EFFECT OF KETOCONAZOLE ON THE PHARMACOKINETICS OF ORNIDAZOLE - A POSSIBLE ROLE OF P-GLYCOPROTEIN AND CYP3A

Srirangam Ramesh, Yamsani Shravan Kumar
and Yamsani Madhusudan Rao*

University College of Pharmaceutical Sciences,
Kaktiya University, Warangal, India

SUMMARY

The influence of ketoconazole, a modulator of P-glycoprotein (P-gp), on the exsorption of ornidazole from everted sacs of rat intestine (duodenum, jejunum and ileum) was investigated. The effect of ketoconazole pretreatment on the pharmacokinetics of ornidazole was also studied in eight healthy human volunteers. After overnight fasting ornidazole 500 mg was administered before and after pretreatment with ketoconazole 200 mg once daily for 7 days. Serum samples were analyzed by reversed phase HPLC. Significant differences were observed in pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max} and clearance. Ornidazole is believed to be metabolized through CYP3A and it has considerable intestinal efflux, which was observed from the *in vitro* study. The altered pharmacokinetic parameters can be attributed to ornidazole efflux from the blood to the intestine and its metabolism by CYP3A in the intestine.

KEY WORDS

ornidazole, ketoconazole, P-glycoprotein, CYP3A, pharmacokinetics

* Author for correspondence:
Prof. Y. Madhusudan Rao
University College of Pharmaceutical Sciences
Kaktiya University
Warangal (A.P.), India 506009
e-mail: ymrao123@yahoo.com

INTRODUCTION

Marked variability in plasma concentration of drugs after administration of a fixed dose is often related to differences in drug absorption and metabolism. Inhibition and induction of hepatic metabolism, and also prehepatic biotransformation in the intestine, can lead to alterations in the pharmacokinetics of the administered drug. Cytochrome P-450 (CYP) 3A is the dominant CYP in the human small intestine and accounts for the majority of total microsomal P-450 found in the intestine /1/. The potential role of P-glycoprotein (P-gp), an efflux transporter that is located on the apical brush membrane of the epithelium of the small intestine, in limiting oral drug absorption has been increasingly appreciated /2/. It has been proposed that the CYP3A system and P-gp may functionally work together in reducing the oral bioavailability of drugs. A literature survey revealed a striking overlap between substrates for CYP3A and P-gp /3/. In addition to similarity in substrate specificity, CYP3A and P-gp appear to be inhibited by similar inhibitors.

Ornidazole is a 5-nitroimidazole derivative used as an antimicrobial agent in the treatment of hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of the urogenital tract, bacterial vaginosis, and other anaerobic infections. Ornidazole is also effective for the prevention of recurrence of Crohn's disease after ileocolonic resection. Ornidazole is mainly metabolized to α-(chloromethyl)-2-hydroxymethyl-5-nitroimidazole-1-ethanol (M1) and 3-(2-methyl-5-nitroimidazole-1-yl)-1,2-propane-diol (M4) in the liver. There are no reports in the literature about which CYP isoenzyme is involved in the metabolism of ornidazole, but the hydroxy metabolite (i.e. M1) is similar to the metabolite of metronidazole, and the formation of this metabolite is catalyzed by CYP3A4. The 5-nitroimidazole derivative, metronidazole, is reported to be a substrate of P-gp and it is metabolized by intestinal CYP3A4. Moreover, cross-resistance to 5-nitroimidazoles in *Trichomonas vaginalis* is because of overexpression of multi-drug resistant (MDR) P-gp, which is similar to mammalian P-gp /4/.

With this theoretical background, the exsorption of ornidazole was investigated *in vitro* in rat everted sacs, alone and in the presence of the known P-gp inhibitor, ketoconazole /5/. Later, we investigated the effect of P-gp and CYP3A on the absorption of ornidazole in an *in*

vivo study in healthy human volunteers, in which the influence of pretreatment with a known P-gp and CYP3A inhibitor, ketoconazole, on the pharmacokinetics of ornidazole was observed.

MATERIALS AND METHODS

Animals and chemicals

Male Wistar rats bred in the animal center of Kaktiya University, Warangal, India, were fasted for 1 day before the experiment; water was given *ad libitum*. Ornidazole 500 mg (Orni®500, Zydus Cadila HC Ltd, Ahmedabad, India), ketoconazole 200 mg (Phyterol® 200 mg, Brown and Burk Ltd, India), tinidazole (gift sample from Aristo pharma, Mumbai, India), Dulbeccos phosphate buffer (pH 7.4) (Hi Media (India) Limited, Mumbai), acetonitrile, methanol (HPLC grade, E. Merck (India) Limited, Mumbai); all other chemicals were of AR grade.

