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Research Article

The Effect of Fasting on the Plasma Disposition of Albendazole in Goats

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Abstract

This study was designed to investigate the effect of fasting on the plasma disposition of albendazole (ABZ) in goats following oral administration. A total of 10 goats, aged 5-6 months were used in this study. The animals were allocated into two groups (fasted and fed groups) of five animals each. ABZ was administered orally to animals in two groups at 10 mg/kg bodyweight. Heparinize blood samples were collected between 1 h and 144 h after treatment and the plasma samples were analysed by high performance liquid chromatography for ABZ, active albendazole sulphoxide (ABZSO) and inactive albendazole sulphone ($ABZSO_2$) metabolites. ABZ is not detected and ABZSO and $ABZSO_2$ metabolites were present in the samples of fed and fasted animals. Feeding was significantly enhanced the plasma concentration of the ABZSO and $ABZSO_2$ metabolites. The area under the curve (AUC) and half-life (t1/2) of both metabolites were significantly larger and longer in fed compared to fasted animals, respectively. Moreover the maximum plasma concentration (C_{max}: 1.06±0.17 µg/ml) of ABZSO2 was also significantly higher in fed group compared with the fasting group (0.72±0.20 µg/ml). The changes in plasma kinetics, reflecting an altered quantitative gastrointestinal absorption or metabolism, were reflected in increased availability of ABZ metabolites in the plasma of fed goats. This could be a strategy to extend the exposure of parasites to the more active metabolite of ABZ and thus to improve the efficacy in goats.

Keywords: *Benzimidazoles, Albendazole, Pharmacokinetics, Fasting, Goat*

Keçilerde Albendazolün Plazma Dağılımına Aç Bırakmanın Etkisi

Özet

Bu çalışma keçilere albendazolün (ABZ) ağız yolu ile uygulanmasını takiben aç bırakmanın etkisini araştırmak amacı ile yapılmıştır. Çalışmada 5-6 aylık yaşta 10 keçi kullanılmıştır. Hayvanlar her grupta (aç ve tok) beş keçi olacak şekilde iki gruba ayrılmıştır. Her iki gruptaki hayvanlara ABZ 10 mg/kg dozda ağız yolu ile uygulanmıştır. Heparinize kan örnekleri ilaç uygulanmasından sonra 1 saat ile 144. saat arasında toplanmıştır ve plazma örnekleri ABZ, aktif albendazol sülfoksit (ABZSO) ve inaktif albendazol sülfon ($ABZSO_2$) metabolitleri için yüksek basınçlı sıvı kromatografisi ile analiz edildi. Aç ve tok hayvanlardan toplanan örneklerde ABZ tespit edilememiş, ABZSO ve $ABZSO_2$ metabolitleri bulunmuştur. Besleme ABZSO ve $ABZSO_2$ metabolitlerinin plazma konsantrasyonunu anlamlı şekilde artırmıştır. Beslenen gruptaki hayvanlarda her iki metabolitin eğri altı alanı (EAA) ve yarılanma ömrü (t1/2) anlamlı bir şekilde geniş ve uzun bulunmuştur. $ABZSO_2$'un doruk plazma yoğunluğu (Y_{doruk}: 1.06±0.17 µg/ml) beslenen gruptaki hayvanlarda, aç bırakılan gruptaki hayvanlardan (Y_{doruk}: 0.72±0.20 µg/ml) anlamlı birşekilde yüksek bulunmuştur. Nicel gastrointestinal emilim yada metabolizmada ki değişimleri yansıtan plazma kinetiğindeki değişimler beslenen keçilerin plazmasında ABZ metabolitlerinin yararlanımının arttığının göstergesidir. Bu durum keçilerde ABZ'un aktif metabolitlerine parazitin daha fazla maruz kalması ve etkinliğinin düzeltilmesi için bir strateji olabilir.

