EFFECT OF RIFAMPICIN PRETREATMENT ON THE TRANSPORT ACROSS RAT INTESTINE AND ORAL PHARMACOKINETICS OF ORNIDAZOLE IN HEALTHY HUMAN VOLUNTEERS

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SUMMARY

Increased exsorption of ornidazole was observed from different parts of the small intestine of the rat after pretreated with rifampicin and sodium butyrate by the everted sac method. Based on the *in vitro* studies the effect of rifampicin pretreatment on the pharmacokinetics of ornidazole was investigated in eight healthy male volunteers. After an overnight fast, 500 mg ornidazole was administered to the volunteers, either alone or after 6 days pretreatment with a once daily dose of 600 mg rifampicin. Serum concentrations of ornidazole were estimated by reverse phase HPLC. Pharmacokinetic parameters were determined based on non-compartmental model analysis using the computer program Win Nonlin 1.1. Rifampicin preteatment resulted in a significant decrease in AUC, C_{max} and $t_{½}$, by 21.16%, 20.43% and 18.11%, respectively. Clearance was increased significantly by 32.14%. This may be due to increased induction of cytochrome P450 enzymes and/or increased expression of P-glycoprotein. This inter-

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action may have clinical significance when ornidazole is co-administered with rifampicin in chronic treatment conditions, such as tuberculosis, leprosy and other infections of joints, bones, etc.

KEY WORDS

rifampicin, ornidazole, pharmacokinetics, P-glycoprotein, cytochrome P450 enzymes, drug interaction

INTRODUCTION

Ornidazole, α-chloromethyl-2-methyl-5-nitroimidazole-1-ethanol, has antiprotozoal and antibacterial properties against anaerobic bacteria. Ornidazole may be used in the treatment of hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of the urogenital tract, and bacterial vaginosis /1,2/. It is also used in the treatment and prophylaxis of susceptible anaerobic infections in dental and gastro-intestinal surgery and in other mixed aerobic-anaerobic infections. Ornidazole is also advocated in combination with other drugs in the management of *Helicobacter pylori* in duodenal ulcers. There are no reports in the literature about the CYP isoenzymes involved in the metabolism of ornidazole. Ornidazole is mainly metabolized to α-chloromethyl-2-hydroxymethyl-5-nitoimidazole-1-ethanol (M1) and 3-(2-methyl-5-nitroimidazole 1-yl)-1,2-propane-diol (M4) in the liver /3/.

Rifampicin is the drug of choice in both mycobacterial (tuberculosis and leprosy) and non-mycobacterial infections (reviewed by Vesley *et al.* /4/). Rifampicin is a useful drug for several types of bacterial infections because of its broad spectrum activity and excellent tissue penetration.

Rifampicin is recognized as a potent inducer of both CYP3A and P-glycoprotein (P-gp). Rifampicin is a known inducer of several drug metabolizing enzymes /5-7/ and decreases the plasma concentration of many drugs, including CYP3A probe drugs, such as digoxin, cyclosporin A and talinolol /8-11/.

The objective of the present investigation was to study the influence of rifampicin on the pharmacokinetics of ornidazole in healthy human volunteers.

MATERIALS AND METHODS

Drugs and chemicals

Ornidazole tablet (500 mg) Orni® and ornidazole pure substance were obtained from Zydus Cadila HC Ltd, Ahmedabad, India; rifampicin capsule (600 mg) R-cin® was obtained from Lupin Ltd, Aurangabad, India; tinidazole pure substance was obtained from Aristo Pharmaceuticals Ltd, Mumbai, India; Dulbecco's phosphate buffer (pH 7.4) was obtained from Hi Media (India) Limited, Mumbai. DMSO and glucose were obtained from E. Merck (India) Limited, Mumbai. All solvents used were of HPLC grade.