In vitro everted sac method

The rat everted sac method reported by Yumoto *et al.* /6/ was followed. 10 mg/ml solution of ornidazole was prepared in pH 7.4 Dulbeccos PBS (D-PBS) containing 25 mM glucose and 4% DMSO. 1 ml of this solution was introduced into everted sacs (serosal side) of the duodenum, jejunum and ileum. The sacs containing drug solution were immersed into 40 ml of D-PBS containing 25 mM glucose and 4% DMSO as that in the serosal side. The medium was pre-warmed at 37°C and pre-oxygenated with 5% CO_2/95% O_2 for 15 minutes. Under bubbling with CO_2/O_2 the transport of ornidazole across the intestine was measured by sampling the mucosal medium for 90 min. Using these media the transport of ornidazole was studied in the absence (control) or presence of inhibitor (200 μM ketoconazole).

In vivo human volunteer study

Subjects

Eight healthy male volunteers with a mean age of 26.75 ± 2.05 years (range 25-30 years), mean height 1.69 ± 2 m (range 1.62-1.73 m) and mean body weight 62.65 ± 5.88 kg (55-69 kg) participated in the

study after undergoing a thorough physical examination. The volunteers were briefed about the study and written informed consent was obtained. The institutional local ethics committee approved the study protocol.

The volunteers had no history of any ill health during the preceding 6 months and none had taken any medication for at least 15 days prior to the administration of ornidazole. They had to avoid nicotine, alcohol, caffeine and citrus fruit products for one week before and throughout the study period. Volunteers were excluded from the study if they had food allergies or were allergic to ornidazole or ketoconazole.

Protocol

After overnight fasting (approximately 12 h), each volunteer received ornidazole 500 mg with 200 ml of water. Venous blood samples (4 ml) were drawn from the antecubital vein at 0, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24 and 36 hours after drug administration. The blood was allowed to clot and centrifuged for 10 min at 3,000 rpm (R&C, Remi Instruments, Mumbai, India). Serum was separated into Eppendorf tubes and stored at -20°C until analysis. After a washout period of 15 days, a once daily dose of ketoconazole 200 mg was given for 7 consecutive days under direct observation. On the seventh day ornidazole was again administered, 1 hour after the ketoconazole administration, and blood sample collection was repeated as above.

Sample assays

Ornidazole in serum samples was estimated by a reverse phase high performance liquid chromatography (HPLC) method /7/. The HPLC system (Shimandzu, Japan) consisted of an LC -10AT solvent delivery module and SPD -10A UV-visible spectrophotometric detector. The mobile phase for the everted sac samples consisted of 0.002 M acetate buffer, acetonitrile and methanol (60:30:10 v/v/v) pH 4.8 with a flow rate of 1 ml/min. For serum samples the mobile phase consisted of 0.002 M acetate buffer, acetonitrile and methanol (70:20:10 v/v/v) pH 4.8. The column used was a Phenomenex C-18 Gemini stainless steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5 μ diameter, 100 Å pore diameter, and the eluent was monitored at 318 nm.

Pharmacokinetic parameters

Various pharmacokinetic parameters: peak plasma concentration (C_{max}), area under the curve (AUC), elimination half life ($T_{½}$), volume of distribution (V/f), total clearance (Cl/f), mean residence time (MRT), area under the moment curve (AUMC) for ornidazole, were obtained for each subject using a non-compartmental pharmacokinetic program, Win Nonlin 1.1 (Pharsight, Palo Alto, CA).

Statistical analysis

The mean pharmacokinetic parameters of ornidazole obtained before and after pretreatment with ketoconazole were compared by Student's paired t-test (paired data) and *in vitro* results were compared by Student's t-test using Sigma Stat Software (Jandel Scientific, Sigma Stat version 1, 1992-94). A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

In the everted sac study, the mean transport of ornidazole from the serosal to the mucosal surface across everted rat intestine in the absence and presence of ketoconazole (a potent CYP3A4 and P-gp inhibitor) was determined in duodenum, jejunum and ileal regions of rat small intestine. The time course of ornidazole transport across everted sacs of the duodenum, jejunum and ileum are shown in Table 1.