Anahtar sözcükler: *Benzimidazol, Albendazol, Farmakokinetik, Aç bırakma, Keçi*

INTRODUCTION

Goats are one of the most important food-producing animal species in developing countries. However, their sensitivity to gastrointestinal nematode infestations causes disease and economic loss. Available benzimidazole anthelmintic derivatives such as albendazole (ABZ) are frequently used in adult and young animals as a primary strategy for controlling gastrointestinal nematodes. ABZ (methyl [5-(propylthio)-1H-benzimidazol-2-yl] carbamate), is highly effective against all stages of gastrointestinal nematodes including lungworms, cestodes and adult liver flukes in cattle, sheep and goats at different dose rates [1]. ABZ is a poorly water-soluble drug (0.2 mg/ml in water

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at 25°C) and, consequently, it is poorly and erratically absorbed from the gastro-intestinal tract. This property which is ideal for its use against luminal infections is a problem in the treatment of systemic helminthiasis [2].

Following oral administration, ABZ absorbed from the gastrointestinal tract is rapidly metabolized into its pharmacologically active metabolite, albendazole sulphoxide (ABZSO) and inactive metabolite albendazole sulphone ($ABZSO_2$) by the liver [3,4]. It is thought that the flavine-containing monooxygenase (FMO) is mainly responsible for sulphoxidation, whereas cytochrome-dependent mono-oxygenase is responsible for sulphonation [5].

Host-related factors, including biotransformation and absorption, affect the kinetic behaviour and resultant clinical efficacy of benzimidazole compounds in animal [6,7]. The gastrointestinal passage rate of digesta is affected by alteration in the quality and quantity of the feed consumed and this could confer variable absorption time and therefore bioavailability of drugs. Because of its poor water solubility, formulation of ABZ is restricted to oral administration. After oral administration of benzimidazole anthelmintics to ruminants, greater bioavailability is observed than in monogastric species because in ruminants, the relatively slower gut transit time conferred by the fore stomach reservoir means that benzimidazoles are more extensively absorbed than in monogastric animals [8].

Reduction of feed intake resulted in increased plasma availability of oxfendazole, albendazole and triclabendazole in sheep and goats [6,8-10]. In cattle, it was demonstrated that fasting or restricted feed intake increased the relative bioavailability of ABZ metabolites [11] whereas in the dog [12] and horse [13] feeding increased the bioavailability of benzimidazoles after oral administration. However, to the best of our knowledge, there is no published report concerning the relationship among feed restriction and plasma disposition of ABZ in goats, the present study was designed to investigate the influence of fasting on the plasma disposition of ABZ in goats following oral administration

MATERIAL and METHODS

The study was approved by the Animal Ethics Committee of Adnan Menderes University (HEK/2006-0010). A total of 10 goats, aged 5-6 months and weighing 16-20 kg were used in this study and identified by specific name tags attached to their ears. The animals were appropriately housed in single pens. Water was supplied *ad libitum*.

Treatments and Sampling

For experiments, the goats were weighted and randomly allocated into 2 groups; fed and fasted, each with 5 goats. Both fed group and fasted group were fed *ad libitum* hay plus a small concentrate ration (5% fat content) but the animals in fasted group were not fed prior 24 h and 8 h after drug administration. Drug amounts to be administered to the animals were prepared from a commercial formulation (Valbazen-K tablet, 76 mg albendazole/tablet, Pfizer) for delivery at a single dose of 10 mg/kg bodyweight.

Heparinized blood samples (5 ml) were collected via the catheter before and 1, 2, 4, 8, 12, 16, 20, 24, 32, 48, 72, 96, 120 and 144 h post-treatment. Blood samples were centrifuged at 3000 g for 20 min and plasma was transferred to plastic tubes. All the plasma samples were stored at -20°C until analysis and there were analysed within 30 days after collection

Analytical Procedures

The analytical procedures involved two phases of measurements with high performance liquid chromatography (HPLC) following a liquid-liquid phase extraction procedure, as described by Marriner and Bogan [14].

A stock solution (100 µg/ml) of pure standard compounds of ABZ, ABZSO and $ABZSO_2$ (SmithKline Beecham, West Sussex, UK) were prepared using acetonitrile as the solvent. The resulting stock was then further diluted to produce 0.5, 1, 5, 10 and 25 µg/ml solutions which were later utilized for the purposes of calibration or determining the recovery of the drug-free plasma samples.

