

A Bioequivalence Study of an Albendazole Oral Suspension Produced in Iran and a Reference Product in Sheep

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ABSTRACT

In a parallel design, a single oral dose of albendazole (ABZ), 5 mg/kg, was administered to 2 groups of 8 sheep to study the bioequivalence of an ABZ oral suspension produced in an Iranian pharmaceutical company and a reference product (Valbazen[®], Pfizer Inc.). A third group of 8 sheep without dosing was used as control. Blood samples of all groups were collected at specified times within 0-72 hours post-dosing. The serum levels of albendazole sulphoxide (ABZ-SO), the main metabolite of ABZ, were determined using a high performance liquid chromatographic method with ultraviolet detection. Peak areas were used for calculating ABZ-SO concentrations and ABZ-SO pharmacokinetic parameters obtained using non-compartmental analysis. Statistical analysis of data pointed out that there were significant differences between the area under the concentration-time curve and peak serum concentration of these products, although there was no significant difference in their time to peak serum

concentration and biologic half-life. It was concluded that these products were not bioequivalent; more studies are needed for evaluating the bioequivalence of veterinary drugs produced in Iran.

INTRODUCTION

Albendazole (ABZ) is a benzimidazole derivative with a broad-spectrum anthelmintic activity that is widely used in veterinary and human medicine.^{1,2} Since 1989, ABZ has been used as a drug of choice for strategic treatment of gastrointestinal (GI) nematodes (at 5 mg/kg) as well as liver flukes and GI cestodes (at 10 mg/kg) for 75 million sheep and goats in Iran. Preliminary comparisons between the anthelmintic efficacy of oral ABZ suspension products obtained from Iranian pharmaceutical companies and those of external sources revealed that the clinical effects of some Iranian products were lower than those of external ones (Eslami, personal communication).

Because of the importance of this drug in veterinary medicine and the lack of reports for blood-level bioequivalence studies on ABZ products in Iran, the present investigation was carried out.

Albendazole is poorly absorbed from the GI tract. This property, which is ideal for its use against luminal parasitic infections, is a problem in the treatment of systemic diseases.³ After oral administration of ABZ, it is metabolized rapidly to a pharmacologically active metabolite, albendazole sulfoxide (ABZ-SO), and it constitutes the main part of drug in blood.^{1,4,5} Because the first-pass metabolism for ABZ is extensive and ABZ serum level is negligible after its oral dosing in sheep,^{1,5} the kinetic profile of ABZ-SO was used for comparison of bioavailability of 2 oral ABZ products in the present study.

MATERIALS AND METHODS

Animals

Twenty-four parasite-free local sheep (35-45 kg) were randomly divided into 3 equal groups. Group I and II received a single dose (5 mg/kg) of oral ABZ suspension product obtained from an Iranian pharmaceutical company (ABZ-test) and from Pfizer Inc. (Valbazen[®], 2.5%; ABZ-reference), respectively. The third group received no treatment and was used as control. The animals were kept indoors and fed high-quality alfalfa hay. Water was provided ad libitum. The experimental protocol was done under internationally accepted animal welfare guidelines.

Sample Collection

Blood samples (5 mL) were taken from the jugular vein of each individual animal, before and at 3, 6, 9, 12, 15, 18, 24, 48, and 72 hours after ABZ administration. Their serum samples were collected and stored at -20° C until analyzed by high performance liquid chromatography (HPLC).

Analytical Procedure

Extraction of serum samples and chromatographic procedures were performed according to Mirfazaelian et al⁶ with some modifications. A liquid-phase extraction was carried out by shaking 0.5 mL serum with 5.0 mL ethyl acetate for 5 minutes. The organic phase was then evaporated to

dryness at 40° C under gentle flow of nitrogen. The residue was dissolved in 0.5 mL of HCl, 0.001 M. It was further cleaned by washing with 5.0 mL n-hexane for 5 minutes. The organic phase was discarded and the samples were re-extracted with ethyl acetate (as above) after alkalization with 1.0 mL NaOH, 0.01 M. The organic phase evaporated to dryness as described; the residue was re-dissolved in 300 µl of the HPLC mobile phase, and 10 µl was injected onto the HPLC system.