In human volunteers, the mean serum concentrations of ornidazole at different time points before and after pretreatment with ketoconazole are shown in Figure 1. Pharmacokinetic parameters are presented in Table 2. There was a statistically significant difference in pharmacokinetic parameters C_{max}, T_{max}, AUC, AUMC, and clearance. Changes were observed in $T_{½}$, V_d, and MRT after pretreatment with ketoconazole, though these did not reach statistical significance. Ketoconazole treatment increased C_{max}, $AUC_{0-\alpha}$, and AUMC by 26.73% ($p < 0.01$), 31.2% ($p < 0.005$), and 60.88% ($p < 0.05$), respectively; T_{max} and clearance were decreased by 45.84% ($p < 0.05$) and 14.15% ($p < 0.01$), respectively. MRT and $T_{½}$ were increased by

TABLE 1

Cumulative efflux concentrations of ornidazole (μg/ml) (mean ± SD) alone and percent change after ketoconazole pretreatment in intestinal everted sacs in albino Wistar rats (n = 6)

Region	Ornidazole alone	With ketoconazole
Duodenum	79.13 ± 1.39	59.03 ± 3.04* (-25.04%)
Jejunum	109.45 ± 2.69	88.09 ± 3.43* (-19.51%)
Ileum	136.36 ± 3.26	102.77 ± 3.58* (-24.36%)

* $p < 0.05$

TABLE 2

Pharmacokinetic parameters of ornidazole (mean ± SD) with percent change after ketoconazole pretreatment in healthy human volunteers (n = 8)

Pharmacokinetic parameter	Ornidazole alone	With ketoconazole
C_{max} (μg/ml)	4.2 ± 0.71	5.31 ± 1.04* (26.73 %)
T_{max} (h)	1.5 ± 0.61	0.81 ± 0.35* (-45.84%)
$AUC_{0-\alpha}$ (μg/ml/h)	96.14 ± 10.36	126.14 ± 10.92* (31.2%)
K_e (h^{-1})	0.0347 ± 0.006	0.0297 ± 0.007 (-14.4%)
$T_{½}$ (h)	20.62 ± 3.55	24.97 ± 7.57 (21.09%)
Cl/F	5.26 ± 0.62	3.99 ± 0.36* (-14.15%)
V_d/F	156.11 ± 31.44	141.22 ± 32.24 (-9.53%)
MRT (h)	29.4 ± 4.72	35.51 ± 10.34 (20.78%)
AUMC (μg/ml/h)	2835.64 ± 568.78	4562.19 ± 1724.2* (60.88%)

* $p < 0.05$.

C_{max} = peak serum concentration; T_{max} = time to reach peak serum concentration; AUC = area under serum concentration-time curve; K_e – elimination rate; $T_{½}$ = elimination half-life; Cl/f = total clearance; V_d/f = volume of distribution; MRT = mean residence time; AUMC = area under the moment curve.

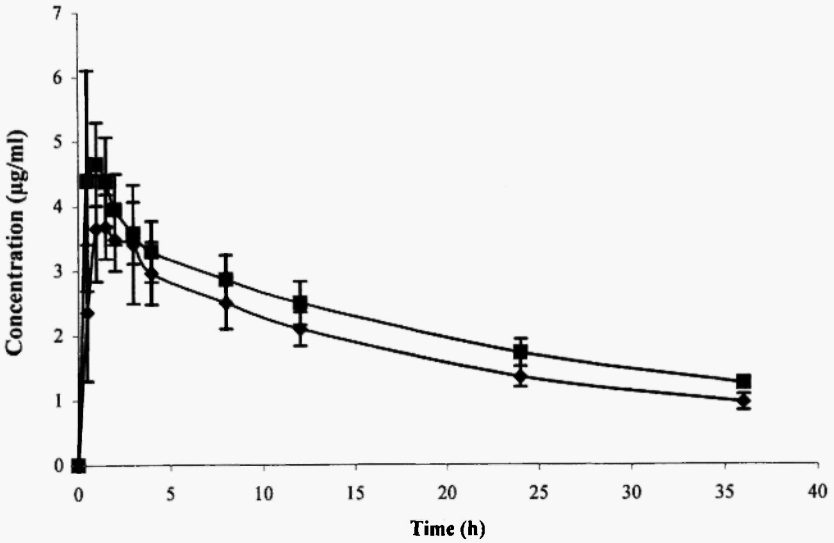

Fig. 1: Serum profile of ornidazole concentration (mean ± SD) before and after pretreatment with ketoconazole (n = 8). ♦ ornidazole; ■ ornidazole after pretreatment with ketoconazole.

20.78%and 21.09%, respectively, and K_e was decreased by 14.27% (all $p > 0.05$).

