IS ALBENDAZOLE SULPHONATION DOSE DEPENDENT IN HUMAN?

MOHAMMAD REZA ROUINI[*], AHMAD MIRFAZAELIAN[*], SIMIN DADASHZADEH[**], MAHMOUD TABATABAIEFAR[***]

[*] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, [**] Department of Pharmaceutics, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [***] Qazvin University of Medical Sciences, Qazvin, Iran

ABSTRACT

Pharmacokinetics of albendazole sulphone in three different single oral doses of albendazole (ABZ) (400, 800 & 1200mg) in 10 healthy human volunteers in a double blind three-way crossover design. were studied. The serum levels of the main metabolites of ABZ (albendazole sulphoxide (ABZ-SO) and albendazole sulphone ($ABZ\text{-}SO_2$)) were analyzed by a modified high-pressure liquid chromatography method.

For both metabolites, there were no significant differences in the biological half life, normalized serum peak concentration (C_{max}/Dose) and time to reach peak concentration (T_{max}). However apparent clearance (Cl_p/F), apparent distribution volume (V_d/F), normalized area under the serum concentration-time curve (AUC/Dose) and normalized area under the first moment curve (AUMC/Dose) of albendazole metabolites were statistically different for various doses, resulting in substantially lower serum concentration and thereafter lower AUC/Dose and AUMC/Dose in higher doses. Ratios of pharmacokinetic parameters of ABZ-SO to those of $ABZ\text{-}SO_2$ were calculated and there were no significant differences for various ABZ doses.

It is concluded that dose dependent pharmacokinetics of $ABZ\text{-}SO_2$ results from dose dependency of the pharmacokinetics of ABZ-SO, on the basis of a change in fraction of absorbed dose (F) as a result of slow and incomplete dissolution of the main drug in the GI tract. It is also concluded that in the subjects under study sulphonation of ABZ-SO was not dose dependent

Key words; Albendazole, Dose-dependency, Pharmacokinetics, Metabolism

INTRODUCTION

Albendazole (ABZ) is a benzimidazole carbamate used as the drug of choice in treatment of echinococcosis (1). Few studies on the disposition, pharmacokinetics, and concentration-effect relationship of ABZ and its metabolites in human have been reported. After oral administration, the drug is quickly and completely oxidized by the first pass metabolism into its pharmacologically active metabolite albendazole sulphoxide (ABZ-SO) (2). Further liver oxidative and hydrolytic metabolism produces albendazole sulphone ($ABZ\text{-}SO_2$) and albendazole amino sulphone ($ABZ\text{-}SO_2\text{-}NH_2$) respectively, which are thought to be inactive (Figure 1). Some pharmacokinetic studies indicate that ABZ-SO is responsible for both anthelmintic and toxic effects of ABZ (3,4).The parent compound is undetectable in the serum after administration to man (5,6), rats (7), sheep (2) cattle (8) and other species. There are various reports on inhibition of microsomal enzyme function or enzyme induction by ABZ or its metabolites (9-12), which are indicative of the nonlinearity in pharmacokinetics of this drug. Our previous study (13) showed nonlinearity in the pharmacokinetics of albendazole sulphoxide, which was explained on the basis of a change in fraction of dose of the main drug absorbed (F) as a result of slow and incomplete dissolution of the main drug in the GI tract. On the basis of the lack of any report on the dose dependency of other metabolites of ABZ such as. $ABZ\text{-}SO_2$. and since conversion of ABZ-SO to $ABZ\text{-}SO_2$ which has a major role in its disposition, knowledge of pharmacokinetic parameters of $ABZ\text{-}SO_2$ at various doses would be of great help in clarification of the certain cause of non-linearity in ABZ-SO kinetics. This study was designed to study the pharmacokinetics of $ABZ\text{-}SO_2$ in various oral single doses (400, 800 & 1200 mg) in human.