Everted sac study

Male Wister rats were fasted overnight with free access to water before the experiments. The whole small intestine was flushed with 50 ml of ice-cold saline with the animal under anesthesia with pento-barbital (30 mg/kg i.p.). The rat was exsanguinated, and the isolated small intestine was divided into three segments of equal length. Each segment was everted, and a 10-cm long everted sac was prepared. 10 mg/ml of ornidazole (probe drug) was dissolved in pH 7.4 isotonic Dulbecco's PBS (D-PBS) containing 25 mM glucose and 4% DMSO. The probe drug solution (1 ml) was introduced into the everted sac (serosal side), and both ends of the sac were ligated tightly. The sac containing probe drug solution was immersed into 40 ml D-PBS containing 25 mM glucose and the same concentration of 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 a CO_2/O_2 mixture, the transport of ornidazole from the serosal to the mucosal surface across the intestine was measured by sampling the mucosal medium periodically for 90 minutes /12/.

In the induction study, rats were pretreated with rifampicin (60 mg/kg p.o. for 7 days) and sodium butyrate (0.5 mg/kg i.p. for 5 days). The transport of the ornidazole in the absence (control) and after induced conditions from the serosal to the mucosal surfaces across the intestine was measured by sampling the mucosal medium periodically for 90 minutes.

Subjects

Eight healthy male volunteers with a mean age of 27 ± 2 years (range 25 to 30 years), mean height 172.21 ± 0.30 cm (range 164.59 to 176.78 cm) and mean body weight 62.6 ± 5.8 kg (55 to 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 ethics committee approved the study protocol.

Protocol

After an overnight fast (approximately 12 h), eight healthy male volunteers participated in the study. The first part of the study consisted of oral administration of 500 mg ornidazole tablet alone. Blood samples (5 ml) were drawn from the antecubital vein at intervals of 0, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24 and 36 hours after administration of ornidazole.

The second part of the study was conducted after a washout period of two weeks. Rifampicin (R-cin® 600 mg, Lupin Limited, India) was administered daily once for 6 days. On day 7, a single tablet each of 500 mg of ornidazole and 600 mg of rifampicin were administered concomitantly. Blood samples were collected as described above and centrifuged at 3,000 rpm for 15 min, and the supernatant serum separated and stored at -20°C until analysis.

Analytical method

Ornidazole in the serum samples was estimated by reverse phase high pressure liquid chromatography (HPLC) /13/. The HPLC system (Shimadzu, Japan) consisted of an LC10AT solvent delivery module, SPD-10AVP UV visible spectrophotometer detector (Shimadzu, Japan), and a rheodyne injection port (Rheodyne, Cotati, CA, USA) with a 20 µl sample loop. The column used was Phenomenex, Gemini® column (USA) (stainless steel, reversed phase C-18 column) of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5µ diameter, 100 Å pore diameter.

The UV absorption of the eluent was monitored at 318 nm. The mobile phase consisted of 0.002 M acetate buffer:acetonitrile: methanol (70:20:10) with a flow rate of 1 ml/min. Sensitivity was set

at 0.001 a.u.f.s (absorbance units full scale). The retention times of the internal standard (tinidazole) and ornidazole in serum were 6.69 and 9.76 minutes, respectively. To 250 µl of serum samples, 20 µl of tinidazole (100 µg/ml) was added as an internal standard and vortexed for 2 min. An equal volume (250 µl) of methanol was added to serum samples for protein precipitation and vortexed on a cyclo-mixer for one minute and centrifuged at 13,000 rpm for 8 min using a Biofuge Fresco centrifuge (Heraeus, Germany). 20 µl of serum supernatant were taken into a Hamilton syringe and injected directly onto the HPLC column.

Pharmacokinetics

The pharmacokinetic parameters, peak serum concentrations (C_{max}) and time to reach peak concentration (t_{max}), were obtained directly from the concentration-time data. In the present study, AUC_{0-t} refers to the area under the curve from 0 to 36 h, which was determined by the linear trapezoidal rule, and $AUC_{0-\infty}$ refers to the AUC from 0 to infinity. The $AUC_{0-\infty}$ was calculated using the formula AUC_{0-t} +[C*/K], where C* is the concentration in µg/ml at the last time point and K is the elimination rate constant. The pharmacokinetic parameters, AUC, elimination half life ($t_{½}$), volume of distribution (V/f) and mean residence time (MRT), for each subject were estimated 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 rifampicin were compared by Student's paired t-test (paired data) 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 under conditions of induced of P-gp with rifampicin (a known CYP3A and

P-gp inducer) or sodium butyrate, a known P-gp inducer, was determined in duodenum, jejunum and ileal regions of rat small intestine. The time course of ornidazole transport across the everted sacs of duodenum, jejunum and ileum are shown in Figures 1-3 and Table 1.