DISCUSSION

The present study presents the effect of ketoconazole (200 mg/p.o., once daily for 7 days), a known P-gp and CYP3A inhibitor, on the oral pharmacokinetics of ornidazole. Ornidazole is mainly used in the treatment of hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of the urogenital tract, bacterial vaginosis, and other anaerobic infections. Ornidazole is also effective for the prevention of recurrence of Crohn's disease after ileocolonic resection. Reports suggest that ornidazole and other nitroimidazoles can induce liver damage but rarely /8,9/. Ketoconazole, a widely used antifungal agent, is recognized as a potent inhibitor of CYP3A. Ketoconazole has been shown to inhibit P-gp in a vinblastine resistant cell line (KB-V1) /5/.

Recently Ward *et al.* developed a monkey screen model using ketoconazole to probe P-gp/CYP3A interactions with compounds of interest in a discovery setting /11/.

In this study, investigations were carried out to see whether transport of ornidazole at the intestinal level is influenced by ketoconazole (a P-gp inhibitor) in rat everted sacs of duodenum, jejunum and ileum. Ketoconazole reduced the mean cumulative transport out of everted sacs by 25.04% ($p < 0.05$), 19.51% ($p < 0.05$) and 24.36% ($p < 0.05$) in the duodenal, jejunal and ileal sacs, respectively. Results of this *in vitro* study revealed that ornidazole transport across the small intestine is much affected by this known P-gp inhibitor. In this study, the mean ± SD cumulative exsorption concentrations of ornidazole in the presence of ketoconazole was decreased. This observation indicates the role of P-gp, an efflux pump, on ornidazole exsorption. These data are in good correlation with those of Trezise *et al.* /12/; they reported that the expression of multidrug resistant *mdr1* mRNA varied in rat intestine, with a moderate expression in the duodenum and jejunum and maximal expression in the ileum. There are reports that several CYP3A and P-gp related compounds, such as ketoconazole, erythromycin, and midazolam, significantly reduced the exsorption of rhodamine 123 (a P-gp substrate) across rat ileum everted sac preparations. Yumoto *et al.* /6/ observed a linear relationship between the *in vitro* everted sac and *in vivo* studies and suggested that P-gp related drug-drug interactions *in vivo* can be predicted by *in vitro* everted sac studies. These workers also suggested that drug-drug interactions related to P-gp mediated transport in human intestine could be predicted by *in vivo* (exsorption across rat ileum) or *in vitro* (everted rat intestine) transport studies using rat ileum as comparable with the transport studies in Caco-2 cell monolayers.

Based on these *in vitro* results and the report that resistance to 5-nitroimidazoles in *T. vaginalis* is because of overexpression of P-gp, a multidrug resistant protein, which is similar to human P-gp /4/, the effect of ketoconazole on the oral pharmacokinetics of ornidazole was observed in healthy human volunteers. All volunteers tolerated the treatments well and there was no dropout. In earlier studies with ornidazole /13/ Ramamurthy *et al.* observed a C_{max} of 32.67 ± 4.45 μg/ml with an AUC_{0-12} of 261.67 ± 77 μg/ml/h with a single dose of 1.5 g, but in this case a similar level with a single dose of 500 mg

(4.1914 ± 0.71 μg/ml and AUC_{0-36} 67.64 ± 8.38) was not observed, i.e. the decrease in these parameters with decrease of dose is not linear. There were no complaints of any severe or minor adverse effects of ornidazole or ketoconazole from the volunteers.

Pretreatment with ketoconazole significantly increased the C_{max}, $AUC_{0-\alpha}$ and AUMC of ornidazole by 26.73%, 31.2% and 60.88%, respectively. Clearance was decreased significantly by -14.15%. Although changes in MRT, V_d and $T_{½}$ did not reach statistical significance, they changed by 20.78%, -9.53% and 21.09%, respectively.

The observed pharmacokinetic data of ornidazole after the pretreatment indicates the influence of the P-gp modulator, ketoconazole, on the oral pharmacokinetics of ornidazole, especially by acting through intestinal P-gp, apart from other regions, such as the liver, biliary system, canaliculi, etc. Intestinal P-gp is known to be localized in the brush border membrane to pump drugs from the serosal side to the luminal side. Inhibition of intestinal P-gp may decrease drug exsorption and thus increase net drug absorption. As ketoconazole decreased the levels of P-gp and CYP3A at the intestinal level, the net absorption was increased. The significant pharmacokinetic changes observed in C_{max}, T_{max} and AUC clearly indicate that the absorption of ornidazole is influenced through first pass metabolism by ketoconazole. Everted sac studies in the rat have found that alterations in the oral pharmacokinetics are due to changes at the absorption site, i.e. in the intestine. Therefore we can attribute this to findings from the human volunteer study, i.e. we observed an increase in C_{max} and AUC and a decrease in T_{max}. Since the efflux of drug from blood to intestine is decreased by ketoconazole, there is rapid absorption of the drug and hence a decrease in T_{max}. Here, the possible role of CYP on the pharmacokinetics cannot be ruled out directly, as it is believed that metabolism of ornidazole is through CYP3A, and ketoconazole is also an inhibitor of CYP3A. Thus, whatever changes occurred are possibly due to inhibition of P-gp *and* CYP3A in the intestine. There are several reports of this sort; Wu *et al.* have reported that in the absence of intestinal metabolism and efflux (by ketoconazole), the bioavailability of cyclosporine is reported as 65 ± 12% in healthy volunteers /14/. Salphati and Benet have reported that ketoconazole increased the plasma concentration of digoxin in rats by inhibiting P-gp and CYP3A /15/.