Correspondance: Mohammad-Reza Rouini, Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences. Email: rouini@sina.tums.ac.ir

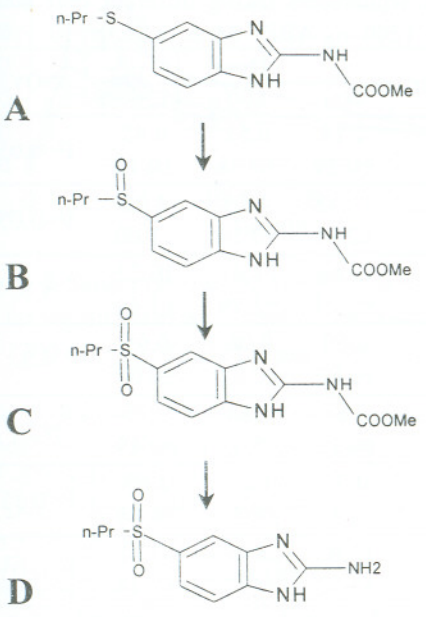

Figure 1- Proposed main metabolic pathways of ABZ in human.
A: Albendazole (ABZ), **B**: Albendazole sulphoxide (ABZ-SO), **C**: Albendazole sulphone (ABZ-SO_2), **D**: Albendazole amino-sulphone (ABZ-SO_2-NH_2)

MATERIALS AND METHODS

Chemicals

Commercial oral dosage forms of ABZ were used. ABZ-SO and ABZ-SO_2 reference standards were donated by SmithKline Beecham (Worthing, UK). ABZ and Mebendazole (MBZ) reference standards were gifted by Daroupakhsh Pharmaceutical Co., Iran. Methanol (HPLC grade, Merck) acetonitrile (HPLC grade, Merck) and glacial acetic acid (analytical grade, Merck) were used for high-pressure liquid chromatography analyses.

Drug administration and Blood Sampling

Ten healthy human volunteers (4 female and 6 male), aged 21-44 years and weighing 51-77Kg were selected. A complete medical history and physical examination, urinanalysis, and hematology tests were obtained for all volunteers prior to initiation of the study period. The volunteers were abstained from taking any medication for one week prior to and during the study period. The drug was administered orally in fasting state with 250 mL of water. The volunteers were given one, two or three tablets of ABZ 400 mg in a randomized double blind three-way crossover design with a washout period of one week between treatments. Blood samples (10 mL) were drawn at 0, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 8, 12 and 24 h post dosing via an indwelling canula in the forearm vein. The samples were then centrifuged for 10 min at 3000 rpm and sera were separated and kept frozen at –20°C until assayed.

High Pressure Liquid Chromatographic analysis of the serum samples

Serum samples were extracted and analyzed according to methods described by Mirfazaelian et al (14)

The extraction of serum samples was performed by liquid phase extraction with ethyl acetate. The organic phase was then evaporated to dryness at 40°C under gentle stream of nitrogen and the residue was dissolved in 0.001M HCl. The samples were further cleaned by back extraction with n-hexane. The n-hexane phase was discarded and the sample was re-extracted with ethyl acetate after alkalization. The organic phase was evaporated to dryness as described; and the residue was re-dissolved in 300 µL of the HPLC solvent and injected onto the HPLC column.

Analysis was performed using a Rheodyne 7725i injector fitted with a 50 µL loop, a high-pressure pump (Perkin-Elmer Series 4, USA), a spectrophotometeric detector (Unicam 4225, USA), a flourescence detector (Perkin-Elmer LS-4, USA) and a dual pen recorder (Philips- PM 8252, USA). The stainless steel column (C_8, Partisil, 5 µm, 100 mm x 4.6 mm, Grom, Germany) was preceded by a (C_8, 5 µm, 30 mm x 4.6 mm, Pye Unicam, USA) precolumn. The mixture of methanol: acetonitrile: acetic acid: water (40:1:10:49) was used as eluent with a flow rate of 0.8 mL/min. The eluent was monitored at 286 nm with UV spectrophotometer for ABZ-SO and MBZ (Internal Standard) and at 286 nm (excitation)– 333 nm (emission) for ABZ-SO_2 with flourescence spectrophotometer.