The HPLC system consisted of a solvent delivery system (Knauer, Germany), a Nucleosil-100, 5 µm, 250 × 4.0 mm ID, C8 column (Macherey-Nagel, Germany), preceded by an RP-C18 precolumn, a UV detector (Waters, USA), and a data acquisition and processing system including software (Autochro-Win Chromatography Data System, Young Lin Instrument Co. Ltd., Korea). Acetonitril:acetic acid:water (30:10:60) was used as the mobile phase with a flow rate of 0.8 mL/min. The metabolite ABZ-SO was detected with a UV detector at 286 nm.

Identification of ABZ-SO peak was undertaken by comparison with retention time of pure reference standard, with 97.3 % purity (supplied by Pfizer Inc., Belgium). Calibration curve for spiked ABZ-SO in serum, in the concentration range of 0, 100, 200, 300, 400, 500, 750, 1000, 1250, 1500, 2000, and 2500 ng/mL, was determined. Unknown serum levels of ABZ-SO were quantified by comparison of ABZ-SO peak area with a calibration curve formula using Microsoft Office Excel 2003 software.

Pharmacokinetic Analysis

The serum concentration vs time curve for each individual animal was fitted with a drug-kinetic computer program.⁷ Pharmacokinetic parameters were determined using non-compartmental analysis. Peak serum concentration (C_{max}) and time to peak serum concentration (T_{max}) values were obtained from observed data on the ABZ-SO serum concentration-time curve for each

individual animal. Elimination or terminal rate constant (k) was calculated from the terminal portion of the serum concentration-time curve using least-square regression analysis of the logarithm of concentration versus time.^{8,9} Biologic half-life ($T_{1/2}$) was calculated by the following relationship: $T_{1/2} = 0.693/k$.

The area under the concentration-time curve (AUC) was calculated by trapezoidal rule to 72 hours and then extrapolated to infinity by dividing the last experimental concentration by the terminal rate constant ($AUC_{0-\infty} = AUC_{0-72} + C_{72}/k$). Area under the first moment of the concentration vs time curve (AUMC) is defined as the area under the curve of the product of time and drug concentration vs time. $AUMC_{0-72}$, $AUMC_{0-\infty}$, and mean residence time (MRT) were calculated through the following equations:

$$AUMC_{t_1-t_n} = [(t_2 - t_1)(C_1t_1 + C_2t_2)/2] + \dots + [(t_n - t_{n-1})(C_{n-1}t_{n-1} + C_n t_n)/2]$$

$$AUMC_{t_n-\infty} = C_n t/k + C_n/k^2$$

$$AUMC_{t_1-\infty} = AUMC_{t_1-t_n} + AUMC_{t_n-\infty}$$

$$MRT = AUMC_{0-\infty}/AUC_{0-\infty}$$

The kinetic parameters used for the bioequivalence comparison between ABZ-test and ABZ-reference products were C_{max} and AUC_{0-72} .

Statistical Analysis

Data from pharmacokinetic analysis are reported as mean \pm SEM ($n = 8$). Student's t -test was used to evaluate the significance of difference between the means of kinetic parameters obtained from the 2 treatment groups. P -values <0.05 were considered significant.

RESULTS

The chromatograms for the extracted serum samples, including a blank serum, a serum spiked with ABZ-SO (500 ng/mL), and a serum sample collected from a sheep following administration of oral ABZ suspension (5 mg/kg), are shown in Figure 1. The retention time for ABZ-SO was 5.8 minutes. As illustrated in Figure 1, the blank serum had no interfering peak at the retention time

of interest. The linearity of the calibration curve for the spiked ABZ-SO in sheep serum was studied at the concentration range of 100-2500 ng/mL. The standard curve showed a good linearity over the range of concentrations examined: $y = 248x + 2381$, $r^2 = 0.998$.

The mean \pm SD serum concentration of ABZ-SO vs time obtained following oral administration of the ABZ-test and -reference products (5 mg/kg) to 2 groups of 8 sheep are plotted in Figure 2. The metabolite ABZ-SO was detected from 3 hours up to 72 hours post-dosing. The C_{max} for ABZ-SO in Group II treated with ABZ-reference product was much higher than that of Group I treated with ABZ-test product. However, T_{max} was almost the same in both products.