The drug-free plasma samples (1 ml) were spiked with standards of ABZ, ABZSO and $ABZSO_2$ to reach final concentrations: 0.05, 0.1, 0.5, 1 and 2.5 µg/ml. FBZ (10 µg/ml) was used as an internal standard for ABZ analysis. After mixing for 15 s, 5 ml ethyl acetate was added. The tubes were shaken on a slow rotary mixer for 10 min. After centrifugation at 3.000 g for 5 min, the organic phase (3.5 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 60°C in a sample concentrator (Maxi-dry plus, HetoLab. Equipment, Denmark). The dry residue was re-suspended with 250 µl mobile phase. Then the tubes were placed in an ultrasonic bath and finally, 35 µl of this solution was injected into the chromatographic system. For ABZ and metabolites, the mobile phase was a mixture of acetonitrile-water (93:07) to which glacial acetic acid was added (0.5%, v/v). It was pumped through the column (Macherey-Nagel, nucleosil C_{18} 4 µm, 250 mm x 4.6 mm, Duren, Germany) with nucleosil C_{18} guard column (Phenomenex, Cheshire, UK) in a linear gradient fashion changing from 93:07 (acetonitrile-water) to 70:30 for 12 min, 70:30 to 90:10 for 1.5 min and the last ratio (93:07) was maintained for 3.5 min. The flow rate was 1.1 ml/min. Samples were processed on a computerized HPLC system (1100 series, Agilent Technologies, GmbH, Germany) comprising a degasser, a quaternary pump (G1354A), an auto sampler (G1313), a column oven (G1316A) and diodearray detector (G1315B) set at 292 nm. The retention times were 7.58 min (ABZSO), 8.52 ($ABZSO_2$), 12.16 min (ABZ) and 12.77 min (FBZ).

Validation Procedures

The analytical method used for ABZ, ABZSO and $ABZSO_2$ in goat plasma was validated prior to the start of the studies. The analytes were identified with the retention times of pure reference standards. Recoveries of the three molecules under study were measured by comparison of the peak areas from spiked plasma samples with the areas resulting from direct injections of standards prepared in acetonitrile. The inter- and intra-assay precisions of the extraction and chromatography procedures were evaluated by processing replicate aliquots of drug-free goat plasma samples containing known amounts of the drugs on different days. Calibration graphs for three molecules were prepared (linear range 0.05-5.0 µg/ml). The slope of the lines between peak areas and drug concentrations were determined by least squares linear regression and showed correlation coefficient 0.999. The detection limits of the three molecules were established with HPLC analysis of blank plasma fortified with the standard, measuring the baseline noise at the retention time of the peak. The mean baseline noise at the peak retention time plus three standard deviations was defined as the detection limit (LOD). The mean baseline noise plus five standard deviations was defined as the limit of quantification (LOQ) [15].

Pharmacokinetics and Statistical Analysis of Data

The plasma concentration versus time curves of each molecules obtained after treatment in individual animal, were fitted with WinNonlin software program (version 5.2, Pharsight Corp., Mountain View, California, US). Pharmacokinetic parameters for each animal were estimated using non-compartmental model analysis extravascular input. The maximum plasma concentration (C_{max}) and time to reach maximum concentration (t_{max}) were obtained from the plotted concentration-time curve. The area under the curve (AUC) and mean residence time (MRT) from time zero to infinity were calculated by trapezoidal rule. Terminal half-life ($t_{1/2\lambda z}$) was calculated as:

$$t_{1/2\lambda z} = -\ln(2)/\lambda_z$$

where λ_z represents the first order rate constant associated with the terminal (log linear) portion of the curve.

The measurements for the above pharmacokinetic parameters were tabulated and statistically analyzed with Mann-Whitney U tests using a software package (SPSS ver. 17.0 for Windows - SPSS Inc., Chicago USA). Statistically significant differences were set at $P<0.05$.