Pretreatment with rifampicin or sodium butyrate increased the mean cumulative concentration in all three regions of rat intestine. Rifampicin pretreatment increased the mean cumulative concentrations from 79.13 ± 1.39 to 110.36 ± 8.78, 109.45 ± 2.69 to 147.74 ± 3.97, and 136.36 ± 3.26 to 175.65 ± 13.73 μg/ml; and with sodium butyrate, 79.13 ± 1.39 to 125.76 ± 12.06, 109.45 ± 2.69 to 138.34 ± 9.4, and 136.36 ± 3.26 to 169.31 ± 6.65 μg/ml, in duodenum, jejunum and ileum, respectively. Rifampicin pretreatment increased the mean cumulative efflux of ornidazole by 39.46% ($p > 0.05$), 34.98% ($p < 0.05$) and 28.81% ($p < 0.05$) from duodenal, jejunal and ileal regions, respectively. Sodium butyrate increased the mean cumulative transport by 58.92% ($p < 0.05$), 26.39% ($p < 0.05$) and 24.16% ($p < 0.05$), respectively, from the duodenal, jejunal and ileal regions.

All volunteers tolerated the treatments well and there were no severe adverse affects during the study period. Mean ornidazole serum concentration before and after pretreatment with rifampicin are shown in Figure 4 and the pharmacokinetic parameters of ornidazole are presented in Table 2. The individual pharmacokinetic data of AUC and C_{max} of ornidazole before and after pretreatment with rifampicin in healthy human volunteers are shown in Table 3.

There was a statistically significant difference in pharmacokinetic parameters C_{max}, $t_{½}$, Vd/f, MRT, AUC and AUMC. No statistically significant difference was observed in t_{max}. However, some change was observed in this parameter after pretreatment with rifampicin, though this was not statistically significant.

After pretreatment with rifampicin, C_{max} decreased from 4.2 ± 0.71 to 3.34 ± 0.66 μg/ml, and $AUC_{0-\infty}$ decreased from 96.14 ± 10.36 to 75.8 ± 9.03 μg/h/ml. AUMC decreased from 2,835.64 ± 568.78 to 1,938.9 ± 602.8, MRT decreased from 29.4 ± 4.72 to 24.49 ± 4.43, $t_{½}$ decreased from 20.62 ± 3.55 to 16.89 ± 3.66 h. t_{max} increased from 1.5 ± 0.6 to 1.68 ± 0.88 h, Ke increased from 0.03 ± 0.01 to 0.04 ± 0.01 h^{-1}.

Rifampicin pretreatment decreased C_{max}, $AUC_{0-\infty}$, AUMC, MRT and $t_{½}$ by 20.43% ($p < 0.0007$), 21.16% ($p < 0.0007$), 31.63% (p