In conclusion, it was observed from this investigation that ornidazole is a P-gp substrate in *in vivo* conditions in human volunteers. The observed pharmacokinetic parameters indicated that the oral absorption of ornidazole was influenced at the intestinal absorptive phase.

REFERENCES

1. Kolars JC, Lown KS, Schmeidlin-Ren P, Ghosh M, Wrighton SA, Merion RM, Watkins PB. CYP3A gene expression in human gut epithelium. Pharmacogenetics 1994; 4: 247-259.
2. Benet LZ, Wu CY, Hebert MF, Wacher VJ Intestinal drug metabolism and antitransport process: a potential shift in oral drug delivery. J Contr Release 1996; 39: 139-143.
3. Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein - implication for drug delivery and activity in cancer chemotherapy. Mol Pharmacol 1995; 13: 129-134.
4. Dunne RL, Dunn LA, Upcroft P, O'Donoghue PJ, Upcroft JA. Drug resistance in the sexually transmitted protozoan *Trichomonas vaginalis*. Cell Res 2003; 13: 239-249.
5. Siegsmund MJ, Cardarelli C, Aksentijevich I, Sugimoto Y, Pastan I, Gottesman MM. Ketoconazole effectively reverses multidrug resistance in highly resistant KB cells. J Urol 1994; 151: 485-491.
6. Yumuto R, Teruo M, Yuko N, Risa H, Junya N, Mikihisa T. Transport of rhodamine 123, a P-glycoprotein substrate, across rat intestine and Caco-2 cell monolayers in the presence of cytochrome P-450 3A related compounds. J Pharmacol Exp Ther 1998; 289: 149-152.
7. Soma Shekar M, Vidya Sagar J, Narsaiah N, Krishna DR. Validated HPLC method for the determination of ornidazole in human serum and urine. Indian J Pharm Sci; in press.
8. Ersoz G, Karasu Z, Akoarca US, Gunsar F, Yuce G, Batur Y. Nitroimidazole-induced chronic hepatitis. Eur J Gastroenterol Hepatol 2001; 13: 963-966.
9. Tabak F, Ozaras R, Erzin Y, Celik AF, Ozbay G, Senturk H. Ornidazole-induced liver damage: report of three cases and review of the literature. Liver Int 2003; 3: 351-354.
10. Raether LW, Hänel LH. Nitroheterocyclic drugs with broad-spectrum activity. Parasitol Res 2003; 90: S19-S39.
11. Ward KW, Stelman GJ, Morgan JA, Zeigler KS, Azzarano LM, Kehler JR, McSurdy-Freed JE, Proksch JW, Smith BR. Development of in vivo preclinical screen model to estimate absorption and first pass hepatic extraction of xenobiotics. II. Use of ketoconazole to identify P-glycoprotein/CYP3A-limited bioavailability in the monkey. Drug Metab Dispos 2004; 32: 172-177.

12. Trezise AE, Romano PR, Gill DR, Hyde SC, Sepulveda FV, Buchwald M, Higgins CF. The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. EMBO J 1992; 11: 4291-4303.
13. Ramamurthy L, Kulkarni RD, et al. Relative bioavailability of two brands of ornidazole in twelve healthy human volunteers. J Assoc Physician India 2002; 50: 1149-1152.
14. Wu CY, Benet LZ, Hebert MF, Gupta SK, Rowland M, Gomez DY, Wacher VJ. Differentiation of absorption and first pass gut and hepatic metabolism in humans: studies with cyclosporine. Clin Pharmacol Ther 1995; 58: 492-497.
15. Salphati L, Benet LZ. Effect of ketoconazole on digoxin absorption and disposition in rat. Pharmacology 1998; 56: 308-313.