Pharmacokinetic parameter profiles of ABZ-SO in each individual animal following oral administration of 2 ABZ products are shown in Table 1. The comparison of the serum kinetic parameter profiles for ABZ-SO in the 2 treatment groups is shown in

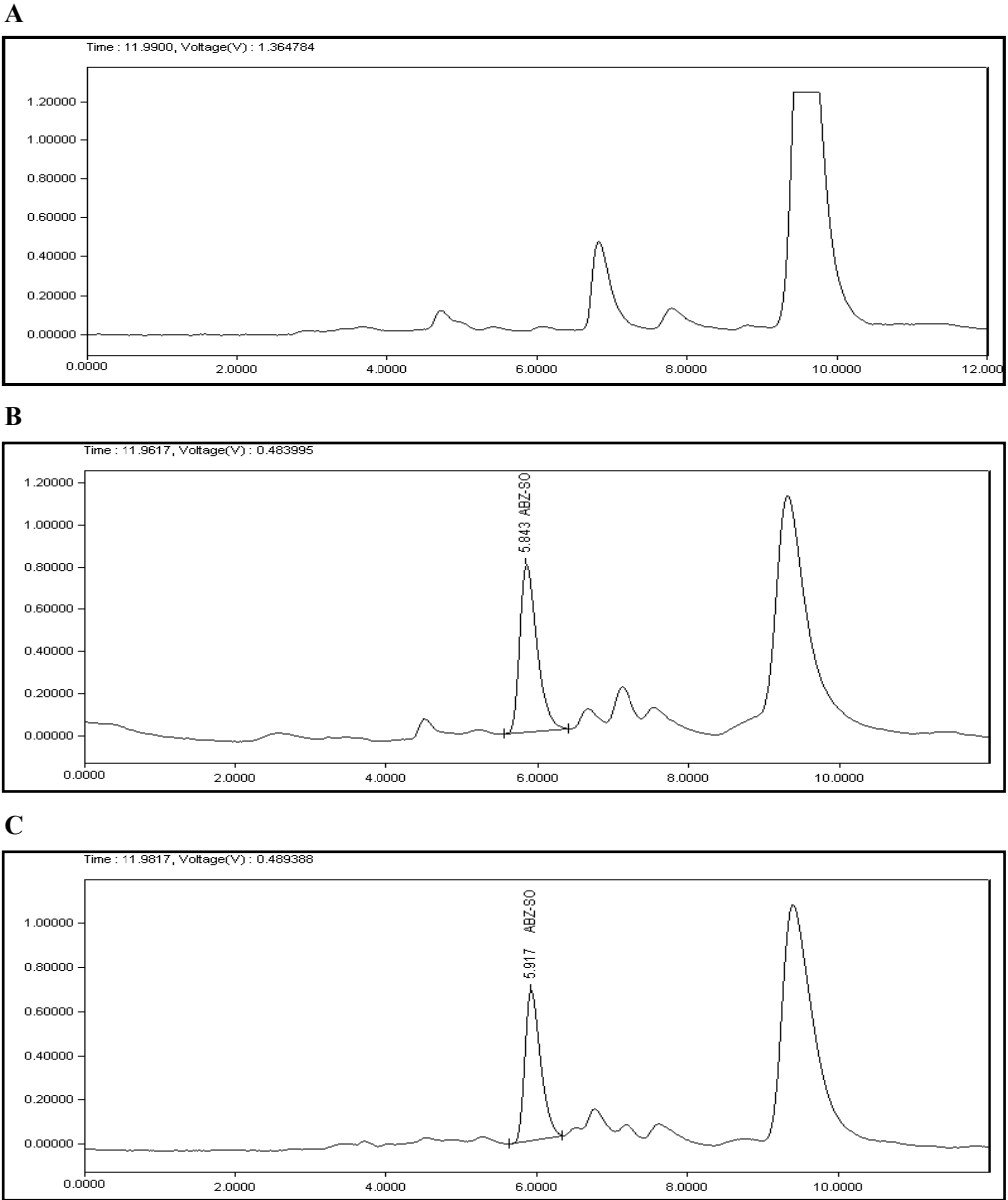
Table 2. Overall, the disposition kinetic data (C_{max} , AUC, and AUMC) for ABZ-SO showed that the serum kinetics were markedly different between the 2 products. Although the MRT values were higher in the ABZ-reference group, there was no significant difference between 2 products.

Using the ratio of the means of AUC_{0-72} and C_{max} for ABZ-test and -reference products for the bioequivalence comparison, it was shown that ABZ -test had significantly lower bioavailability than that of ABZ-reference. The ratios for $AUC_{test}/AUC_{reference}$ and $C_{max-test}/C_{max-reference}$ were 0.55 and 0.61, respectively.