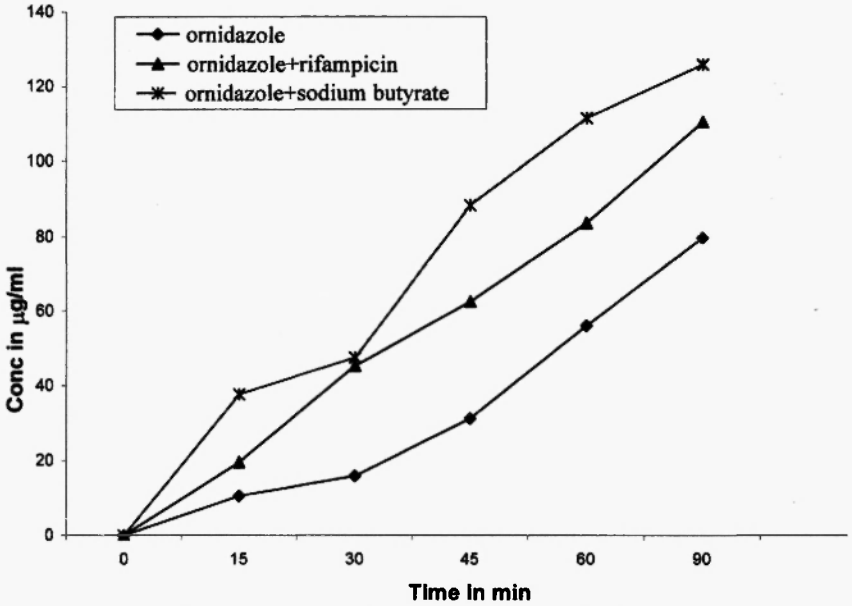

Fig. 1: Mean cumulative transport of ornidazole in duodenal everted sacs in Wistar rats.

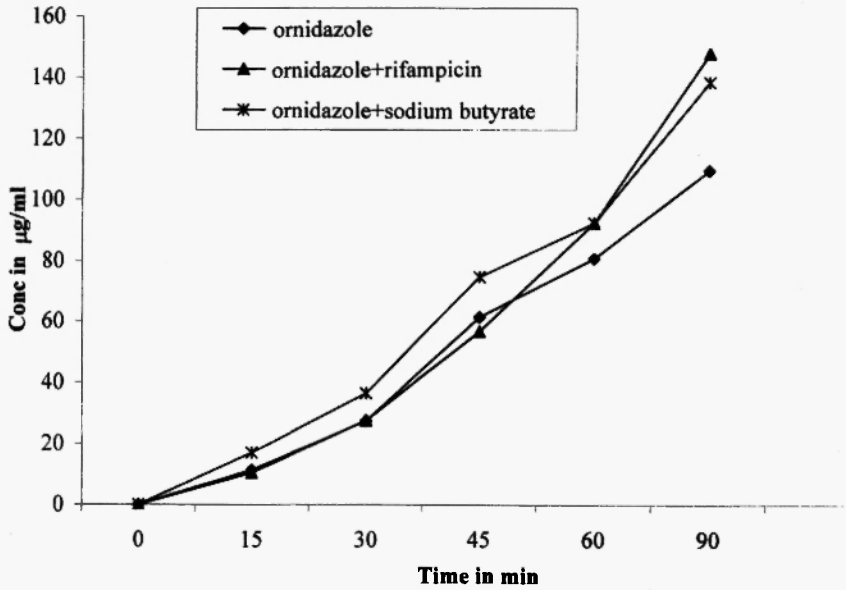

Fig. 2: Mean cumulative transport of ornidazole in jejunum everted sacs in Wistar rats

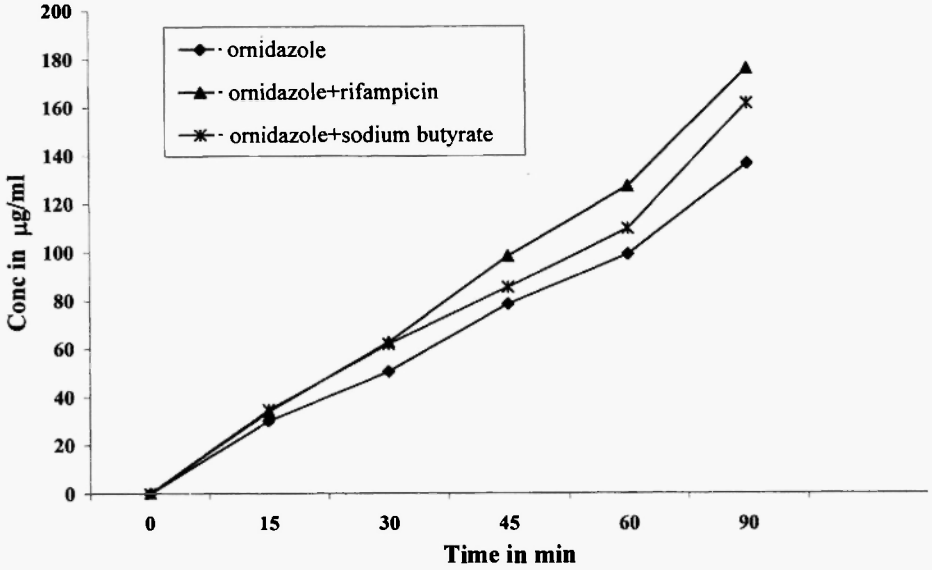

Fig. 3: Mean cumulative transport of ornidazole in ileal everted sacs in Wistar rats.