Pharmacokinetic analysis

Pharmacokinetic parameters were obtained by non-compartmental analysis. Apparent first order terminal rate constant (k) was calculated from the terminal portion of the log of the plasma concentration-time curve using least square regression analysis. Biological half-life ($t_{1/2}$) was calculated by the following relationship:

$T_{1/2} = \ln(2)/k$

The area under the concentration-time curve (AUC) was calculated to 24 h by the trapezoidal rule and then extrapolated to infinity using the terminal rate constant value.

Apparent oral clearance (Cl_p/F) of the two metabolites were calculated by division of the dose of the main drug to $AUC_{0-\infty}$ of each metabolite. Apparent distribution volume (V_d/F)

Table 1 Pharmacokinetic parameters of albendazole sulphoxide in volunteeers taking different oral doses (400, 800 & 1200 mg ABZ). Mean ± SE, n=10

Parameter	Dose (mg)			ANOVA
	400	800	1200	
AUC^{*}_{0-24} ($h.m^{-3}$)	17.74 ±3.70	8.80 ±1.37	8.51 ±2.14	P<0.05
$AUMC^{*}_{0-24}$ ($h^2.m^{-3}$)	175.25 ±36.27	80.93 ±10.35	73.90 ±17.30	P<0.05
$T_{1/2}$ (h)	17.63 ±2.49	13.94 ±1.94	11.54 ±1.54	N.S.
C_{max}^{*} (m^{-3})	1.61 ±0.29	1.21 ±0.28	1.12 ±0.45	N.S.
T_{max} (h)	3.06 ±0.44	3.3 ±0.06	3.65 ±0.51	N.S.
$Cl_p/F1$ ($m^3.h^{-1}$)	0.04 ±0.01	0.09 ±0.013	0.14 ±0.03	P<0.05
$V_d/F1$ (m^3)	1.05 ±0.19	1.73 ±0.79	2.08 ±0.38	P<0.05

AUC^{*}: Area under the curve of metabolite normalized to dose of the main drug. $AUMC^{*}$: Area under the first moment curve of metabolite normalized to dose of the main drug. C_{max}^{*}: Peak serum concentration of metabolite normalized to dose of the main drug.

Table 2 Pharmacokinetic parameters of albendazole sulphone in volunteeers taking different oral doses (400, 800 & 1200 mg ABZ). Mean ± SE, n=10

Parameter	Dose			ANOVA
	400	800	1200	
AUC^{*}_{0-24} ($h.m^{-3}$)	1.14 ±0.29	0.51 ±0.08	0.45 ±0.12	P<0.05
$AUMC^{*}_{0-24}$ ($h^2.m^{-3}$)	11.48 ±2.91	4.76 ±0.74	4.05 ±1.00	P<0.05
$T_{1/2}$ (h)	15.68 ±1.97	13.41 ±2.79	10.23 ±1.49	N.S.
C_{max}^{*} (m^{-3})	0.09 ±0.02	0.06 ±0.01	0.05 ±0.02	N.S.
T_{max} (h)	3.63 ±0.73	3.34 ±0.52	3.75 ±0.49	N.S.
$Cl_p/F2$ ($m^3.h^{-1}$)	0.76 ±0.11	1.63 ±0.21	2.68 ±0.54	P<0.05
$V_d/F2$ (m^3)	17.92 ±3.22	28.04 ±4.62	37.31 ±7.43	P<0.05

AUC^{*}: Area under the curve of metabolite normalized to dose of the main drug. $AUMC^{*}$: Area under the first moment curve of metabolite normalized to dose of the main drug. C_{max}^{*}: Peak serum concentration of metabolite normalized to dose of the main drug.

was obtained by dividing apparent oral clearance (Cl_p/F) to the terminal rate constant (k). F was defined as the fraction of the dose which was transformed to each metabolite after absorption and reached to the general circulation; F for ABZ-SO (F_1) was defined as fraction of the dose of the parent drug absorbed (F_a) and then turned to the active metabolite (F_{m1}) (i.e. $F_1=F_a \times F_{m1}$) and F of ABZ-SO_2 (F_2) was defined as fraction of dose of the parent drug absorbed (F_a) and then turned to the active metabolite (F_{m1}) and thereafter metabolized to ABZ-SO_2 (F_{m2}) (i.e. $F_2=F_a \times F_{m1} \times F_{m2} = F_1 \times F_{m2}$).