DISCUSSION

The pharmacokinetic data (C_{max} , T_{max} , $T_{1/2}$) for ABZ-SO following oral administration of ABZ-test product in sheep obtained in the present study were comparable with results reported by Lanusse et al¹ and Edvard et al³ after the administration of

Figure 1. The chromatograms of the extracted serum samples: (A) a blank serum, (B) a serum spiked with ABZ-SO, and (C) a serum sample collected from a sheep following administration of oral ABZ suspension (5 mg/kg).



ABZ at the same dose rate (5 mg/kg) to adult sheep. However, the pharmacokinetic profile obtained following oral administration of ABZ-reference product was significantly different from those of ABZ-test. As indicated in Table 1, C_{max} and AUC were significantly higher in ABZ-reference product than ABZ-test. Accordingly, ABZ-

reference product had about 2 times more bioavailability than ABZ-test. Therefore, the difference in bioavailability profiles can be regarded as an important contributing factor for different clinical effectiveness of ABZ products in preliminary studies done by Eslami et al (Eslami, personal communication).

Table 1. Pharmacokinetic Parameter Profiles of ABZ-SO Following Oral Administration of 2 ABZ Products (5 mg/kg) to 2 Groups of 8 Sheep.

Drug	No.	C_{max}	T_{max}	k	$T_{1/2}$	AUC_{0-72}	$AUC_{0-\infty}$	$AUMC_{0-72}$	$AUMC_{0-\infty}$	MRT
		(ng/mL)	(hours)		(hours)	(ng·h/mL)	(ng·h/mL)	(ng·h ² /mL)	(ng·h ² /mL)	
ABZ-Test	1	2844	9	0.093	7.5	58018	58340	988524	1015218	17.4
	2	1809	9	0.058	12.1	72586	73931	1797093	1917107	25.9
	3	1408	12	0.079	8.9	33886	34076	771282	787356	23.1
	4	2571	9	0.074	9.5	46496	46780	876699	900966	19.3
	5	3300	12	0.083	8.4	84064	84365	1775871	1801187	21.3
	6	1099	6	0.083	8.4	32644	33126	852714	893219	27
	7	1172	6	0.064	11.0	40600	41381	1255563	1324020	32
	8	2638	6	0.078	9.0	74830	75920	1662381	1754814	23.1
ABZ-Reference	1	3881	9	0.082	8.5	90565	91175	2329677	2381015	26.1
	2	3509	9	0.057	12.2	115929	119437	3000492	3314681	27.7
	3	3055	9	0.055	12.7	120780	124870	3326931	3695857	29.6
	4	3639	6	0.056	12.5	91839	93714	2294244	2462726	26.3
	5	3467	9	0.065	10.7	101455	104686	2720907	3003227	28.7
	6	2510	15	0.065	10.8	99478	101768	2925882	3127539	30.7
	7	3888	12	0.060	11.7	100437	104604	2592981	2925481	28
	8	3832	6	0.080	8.7	84789	86352	1617813	1749844	20.3

C_{max} = peak serum concentration; T_{max} = time to peak serum concentration; $T_{1/2}$ = elimination half-life; AUC_{0-72} = area under the concentration vs time curve between drug administration and 72 hours post-dosing; $AUC_{0-\infty}$ = area under the concentration vs time curve extrapolated to infinity; $AUMC_{0-72}$ = area under the first moment curve between drug administration and 72 hours post-dosing; $AUMC_{0-\infty}$ = area under the first moment curve extrapolated to infinity; MRT = mean residence time.

Table 2. Comparison of Serum Kinetic Parameter Profiles for ABZ-SO Obtained Following Oral Administrations of 2 ABZ Products (5 mg/mL) to 2 Groups of 8 Sheep.