TABLE 1

Cumulative efflux concentrations (µg/ml) and percentage change in transport in intestinal everted sacs in Albino Wister rats

Region	Ornidazole alone	Ornidazole + rifampicin	Ornidazole + sodium butyrate
Duodenum	79.13 ± 1.39	110.36 ± 8.78 (39.46%)	125.76 ± 12.06* (58.92%)
Jejunum	109.45 ± 2.69	147.74 ± 3.97* (34.98%)	138.34 ± 9.4* (26.39%)
Ileum	136.36 ± 3.26	175.65 ± 13.73* (28.81%)	169.31 ± 6.65* (24.16%)

Means ± SD (n = 3).
* $p < 0.05$ (t-test).

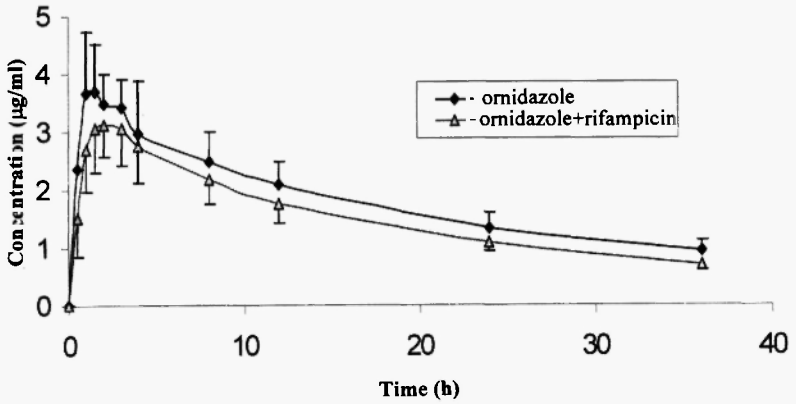

Fig. 4: Mean ornidazole serum concentrations before and after pretreatment with rifampicin in healthy human volunteers (n = 8).

TABLE 2

Pharmacokinetic parameters of ornidazole with percent change in healthy human volunteers alone and with pretreatment

Pharmacokinetic parameter	Ornidazole alone	Ornidazole + rifampicin
C_{max} (µg/ml)	4.2 ± 0.71	3.342 ± 0.66 * (-20.43 %)
t_{max} (h)	1.5 ± 0.61	1.68 ± 0.88 (12%)
$AUC_{0-\infty}$ (µg/ml/h)	96.14 ± 10.36	75.8 ± 9.03* (-21.16%)
K_e (h^{-1})	0.03 ± 0.01	0.04 ± 0.01* (14%)
$t_{½}$ (h)	20.62 ± 3.55	16.89 ± 3.66* (-18.11%)
$V_{d/f}$	156.11 ± 31.44	175. 3 ± 45.3* (12.29 %)
MRT (h)	29.4 ± 4.72	24.49 ± 4.43* (-16.71%)
AUMC (µg/ml/h)	2,835.64 ± 568.78	1938.9 ± 602.8* (-31.63%)

Means ± SD (n = 8).
* $p < 0.05$ (paired t-test).

TABLE 3

Individual pharmacokinetic data of ornidazole before and after pretreatment with rifampicin in healthy human volunteers

Volunteer	C_{max} (μg/ml)		$AUC_{0-\infty}$ (μg/ml/h)	
	Before pretreatment	**After pretreatment**	**Before pretreatment**	**After pretreatment**
1	5.4807	3.876	94.33	72.94
2	4.2875	3.454	107.21	84.63
3	4.2875	3.4713	106.41	78.18
4	3.7135	2.6153	100.79	78.41
5	3.516	2.3850	78.65	57.23
6	3.2067	2.8385	80.53	55.65
7	5.0185	4.2736	103.46	80.37
8	4.0214	3.5785	97.72	74.25

<0.0040), 20.78% ($p > 0.05$) and 21.09% ($p > 0.05$), respectively, and increased Ke by 14% ($p > 0.0017$). t_{max} increased by 12%, but did not reach statistical significance

DISCUSSION

The 5-nitroimidazole derivative, metronidazole, is reported to be a substrate of P-gp, and it is metabolized by CYP3A4 of the intestine. The same is reported for tinidazole. So it was assumed that ornidazole might also be a substrate of P-gp. There is a report that resistance of *T. vaginalis* to 5-nitroimidazoles is due to overexpression of P-gp /14/.

