome these problems is to use prodrugs, such as netobimin (NTB), *N*-methoxycarbonyl-*N'*-(2-nitro-5-propylphenylthio)-*N''*-(2-ethyl sulfonic acid) guanidine (Lanusse et al. 1993). NTB is an inactive soluble prodrug, which is reduced and cyclised to albendazole (ABZ) by the gastrointestinal flora (Delatour et al. 1986). The ABZ thus formed is oxidised to albendazole sulfoxide (ABZSO) and then to albendazole sulfone (ABZSO<sub>2</sub>). The activity is mainly due to ABZ and ABZSO (Lanusse and Prichard 1993; McKellar et al. 1993).

Most pharmacokinetic studies have been performed in healthy animals, in which the potential for parasitism to induce changes in the pharmacokinetic behaviour has not been considered (Lanusse and Prichard 1993). Different parasitic infections can modify the host's ability to metabolise drugs by altering the microsomal enzymatic system, which has physiological, toxicological and pharmacological consequences in the infected host and affects drug efficiency (Tekwani et al. 1988; Calleja et al. 1997).

Secondary hydatid disease was developed as an animal model to study this parasitic infection and its chemotherapy. The gerbil (*Meriones unguiculatus*) has been described as a suitable experimental animal to reproduce the hydatid disease from *Echinococcus granulosus* metacestodes (Schwabe et al. 1970; Thompson 1976). In order to investigate the effect of parasitism on the pharmacokinetic behaviour of NTB and its metabolites, gerbils with secondary hydatid disease were treated with NTB. The albendazole sulfoxidase in vitro activity was compared in liver and intestinal microsomes from non-infected and infected gerbils.

### Materials and methods

#### Chemicals

NTB was supplied by Schering-Plough (Madrid, Spain). ABZ, ABZSO and ABZSO<sub>2</sub> were supplied by SmithKline & Beecham (Madrid, Spain). The mebendazole (MBZ) used as internal standard was supplied by Lab. Dr. Esteve (Barcelona, Spain).

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## Animals

Female gerbils (*Meriones unguiculatus*;  $65 \pm 5$  g) were infected by intraperitoneal inoculation of 1,200–1,500 protoscolices of *Echinococcus granulosus* obtained from infected offal of slaughtered sheep (García-Llamazares et al. 1997).

## Pharmacokinetic study

Fifteen non-infected gerbils and fifteen gerbils with a demonstrable abdominal distension consistent with hydatid disease (Fig. 1) were treated with 50 mg NTB  $\text{kg}^{-1}$ . Treatments were administered as a single dose by gastric intubation. Blood samples were drawn by retro-orbital venous plexus puncture. To carry out the sample collection, gerbils were anaesthetised with ethyl ether. The samples were collected into heparinised Eppendorf tubes at 0.5, 1, 1.5, 2, 4, 6, 8 and 10 h after drug administration and were centrifuged at 600g for 30 min to obtain the plasma. The samples were stored at  $-20^\circ\text{C}$  until analysis.

## Microsomal metabolism

The sulfoxidase activity was measured in liver and intestinal microsomes from infected and non-infected gerbils. The microsomal fraction was obtained from liver (1 g; Redondo et al. 1998) and isolated enterocytes (Weisser 1973; Villaverde et al. 1995) which were homogenised with ice-cold 0.3 M sucrose (proportion 1:4). The homogenate was centrifuged at 12,000g for 10 min at  $4^\circ\text{C}$ , the supernatant fraction collected and centrifuged at 105,000g for 60 min at  $4^\circ\text{C}$ . The microsomal pellet was suspended in 100 mM phosphate buffer, pH 7.2, with 1 mM EDTA and 20% glycerol and stored at  $-80^\circ\text{C}$  until used for assays. The protein content was determined according to Lowry et al. (1951).

The sulfoxidase activity was assessed by the amount of ABZSO produced in the presence of a NADPH-regenerating system (Moroni et al. 1995). The incubation mixtures contained the microsomal fraction (1 mg protein) and ABZ as substrate (dissolved in dimethyl-sulfoxide, 1.5% final concentration) in a final volume of 1 ml. Incubations without microsomes were used as controls. The incubations were conducted in a shaking water bath at  $37^\circ\text{C}$  under aerobic conditions.

Hepatic sulfoxidase activity was characterised by the Michaelis–Menten constant in non-infected and infected gerbils. The ABZ concentration ranged over 5–50  $\mu\text{M}$  and the samples were incubated for 3 min. Preliminary incubations showed product formation to be linear over this reaction period.