Kinetic Parameter	ABZ-Test	ABZ-Reference	P-Value
C_{max} (ng/mL)	2105.1 ± 296.8	3472.6 ± 169.0	0.002*
T_{max} (hours)	8.6 ± 0.88	9.4 ± 1.05	0.59
$T_{1/2}$ (hours)	9.4 ± 0.53	11.0 ± 0.58	0.06
k (hours ⁻¹)	0.076 ± 0.004	0.065 ± 0.004	0.06
AUC_{0-72} (ng·h/mL)	55390 ± 7042	100659 ± 4372	0.0002*
$AUC_{0-\infty}$ (ng·h/mL)	55990 ± 7106	103326 ± 4736	0.0001*
$AUMC_{0-72}$ (ng·h ² /mL)	1247516 ± 154637	2601116 ± 186067	0.00007*
$AUMC_{0-\infty}$ (ng·h ² /mL)	1299236 ± 164010	2835546 ± 215992	0.00008*
MRT (hours)	23.6 ± 1.64	27.2 ± 1.13	0.10

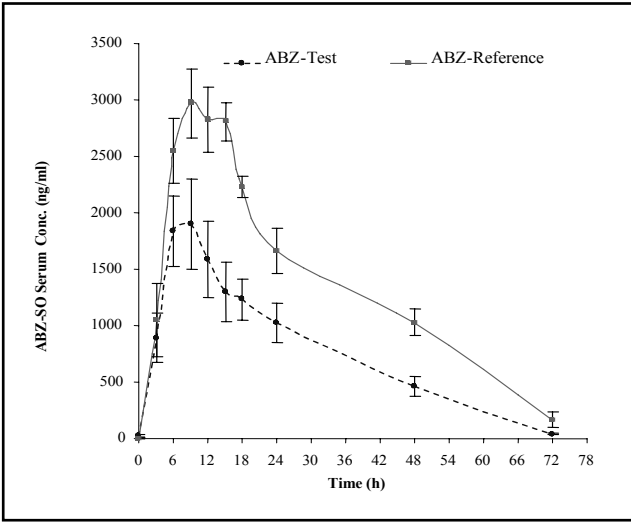
*Significant difference.

Values are expressed as mean ± SEM (n = 8).

C_{max} = peak serum concentration; T_{max} = time to peak serum concentration; $T_{1/2}$ = elimination half-life; AUC_{0-72} = area under the concentration vs time curve between drug administration and 72 hours post-dosing; $AUC_{0-\infty}$ = area under the concentration vs time curve extrapolated to infinity; $AUMC_{0-72}$ = area under the first moment curve between drug administration and 72 hours post-dosing; $AUMC_{0-\infty}$ = area under the first moment curve extrapolated to infinity; MRT = mean residence time.

The metabolite ABZ-SO is reversibly exchanged between blood and different GI compartments in a pH gradient-mediated distribution process and can be reduced back to ABZ by ruminal and intestinal flora.¹ This bacteria-mediated metabolic reduction may occur only in the GI tract.¹ Binding to parasite tubulin is the putative mechanism of action for benzimidazole compounds. Because ABZ has a greater affinity for parasite tubulin than ABZ-SO, the reduction of the sulphoxide metabo-

Figure 2. The mean \pm SD serum concentration of ABZ-SO vs time obtained following oral administration of the ABZ-test and -reference products (5 mg/kg) to 2 groups of 8 sheep.



lite to its parent drug may be important for efficacy against GI parasites.⁵ Thus, the high anti-parasitic activity of ABZ-SO against GI parasites, compared with tissue-dwelling parasites, may depend on this bacterial reduction.^{1,5}

The differences in pharmacokinetic parameters of the anthelmintically active metabolite (ABZ-SO) in the present study may result in important differences in efficacy of these ABZ products, especially against those parasites located in tissues where the presence of high drug concentration for an extended period of time is usually important to achieve optimal clinical effectiveness. For determining the bioequivalence of 2 pharmaceutical products, regulatory agencies have set a statistical criterion. It specifies that the mean AUC and C_{max} values of the test product should not be more than 20% different from the corresponding mean values of the reference product.^{8,10} This can be expressed as $0.80 < \mu_T/\mu_R < 1.20$, where μ_T and μ_R denote the mean of pharmacokinetic parameters for test and reference formulations, respectively. In the present study, the ratio of μ_T/μ_R for AUC_{0-72} and C_{max} for 2 oral ABZ suspension products were 0.55 and 0.61, respectively, which indicate that they can not be assumed to be bioequivalent.

It is concluded that the pivotal bioequivalence parameters (C_{max} and AUC) obtained after oral administration of ABZ-test and ABZ-reference were statistically different and more studies are needed for evaluating the bioequivalence of veterinary drugs produced in Iran.

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