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Pharmacological assessment of netobimin as a potential anthelmintic for use in horses: Plasma disposition, faecal excretion and efficacy

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ABSTRACT

This study aimed to determine the plasma disposition and faecal excretion of netobimin (NTB) and its respective metabolites as well as the efficacy against strongyles in horses following oral administration. Netobimin (10 mg/kg) was administered orally to 8 horses. Blood and faecal samples were collected from 1 to 120 h post-treatment and analysed by high performance liquid chromatography (HPLC). Using a chiral phase-based HPLC, plasma disposition of ABZSO enantiomers produced was also determined. Faecal strongyle egg counts (EPG) were performed by a modified McMaster's technique before and after the treatment. Neither NTB nor ABZ were present and only albendazole sulphoxide (ABZSO) and sulphone metabolites ($ABZSO_2$) were detected in the plasma samples. Maximum plasma concentration of ABZSO (0.53 ± 0.14 μg/ml) and $ABZSO_2$ (0.36 ± 0.09 μg/ml) were observed at (t_{max}) 10.50 and 19.50 h, respectively following administration of NTB. The area under the curve (AUC) of the two metabolites was similar to each other. Netobimin was not detected, and ABZ was predominant in faecal samples. The maximum plasma concentration (C_{max}) of (−)ABZSO was significantly higher than (+)ABZSO, but the area under the curves (AUCs) of the enantiomer were not significantly different each other in plasma samples. The enantiomers of ABZSO were close to racemate in the faecal samples analyzed. Netobimin reduced the EPG by 100%, 100%, 77%, 80% and 75% 2, 4, 6, 8 and 10 weeks post-treatment, respectively. The specific behaviour of the two enantiomers probably reflects different enantioselectivity of the enzymatic systems of the liver which are responsible for sulphoxidation and sulphonation of ABZ. Considering the pharmacokinetic and efficacy parameters NTB could be used as an anthelmintic in horses.

1. Introduction

Netobimin (NTB) is a pro-drug of the benzimidazole sulphide, albendazole. It is used against liver flukes, tapeworms and gastrointestinal and lung nematodes in ruminants. Netobimin is converted into albendazole (ABZ) by gastrointestinal microflora following oral or intra-ruminal administration to sheep and cattle (Delatour et al., 1986; Lanusse and Prichard, 1990). Almost all of the ABZ absorbed from the intestine in ruminants is rapidly metabolized into its anthelmintically active albendazole sulphoxide (ABZSO) and inactive sulphone ($ABZSO_2$) metabolites by liver enzymes (Delatour et al., 1986, Fig. 1).

Sulphoxide benzimidazoles (ABZSO and oxfendazole-OFZ), which have a chiral centre about the sulphur atom, are formed as metabolites of sulphides and are metabolised into sulphones (Fig. 1). The sulphones are anthelmintically inactive, whereas sulphides and sulphoxides are both active (Lacey et al., 1987). The plasma dispositions of the two enantiomers of ABZSO and fenbendazole sulphoxide (FBZSO) have been investigated in many species after oral administration of pro-chiral ABZ and fenbendazole (FBZ) (Delatour et al., 1990, 1991a,b; McKellar et al., 2002; Goudah, 2003). Moreover the *in vitro* enantioselectivity of ruminal and intestinal flora in sulphoxidation of ABZ, sulphonation and sulphoreduction of ABZSO has been also shown by using the ruminal and intestinal fluids of sheep and cattle (Virkel et al., 2002).

Previous studies have reported that the gastrointestinal environment of equine species probably has a great reductive capacity for benzimidazole drugs (McKellar et al., 2002; Gokbulut et al., 2002, 2006a). It has been shown that the benzimidazoles licensed for use in horses in some countries (e.g. FBZ, OFZ and oxibendazole-OXB) have relatively poor bioavailability, since they are extensively metabolised to their inactive metabolites by gastrointestinal microflora and/or first-pass metabolism compared to ruminant