RESULTS

The analytical method used to extract and quantify the plasma concentration of ABZ, ABZSO and $ABZSO_2$ by chromatographic analysis was validated before processing the experimental samples. This analysis yielded linear regression lines ranging from 0.05 to 5.0 µg/ml of ABZ, ABZSO and $ABZSO_2$ with a high correlation coefficient of 0.999. The limits of quantification (LOQ) of the assay were 0.02 for ABZ and 0.05 µg/ml for ABZSO and $ABZSO_2$. With six replicates of aliquots, the precision at each concentration level was lower than 15% of the coefficient of variation (CV), and the accuracy ranged from 85% to 98% for molecules. The inter-assay precision showed variation between 4.46 and 7.31%. The mean extraction recoveries were 75.4±6.3% for ABZ, 94.18±4.5% for ABZSO and 90.43±6.6% for $ABZSO_2$.

Clinically no adverse effects were observed in the goats following the oral administration of ABZ. After treatment ABZ were not detected parent molecule in plasma samples but ABZSO and $ABZSO_2$ metabolites were found over the detection limit level of fed and fasted animals. *Fig. 1* and *Fig. 2* shows the mean a (±SD) plasma concentration versus time curves and *Table 1* shows the mean (±SD) pharmacokinetic parameters of ABZSO and $ABZSO_2$. Feeding was significantly enhanced the plasma concentration of the sulphoxide and sulphone metabolite of ABZ. The area under the curve (AUC) and half-life ($t_{1/2}$) of both metabolites were significantly larger and longer in fed (AUC: 38.48±5.13

Fig 1. Comparative mean (n=5) plasma concentration profiles albendazole sulphoxide (ABZSO) in goats being offered of two different diets following an oral administration of albendazole to at 10 mg/kg bodyweight

Şekil 1. Keçilere Albendazolün 10 mg/kg dozda ağız yolu ile uygulanmasını takiben iki farklı diyet grubunda albendazol sülfoksitin (ABZSO) ortalama plazma konsantrasyonunun karşılaştırılması (n=5)

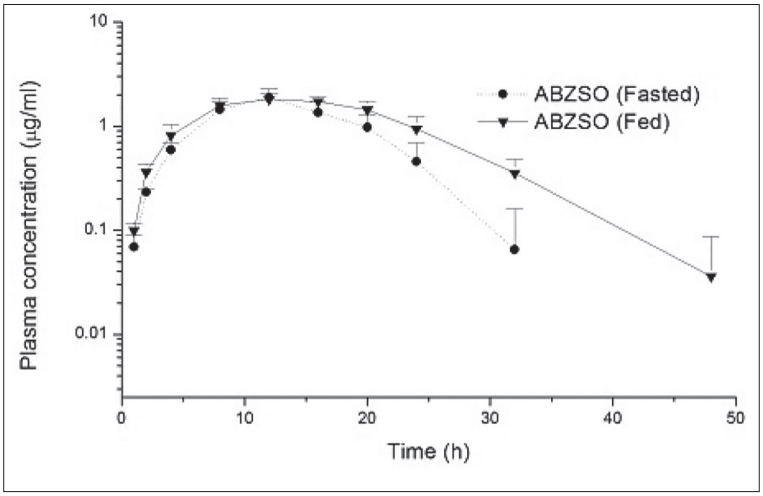

Fig 2. Comparative mean (n=5) plasma concentration profiles albendazole sulphone ($ABZSO_2$) in goats being offered of two different diets following an oral administration of albendazole to at 10 mg/kg bodyweight

Şekil 2. Keçilere Albendazolün 10 mg/kg dozda ağız yolu ile uygulanmasını takiben iki farklı diyet grubunda albendazol sülfonun ($ABZSO_2$) ortalama plazma konsantrasyonunun karşılaştırılması (n=5)