**Fig. 1** Photograph of gerbil with secondary hydatid disease produced 9–12 months after intraperitoneal inoculation of protoscolices of *Echinococcus granulosus*

## Chromatographic analysis

The plasma aliquots (100  $\mu\text{l}$ ) were added in a propylene tube to 1 ml ethylacetate containing 0.2  $\mu\text{g}$  MBZ as internal standard. After being shaken for 15 min and centrifuged for 5 min at 7,000g, the organic phase was evaporated to dryness under a nitrogen stream. The residue was dissolved in 100  $\mu\text{l}$  methanol, shaken on a vortex and then analysed by HPLC (Redondo et al. 1998). The samples of microsomal incubations with internal standard were shaken for 2 min and centrifuged at 754g for 6 min at  $4^\circ\text{C}$ . The organic phase was evaporated to dryness under a nitrogen stream and the residue was dissolved in 100  $\mu\text{l}$  methanol for HPLC analysis.

NTB and ABZ metabolites were quantified by HPLC in reverse phase on a nucleosil C-18 column with a mobile phase composed of acetonitrile and deionised water under gradient conditions. Acetic acid (5%) was added to both solvents. The flow rate was 1  $\text{ml min}^{-1}$  and the absorbance detector was set at a wavelength of 292 nm. Under these chromatographic conditions, the retention times were 5.0 min for ABZSO, 5.8 min for NTB, 6.6 min for ABZSO<sub>2</sub>, 10.4 min for MBZ and 12.8 min for ABZ. Detection limits of the method were 0.05  $\mu\text{g ml}^{-1}$ .

## Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of NTB and its metabolites were analysed by a non-compartmental model (Gibaldi 1991), using PKCALC software (Shumaker 1986). The kinetic constants,  $V_{\text{max}}$  and  $K_m$ , were determined by the SIMFIT v.5.0 program (W.G. Bardsley, University of Manchester, U.K.).

Data are presented as the mean  $\pm$  S.D. of five determinations. All statistical calculations were based on analysis of variance (ANOVA). Whenever a significant F value was obtained, a Newman–Keuls multiple range test was performed to indicate the order of significance. Values of  $P < 0.05$  were considered statistically significant.

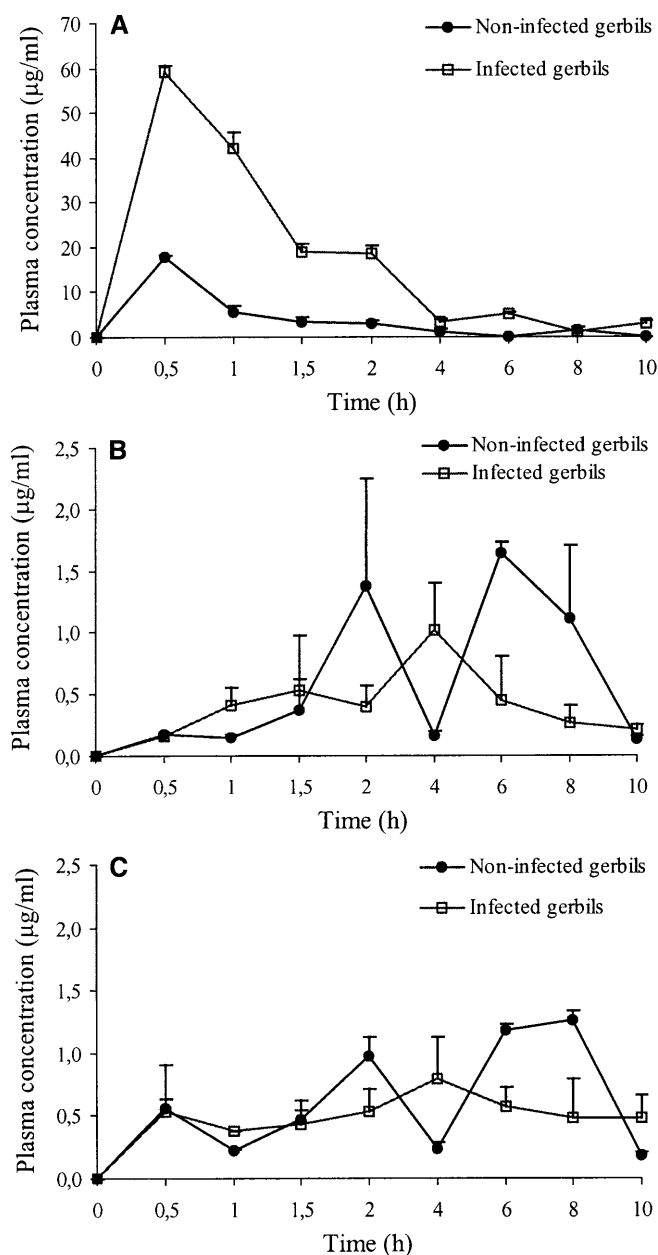
## Results

NTB, ABZSO and ABZSO<sub>2</sub> were detected in the plasma samples. As ABZ was undetected in plasma samples, the pharmacokinetic results refer to NTB, ABZSO and ABZSO<sub>2</sub>. Their plasma concentrations versus time obtained after oral administration of 50 mg NTB  $\text{kg}^{-1}$  in non-infected and infected gerbils are shown in Fig. 2. The pharmacokinetic parameters are presented in Table 1.