This study was planned to investigate whether the transport of ornidazole at the intestinal level is influenced by rifampicin or sodium butyrate (P-gp inducers) in rat everted sacs of duodenum, jejunum and ileum. Results of the *in vitro* study revealed that ornidazole transport across the small intestine is affected by these known P-gp inducers. In this study, the mean ± SD cumulative exsorption concentrations of ornidazole were increased after pretreatment with rifampicin or

sodium butyrate. This observation indicates the role of P-gp, an efflux pump, in ornidazole absorption. Yumoto *et al.* /12/ observed a linear relationship between *in vitro* everted sac and *in vivo* results, and suggested that P-gp related drug-drug interactions *in vivo* can be predicted by *in vitro* everted sac studies. These authors 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 a report that resistance to 5-nitroimidazoles in *T. vaginalis* is because of overexpression of P-gp, a multidrug resistance protein which is similar to human P-gp /13/, this study was conducted to investigate the effect of rifampicin on the oral pharmacokinetic parameters of ornidazole.

We observed the effect of rifampicin (600 mg p.o. once daily for 7 days), a known P-gp and CYP3A inducer, on the oral pharmacokinetics of ornidazole. In the study of healthy human volunteers, all volunteers tolerated the treatment well and there were no dropouts or complaints of any severe or minor adverse effects of ornidazole or rifampicins. In earlier studies with ornidazole /15/, C_{max} 32.67 ± 4.45 μg/ml with $AUC_{0\text{-}12}$ of 261.67 ± 77 μg/ml/h were observed after a single 1.5 g dose. In the present study, a similar level was not observed with a single 500 mg dose (C_{max} 4.1914 ± 0.71μg/ml and $AUC_{0\text{-}36}$ of 67.64 ± 8.38), i.e. the decrease in these parameters with decrease of dose is not linear.

The observed pharmacokinetic data of ornidazole, i.e. decrease of C_{max}, AUC, $t_{½}$, and MRT, after pretreatment with rifampicin may be due to rifampicin's action as a P-gp inducer. Intestinal P-gp is known to be localized in the brush border membrane to pump drugs from the serosal side into the luminal side. Induction of intestinal P-gp may increase drug exsorption and thus decrease net drug absorption. The significant pharmacokinetic changes observed in C_{max} have clearly indicated that the absorption of ornidazole is influenced by rifampicin. Here, the possible role of CYP3A on the pharmacokinetics of ornidazole cannot be ruled out directly, as rifampicin is also an inducer of CYP3A. Thus, the observed changes might be due to induction of P-gp and/or CYP3A in the intestine. There are several similar reports: Greiner *et al.* studied the role of intestinal P-gp in the interaction of digoxin and rifampicin, and found that concomitant

rifampicin therapy may affect digoxin disposition in humans by induction of P-gp /8/.

The everted sac studies in the rat have clearly indicated that the alterations in the oral pharmacokinetics could be due to changes at the absorption site, i.e. in the intestine. In the human volunteer study, we observed a decrease in C_{max} and increase in t_{max}. Since the efflux of ornidazole from blood to intestine is increased by rifampicin, there is slower absorption of the drug, and hence an increase in t_{max}.

CONCLUSION

Based on these results, it is proposed that rifampicin may alter the pharmacokinetics of ornidazole by increased expression of P-gp and/or induction of cytochrome P450 (CYP) enzymes. This interaction may be of clinical significance when ornidazole is co-administered with rifampicin in chronic treatment conditions, such as tuberculosis, leprosy and other infections of joints, bones, etc.

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