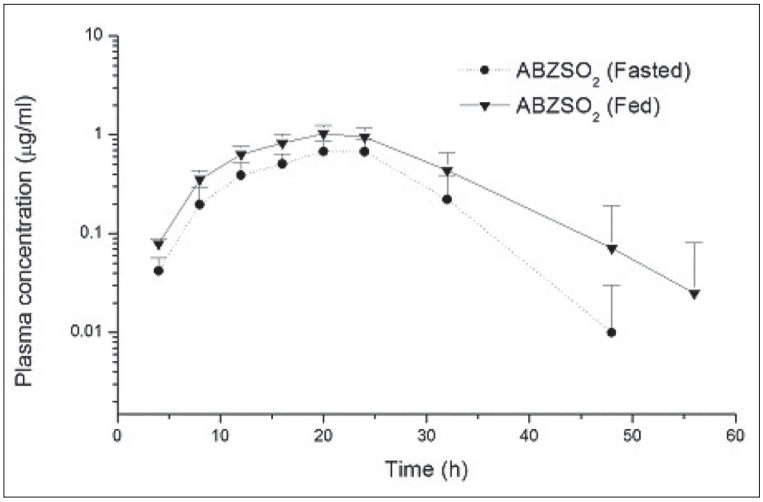

Table 1. *Mean (±SD) pharmacokinetic parameters of albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in plasma following an oral administration of albendazole (10 mg/kg) to fasted and fed goats*

Tablo 1. *Aç ve tok keçilere Albendazolün (10 mg/kg) ağız yolu ile uygulanmasını takiben Albendazol sülfoksit (ABZSO) ve Albendazol sülfonun ($ABZSO_2$) ortalama (±SD) farmakokinetik parametreleri*

Parameters	ABZSO		$ABZSO_2$	
	Fasted	Fed	Fasted	Fed
T_{max} (h)	11.20±1.79	13.60±2.19	22.40±2.19	21.60±2.19
C_{max} (µg/ml)	1.89±0.40	1.90±0.22	0.72±0.20*	1.06±0.17
AUC_{last} (µg.h/ml)	27.00±7.09*	38.48±5.13	11.29±3.95*	22.48±5.59
$t_{1/2\lambda z}$ (h)	3.78±1.11*	6.73±0.62	4.30±1.06*	5.70±0.45
$AUMC_{last}$ (µg.h²/ml)	363.22±118.3*	611.26±151.7	216.93±89.5*	511.07±256.5
MRT_{last} (h)	13.25±1.03*	15.90±1.68	18.64±1.97	22.21±4.84

T_{max}: *time to reach peak plasma concentration,* ***C_{max}:*** *peak plasma concentration,* ***AUC_{last}:*** *area under the (zero moment) curve from time 0 to the last detectable concentration,* ***$t_{1/2\lambda z}$:*** *terminal half-life,* ***$AUMC_{last}$:*** *area under the moment curve from time 0 to t last detectable concentration,* ***MRT_{last}:*** *mean residence time; *The values in fasted group are significantly different from the values in fed group for both ABZSO and $ABZSO_2$ ($P<0.05$)*

µg.h/ml, $t_{1/2}$: 6.73±0.62 h for ABZSO; AUC: 22.48±5.59 µg.h/ml, $t_{1/2}$: 5.70±0.45 h for $ABZSO_2$) compared to fasted (AUC: 27.00±7.09 µg.h/ml, $t_{1/2}$: 3.78±1.11 for ABZSO; AUC: 11.29±3.95 µg.h/ml, $t_{1/2}$: 4.30±1.06 h for $ABZSO_2$) animals, respectively. The maximum plasma concentration (C_{max}: 1.06±0.17 µg/ml) of sulphone metabolite was also significantly higher in fed group compared with the fasting group (C_{max}: 0.72±0.20 µg/ml). Moreover, the mean residence time (MRT: 15.90±1.68 h) of albendazole sulphoxide in fed group were significantly different from those of fast group (MRT: 13.25±1.03 h).

DISCUSSION

The pharmacokinetics, bioavailability and activity of benzimidazoles are particularly influenced by the physico-chemical properties of drug, route of administration, sex, age, metabolic pathways of the active molecules, diet type and quality and quantity of diet. It was indicated that variations in the quality and quantity of diet could affect the bioavailability of benzimidazole anthelmintics in different animal species [8-11,16-20]. In ruminants, administration of benzimidazoles following a period of feed restriction greatly improves their bioavailability and this is thought to be associated with reduced absorbance to food and reduced reticulo-ruminal turnover and thus slower gut transit [10]. In addition, Singh et al.[20] observed reduced sulphoxide metabolite of albendazole in sheep fed compared to fast animals. The findings of the present study in goats differ substantially from those previous studies. In this study, the availability of ABZSO and $ABZSO_2$ in the plasma samples of fed goats was markedly greater compared to those of fasted goats, respectively. The C_{max} and AUC values of ABZ metabolites (ABZSO and $ABZSO_2$) in fed group are significantly higher and larger compared with those in fasted group. The apparent increased absorption in unfed goat is perhaps surprising since goats might be expected to behave like ruminants. The findings of the present study were suggested that feeding may delay gastric emptying in the goats thus slowing gut transport and improving absorption. These differences may be associated with prolonged absorption time of the drug from fed group compared with the fasted group. The physical form and quality of diet may affect the digestibility, flow rate into the gastrointestinal tract, the binding ability of content to the drug and the quality,