A high concentration of NTB can be observed in the plasma of gerbils at 0.5 h after administration. The area under the curve (AUC) and the peak plasma concentration ( $C_{\text{max}}$ ) of NTB were significantly increased in infected gerbils, whereas there were no significant differences in the ABZSO and ABZSO<sub>2</sub> values (Table 1).

The mean ratio of the AUC for ABZSO to the AUC for ABZSO<sub>2</sub> was calculated in infected and non-infected gerbils, to see whether parasitism affected oxidative metabolism (Table 1). The ratios were smaller in the infected than non-infected gerbils ( $0.55 \pm 0.06$  vs  $0.70 \pm 0.36$ ).

Figure 3 shows the sulfoxidase activity obtained in incubations with hepatic microsomes.  $V_{\text{max}}$  for sulfoxidation activity increased from  $1.56 \pm 0.08$   $\text{nmol min}^{-1} \text{mg}^{-1}$  protein in non-infected gerbils to  $1.78 \pm 0.05$   $\text{nmol min}^{-1} \text{mg}^{-1}$  protein in infected gerbils. The albendazole sulfoxidase affinity increased, since



**Fig. 2** Plasma concentration of netobimin (A), albendazole sulfoxide (B) and albendazole sulfone (C), following the oral administration of 50 mg NTB kg<sup>-1</sup> to non-infected and infected gerbils. Each point represents the mean  $\pm$  SD

$K_m$  decreased from  $19.44 \pm 2.15 \mu M$  to  $12.23 \pm 0.91 \mu M$ . The intrinsic clearance ( $cL_{int} = V_{max}/K_m$ ) is a measure of drug-metabolising activity. The results showed a  $cL_{int}$  value of  $0.081 \pm 0.09 \text{ ml min}^{-1}\text{mg}^{-1}$  in non-infected gerbils and  $0.146 \pm 0.011 \text{ ml min}^{-1}\text{mg}^{-1}$  in infected gerbils; and this value was significantly higher than in non-infected gerbils ( $P < 0.05$ ).

To assess differences in ABZ microsomal metabolism performed by hepatic and intestinal sulfoxidase activities, incubations were conducted with  $15 \mu M$  (ABZ concentration near to  $K_m$ ). Sulfoxidase activity increased significantly in the liver microsomes of infected gerbils ( $0.95 \pm 0.02 \text{ nmol min}^{-1}\text{mg}^{-1}$ ), compared to the activity in non-infected animals ( $0.67 \pm 0.06 \text{ nmol min}^{-1}\text{mg}^{-1}$ ). When sulfoxidation was studied in intestinal microsomes, this activity decreased significantly ( $0.24 \pm 0.04 \text{ nmol min}^{-1}\text{mg}^{-1}$  vs  $0.40 \pm 0.04 \text{ nmol min}^{-1}\text{mg}^{-1}$ ) in infected gerbils (Table 2).

## Discussion

Using NTB to treat gerbils with intra-abdominal secondary hydatid disease could be successful, since ABZSO reached therapeutic concentrations in the plasma. Morris et al. (1985, 1987) reported that effective ABZSO plasma concentrations were  $0.24\text{--}1.27 \mu\text{g ml}^{-1}$ . The biological activity of NTB is dependent upon its reduction by the gastrointestinal microflora, which may be a limiting factor. As the rumen microflora is extremely active, the systemic availability of ABZSO after oral administration of NTB is higher in ruminants than in monogastric species (Delatour et al. 1986; Baggot and McKellar 1994).

The appearance of NTB in the plasma indicated that the rate of metabolism of NTB was not sufficiently complete over the experimental time. Walchshofer et al. (1991) observed the same results in gerbil and mouse treated with *N*-methoxycarbonyl-*N'*-2-nitro-4-trifluoromethyl phenyl thiourea, another nitro-prodrug similar to NTB, but did not observe the prodrug in the plasma of sheep.