Peak serum concentration (C_{max}), area under serum concentration-time curve (AUC) and the area under the first moment curve (AUMC) of different doses were normalized to their relative doses and the derived parameters are shown as C_{max}^{*}, AUC^{*} and $AUMC^{*}$ respectively. The derived parameters were subjected to Two-Way ANOVA (Repeated measures) to evaluate the significance of differences. Further assessment of differences were achived by Tukey-Kramer multiple comparisons post hoc test (2). P-value of less than 0.05 was considered significant.

Serum concentration-time profiles of ABZ main metabolites, ABZ-SO and ABZ-SO_2, at three dose levels are shown in Figures-2 and 3 respectively. Pharmacokinetic parameters of ABZ-SO and ABZ-SO_2 at different doses are summarized in tables 1 and 2 respectively.

RESULTS

In order to clarify the main cause(s) of nonlinearity in ABZ-SO kinetics, pharmacokinetics of the various oral single doses (400, 800 & 1200 mg) of the drug in human were studied.

Serum concentration-time profiles of ABZ main metabolites, ABZ-SO and ABZ-SO_2, at three dose levels are shown in Figures-2 and 3 respectively. Pharmacokinetic parameters of ABZ-SO and ABZ-SO_2 at different doses are summarized in tables 1 and 2 respectively.

No significant differences are observed in the $t_{1/2}$, C_{max}^{*} and T_{max}, whereas AUC^{*} and $AUMC^{*}$ reduced significantly. Apparent oral clearance (Cl_p/F) and apparent oral distribution volume (V_d/F) were significantly increased with the increase in administered doses of both metabolites (ABZ-SO and ABZ-SO_2). These findings are in contrast with linear pharmacokinetic in which values of these parameters remain constant with different doses.

Table 3 demonstrates the ratios of pharmacokinetic parameters of ABZ-SO to those of ABZ-SO_2 at three different dose levels. These ratios were not significantly different in various doses.

DISCUSSION

The values of the pharmacokinetic parameters of ABZ metabolites are in accord with the reported values (15). For example, although statistically non-different, mean C_{max}^{*} of ABZ-

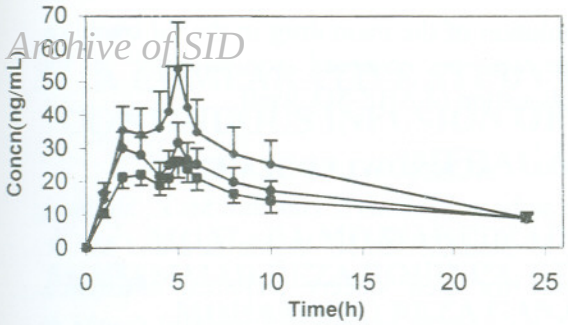

Figure 2 Serum profiles of albendazole sulphoxide after taking different oral single doses [400 (▪), 800 (•)& 1200 mg (♦) of ABZ] Mean ± SE, n=10

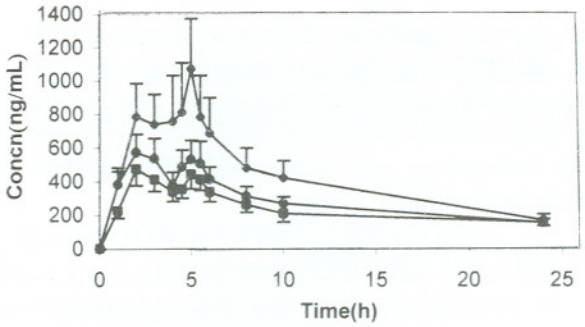

Figure 3 Serum profiles of albendazole sulphone after taking different oral single doses [400 (▪), 800 (•)& 1200 mg (♦) of ABZ] Mean ± SE, n=10

Table 3 Ratio of pharmacokinetic parameters of albendazole sulphoxide to albendazole sulphone (ABZ-SO/ABZ-SO_2) in volunteeers taking different oral single doses (400, 800 & 1200 mg Tablets) Mean ± SE, n=10