quantity and reductive capacity of the gastrointestinal microflora [21].

In the groups of this study, inter individual variations were observed in the plasma pharmacokinetic parameters. The origin of these variations was unclear. The animals were clinically healthy, possible chronic renal/hepatic problems may have contributed to these variations; since no biochemical tests were performed in the animals before starting the experiments in the present study. Albendazole, fenbendazole, triclabendazole and the pro-benzimidazoles (febantel and netobimin), which are commercially available thioether and sulphide benzimidazoles; commonly undergo microsomal sulphoxidation in liver. They are reversibly metabolised to their sulphoxide derivatives [13,22-24]. Irreversible sulphonation follows sulphoxidation and is a slower oxidative step resulting in a sulphone metabolite [25]. Sulphide and sulphoxide benzimidazoles are known to bind nematode tubulin [26] and therefore have activity against nematodes although sulphides exert inhibitory activity on tubulin at lower concentrations than sulphoxides.

In the present study, the parent ABZ was not determined, and ABZSO and $ABZSO_2$ were the main metabolites detected in the plasma samples during the experiment period following oral administration of ABZ. It is known that ABZ was rapidly and almost completely converted to ABZSO and $ABZSO_2$ in ruminant species. Although xenobiotic metabolism occurs mainly in the liver, extra-hepatic metabolism including gastrointestinal tract are also involved. The reductive environment of the gastro-intestinal tract of the ruminants could contribute to the metabolic reduction of the benzimidazole drugs. The reduction of netobimin into albendazole and oxidation of albendazole into albendazole sulphoxide were shown in an artificial rumen [27] and in the in vitro ruminal and intestinal fluids of sheep and cattle [28]. Furthermore, it was reported that the metabolism of oxfendazole to sulphide (fenbendazole) occurred in ruminal fluid in sheep and cattle [29].

In recent years, anthelmintic resistance to broad spectrum chemotherapeutic agents including benzimi-dazoles has become an increasing problem in domestic animals including goats, sheep, cattle, swine and horses throughout the world. Generally, irreversible resistance develops in helminths, usually within 5 years of introduction of the anthelmintic drugs [30]. There are many reports indicating that anthelmintic resistance to important nematodes in ruminants is emerging in many countries [31]. More intensive or often treatments with decreasing anthelmintic efficacy are likely to be served, which could increase the intensity the resistance problem. The progressive decline in the anthelmintic efficacy of BZD drugs because of the development of resistance in sheep and goats has forced to search new strategies: technics to increase efficacy of drugs available. Decreased drug availability in the systemic circulation and/or target tissues, resulting in a subtherapeutic exposure of those helminths having resistant genes, may facilitate the progressive development of anthelmintic resistance [32]. Modifying of diet before or after drug administration has been recommended to enhance the plasma concentration of active compounds thus improve the anthelmintic efficacy of those compounds and delay the development of anthelmintic resistance. It has been indicated that feed restriction caused increasing of the plasma levels of oxfendazole in sheep accounted for elevated efficacy of the drug against BZD-resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* [7,33].

It is concluded that the availabilities of ABZSO and $ABZSO_2$ in the plasma samples of fed goats were markedly greater than in those fasted goats. The changes in plasma kinetics, reflecting an altered quantitative gastrointestinal absorption or metabolism and increased availability of ABZ metabolites in the plasma of fed goats. This could be a strategy to extend the exposure of parasites to the more active metabolite of ABZ and thus to improve the efficacy and to delay the development of anthelmintic resistance in goats.

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