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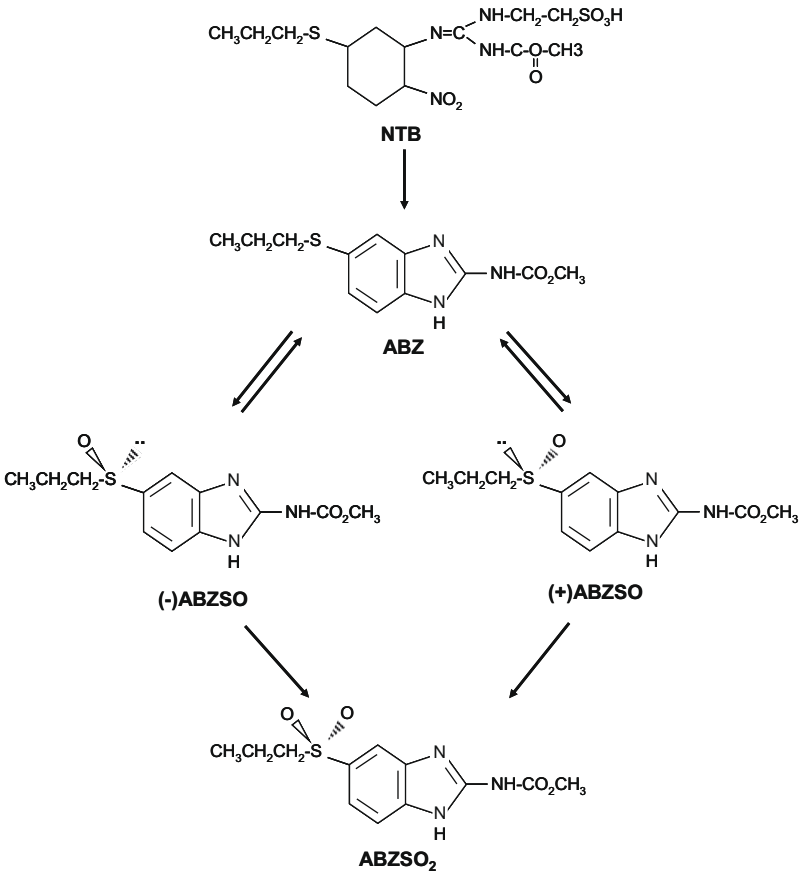

Fig. 1. Metabolic pathways of netobimin (NTB), albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$).

species. The detailed characterization of the kinetic behaviour and metabolic pathways of NTB, has markedly contributed to its optimized use in ruminants. However, there is no information available on the pharmacological behaviour or efficacy of NTB in equine species. Therefore, the aims of this study were to determine the plasma disposition and faecal excretion of NTB, and its metabolites (ABZ, ABZSO and $ABZSO_2$) including the enantiospecific production of ABZSO and to evaluate the efficacy against strongyles following oral NTB administration to horses.

2. Materials and methods

2.1. Experimental animals

Eight thoroughbred horses with a mean weight of 520 (516–550 kg) were used in the study. The animals had a history of grazing pasture contaminated with equine nematode parasites but were kept indoors and fed with hay and concentrated horse feed during the study. They had not been treated with anthelmintics during the previous three months. Water was provided *ad libitum* throughout the course of the study.

2.2. Treatments and sampling

A commercially available formulation of NTB (Hapadex, 15% oral suspension, Schering–Plough) was orally administered to animals at a dose rate of 10 mg/kg bodyweight in the study. Heparinized blood samples were collected by jugular venipuncture prior to drug administration and 1, 2, 4, 8, 12, 16, 20, 24, 32, 48, 56, 72, 96 and 120 h thereafter. Faecal samples (>5 g) were collected per rectum throughout the blood-sampling period, before drug administration and then at 4, 8, 12, 20, 24, 32, 48, 56, 72 and 96 h in order to determine faecal excretion of the NTB and its respective metabolites during the study. Blood samples were centrifuged at 3000*g* for 20 min and plasma was transferred to plastic tubes. All the plasma and faecal samples were stored at −20 °C until estimation of drug concentration.

2.3. Analytical procedures

Plasma concentrations of NTB, ABZ, ABZSO and $ABZSO_2$ were estimated by high performance liquid chromatography (HPLC) with a liquid–liquid phase extraction procedure adapted from that described by Marriner and Bogan (1980).

2.4. Extraction from plasma

Pure standard compounds of NTB (Schering Plough Ltd., Welwyn Garden City, UK), ABZ, rac-ABZSO and $ABZSO_2$ (SmithKline Beecham, West Sussex, UK) and internal standard OFZ were obtained from Sigma Chemical Co. (St Louis, USA). These were diluted with acetonitrile to give 0.5, 1, 2.5, 5, 10 and 10, 50, 500 μg/ml standard solutions for plasma and faecal samples, respectively for calibration as standard curves and to add to drug-free plasma and faecal samples to determine the recovery.

Drug-free plasma samples (1 ml) were spiked with standards of NTB, ABZ, ABZSO and $ABZSO_2$ to reach the following final concentrations: 0.05, 0.1, 0.5, 1 and 5 μg/ml. Ammonium hydroxide (100 μl, 0.1 N, pH 10) was added to 10 ml-ground glass tubes containing 1 ml spiked or experimental plasma samples. Oxfendazole (0.5 μg/ml) was used as an internal standard. After mixing for 15 s, 6 ml ethyl acetate was added. The sample tubes were