Parameter[a]	Dose (mg)			ANOVA
	400	800	1200	
AUC^*_{0-24} ($h.m^{-3}$)	16.35 ±0.75	17.69 ±0.92	18.91 ±0.71	N.S.
$AUMC^*_{0-24}$ ($h^2.m^{-3}$)	16.24 ±0.90	17.60 ±1.049	18.47 ±0.66	N.S.
$T_{1/2}$ (h)	1.23 ±0.08	1.03 ±0.27	1.06 ±0.05	N.S.
C_{max}^* (m^{-3})	18.32 ±1.17	18.8 ±1.28	21.13 ±0.81	N.S.
T_{max} (h)	0.83 ±0.07	0.98 ±0.12	0.97 ±0.03	N.S.
Cl_p/F ($m^3.h^{-1}$)	0.06 ±0.004	0.06 ±0.004	0.05 ±0.002	N.S.
V_d/F (m^3)	0.07 ±0.003	0.06 ±0.004	0.06 ±0.003	N.S.

[a]: Ratio of parameters are dimentionless. Units refer to the parameters themselves. AUC^*: Area under the curve of metabolite normalized to dose of the main drug. $AUMC^*$: Area under the first moment curve of metabolite normalized to dose of the main drug. C_{max}^*: Peak serum concentration of metabolite normalized to dose of the main drug.

SO_2 decreased at the dose of 1200 mg 49% and at the dose of 800 mg 39% in comparison with the dose of 400 mg and mean AUC^* of ABZ-SO_2 decreased 55.64% when the dose was doubled from 400 mg to 800 mg and decreased to 66.65% when it was tripled to 1200 mg. Statistical non-significance in the C_{max}^* values of different doses was attributed to intersubject variations. These findings indicate that the pharmaco-kinetics of both metabolites (ABZ-SO & ABZ-SO_2) depends to the dose of the parent drug and therefore follows a nonlinear pattern. Dose dependency of the pharmaco-kinetics of ABZ-SO which was observed in our study, has previously been explained on the basis of a change in fraction of the dose of the main drug which is absorbed (F_a) as a result of slow and incomplete dissolution of the parent drug in the GI tract (13). Increase in Cl_p/F_2 and V_d/F_2 values by increase in doses, are assumed to arises from increase in Cl_p/F_2 and V_d/F_2 of ABZ-SO_2, which in turn may results by either reduction in ABZ-SO formation by increase in the doses and/or reduction in sulphone formation from (ABZ-SO).

In order to evalute the second hypothesis, ratios of the relative pharmacokinetic parameters of ABZ-SO to ABZ-SO_2 (ABZ-SO/ABZ-SO_2) were calculated and it was found that there were not significantly differences in various doses studied (Table 3). Therefore it was concluded that there is no reduction in sulphone formation from the available ABZ-SO, which indicates in the dose range which were studied, sulphonation of ABZ-SO is not a dose dependent process. As a result, the observed dose dependency in pharmacokinetics of ABZ-SO_2 may be related to the dose dependency of ABZ-SO pharmaco-kinetics and thereby may be attributed to insufficint in vivo dissolution of the different doses of the main drug and not to the proposed nonlinear sulphonation of ABZ-SO. Thereafter, as for ABZ-SO, partial absorption of the main drug (decreased F_a) is due to incomplete dissolution of the administered dose and excretion of a greater portion of the unabsorbed drug in higher doses which results in decrease of F_1 and thereby F_2, and concequently increase in value of V_d/F_2 and Cl_p/F_2 of ABZ-SO_2.

As noted previously an overall significant differences were observed in AUC^*, $AUMC^*$, Cl_p/F_2 and V_d/F_2 of ABZ-SO_2 in different doses in the repeated measures Two-Way ANOVA. However, in spite of the obvious changes in trend of their values from lower to higher doses, Tukey-Kramer multiple comparisons post hoc

test showed significant differences between 400 mg & 800 mg but no significant changes between 800 mg & 1200 mg doses. The results are quite similar to those of ABZ-SO (13) which may indicate *in vivo* saturation of dissolution media in the higher doses.