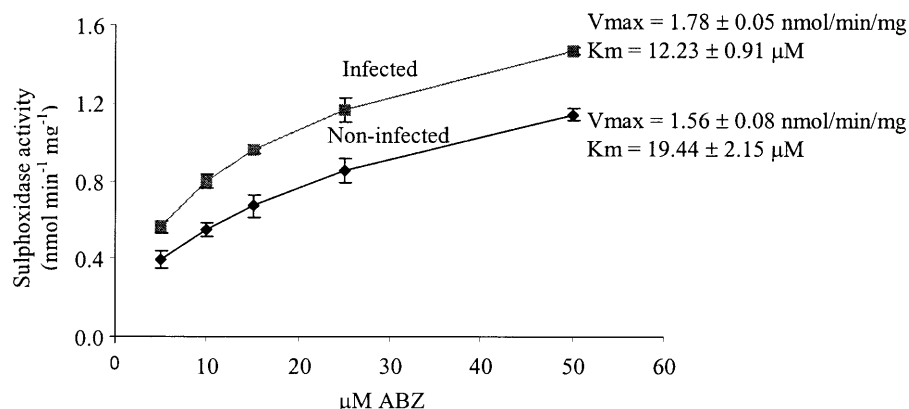
A significant increase in the AUC and  $C_{max}$  of NTB was observed in infected animals. This result could be due to a compression effect of hydatid cysts on the abdominal organs.

**Table 1** Pharmacokinetic data (mean  $\pm$  SD) corresponding to netobimin (NTB), albendazole sulfoxide (ABZSO) and albendazole sulfone (ABZSO<sub>2</sub>) after oral administration of 50 mg NTB kg<sup>-1</sup> to non-infected and infected gerbils. AUC Area under curve

	NTB		ABZSO		ABZSO <sub>2</sub>	
	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected
AUC <sub>0<math>\rightarrow</math><math>\infty</math></sub> (µg/h/ml)	28.01 $\pm$ 16.23	103.94 $\pm$ 10.54*	7.68 $\pm$ 1.97	6.35 $\pm$ 1.41	10.74 $\pm$ 7.99	12.78 $\pm$ 1.66
C <sub>max</sub> (µg ml <sup>-1</sup> )	17.91 $\pm$ 0.06	58.96 $\pm$ 1.75*	1.75 $\pm$ 0.18	1.02 $\pm$ 0.39	1.31 $\pm$ 0.17	0.82 $\pm$ 0.31
t <sub>max</sub> (h)	0.75 $\pm$ 0.29	0.75 $\pm$ 0.50	5.50 $\pm$ 2.52	4.00 $\pm$ 0.00	8.50 $\pm$ 2.52	3.50 $\pm$ 1.00*
AUC ABZSO/AUC ABZSO <sub>2</sub>	0.70 $\pm$ 0.36	0.55 $\pm$ 0.06				

\*Significantly different from non-infected gerbils ( $P < 0.05$ )

**Fig. 3** Sulfoxidase activity in hepatic microsomes from infected and non-infected gerbils. ABZ Albendazole



**Table 2** Sulfoxidase activity (nmol ABZSO min<sup>-1</sup> mg<sup>-1</sup> protein, mean ± SD, n = 5) observed in liver and intestinal microsomes

		15 µM ABZ
Liver microsomes	Non-infected	0.67 ± 0.06
	Infected	0.95 ± 0.02*
Intestinal microsomes	Non-infected	0.40 ± 0.04
	Infected	0.24 ± 0.04*

\* Significantly different from the respective non-infected group ( $P < 0.05$ )

There were no significant differences in ABZSO bio-transformation, since ABZSO and ABZSO<sub>2</sub> plasma profiles were unaltered by the parasitism. McKellar et al. (1991, 1993) did not find significant differences in ABZSO and ABZSO<sub>2</sub> pharmacokinetic variables between non-infected sheep and sheep infected with *Nematodirus battus* after treatment with NTB. In the ratio of ABZSO to ABZSO<sub>2</sub>, there was relatively more ABZSO<sub>2</sub> compared to ABZSO in infected gerbils, as was also observed by McKellar et al. (1993).

Regarding the effect of experimental parasitism on the activity of microsomal drug-metabolising enzymes, a significant decrease has been observed in the liver of infected animals (Tekwani et al. 1988; Calleja et al. 1997), but the increase of some monooxygenase activities has been described as a compensatory mechanism in infected rats (Biro-Sauveur et al. 1995). Our study shows that microsomal sulfoxidase activity in the small intestine of infected gerbils decreased, whereas it increased in the liver. Babany et al. (1985) reported a possible induction of hepatic P450 isoenzymes by endogenous substrates coming from altered intestinal metabolism, as result of a compression effect of hydatid cysts on the gastrointestinal tract.

The activity of liver microsomal sulfoxidase, and presumably liver sulfonase, in gerbil was higher than that reported in rat (Moroni et al. 1995) and sheep (Galtier et al. 1986) and are in agreement with the high P450 content in the hepatic microsomes of gerbil (Fenten and Fry 1991). The presence of cysts in the abdominal cavity produced a decrease in intestinal

microsomal sulfoxidation but induced hepatic sulfoxidation. This result may be a compensatory effect that can keep the ABZSO plasma levels steady in parasitised animals. However, additional investigations are required to improve the knowledge about the effect of parasitism on enzymatic systems of ABZ metabolism in different tissues, since it could increase bioavailability and efficiency against hydatid cysts which have variable locations in their host.

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