CONCLUSIONS

It is concluded that the dose dependent pharmacokinetics of ABZ-SO_2 may results from the dose dependent pharmacokinetics of ABZ-SO, on the basis of a change in fraction of dose absorbed (F) as a result of slow and incomplete dissolution of the main drug in the GI tract, i.e. there was no observed non-linearity in the sulphonation of ABZ-SO, itself.

ACKNOWLEDGEMENTS

The authors thank Ms L. Hakemi, Ms Z. Shabani, Ms. M. Joneidi, Mr. N. Asgharifard and Mr. R. Shokri for their sincere technical assistance. Also we wish to express our grateful thanks to Dr. S. Rezaeifar for his sincere statistical assistance and to acknowledge kind donation of ABZ metabolites by SmithKline & Beecham.

REFERENCES

1. Goldsmith, R.S. (1998) Clinical Pharmacology of the Anthelmintic Drugs. In: Katzung B.G. (ed), Basic and Clinical Pharmacology, 7th Edition. Appleton & Lange, Stamford, pp. 869-870.
2. Marriner, S.E., Bogan, J.A. (1980) Pharmacokinetics of albendazole in sheep. Am. J. Vet. Res. 41: 1126-1129.
3. Delatour, P., Parish, R.C., Gyurik, R.J. (1981) Albendazole: A comparison of relay embryotoxicity with embryotoxicity of individual metabolites. Ann. Rech. Vet. 12: 159-167.
4. Villaverde, C., Alvarez, S., Redondo, P. (1995) Small intestinal sulphoxidation of albendazole . Xenobiotica 25, 433-441.
5. Penicaut, B., Maugein, H., Maisonneuve, H., Rossignol, J.F. (1983) Pharmacocinetique et metabolisme urinaire de lalbendazole chez lhomme. Bull. Soc. Pathol. Exot. 76: 698-708.
6. Marriner, S.E., Morris, D.L., Dickson, B., Bogan, J.A. (1986) Pharmacokinetics of albendazole in man. Eur. J. Pharmacol. 30: 705-708.
7. Delatour, P., Garnier, F., Benoit, E., Longin, C. (1984) A correlation of toxicity of albendazole and of oxfendazole with their free metabolites and bound residues. J. Vet. Pharmacol. Ther. 7: 139-145.
8. Prichard, R.K., Hennessy, D.R., Steel, J.W., Lacet, E. (1985) Metabolite concentrations in plasma following treatment of cattle with five anthelminitics. Res. Vet. Sci. 39: 173-178.
9. Rolin, S., Souhaili-El Amri, H., Batt, A.M., Levy, M., Bagrel, D., Siest, G. (1989) Study of the in vitro bioactivaction of albendazole in human liver microsomes and hepatoma cell lines. Cell Biol. Toxicol. 5: 1-14.
10. Steiger, U., Cotting, J., Reichen, J. (1990) Albendazole treatment of echinococcosis in humans: effects on microsomal metabolism and drug tolerance. Clin. Pharmacol. Ther. 47: 347-53.
11. Souhaili-El Amri, H., Mothe, O., Totis, M., Masson, C., Batt, A.M., Delatour, P., Siest, G. (1988) Albendazole sulfonation by rat liver cytochrome P-450c. J. Pharmacol. Exp. Ther., 246, 758-764.
12. Souhaili-El Amri, H., Fargetton, X., Benoit, E., Totis, M., Batt, A.M. (1988) Inducing effect of albendazole on rat liver drug-metabolizing enzymes and metabolite pharmacokinetics. Toxicol. Apppl. Pharmacol. 92: 141-149.
13. Mirfazaelian A., Dadashzadeh S., Rouini M.R. (2002) Dose dependent pharmacokinetics of albendazole in human. Biopharm. Drug Disp. 23: 379-383.
14. Mirfazaelian A., Dadashzadeh S., Rouini M.R. (2002) A high performance liquid chromatography method for simultaneous determination of albendazole metabolites in human serum. J. Pharm. Biomed Anal. 30: 1249-1254.
15. Jung, H., Hurtado, M., Sanchez, M., Medina, M.T., Sotelo J. (1992) Clinical pharmacokinetics of albendazole in patients with brain cysticerocosis. Clin. Pharmacol. 32: 28-31.