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Influence of Rifampicin Pretreatment on the Pharmacokinetics of Tinidazole in Healthy Male Volunteers

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Abstract

Objective: To investigate the effect of pretreatment with rifampicin (rifampin) on the pharmacokinetics of tinidazole in healthy male volunteers.

Design: Before/after non-blinded investigation conducted in healthy volunteers.

Study Participants: 12 healthy male volunteers with a mean age of 24 ± 3 years.

Methods: After an overnight fast, tinidazole (500mg tablet) was administered to the volunteers, either alone or after a 5-day pretreatment period with a once-daily dose of rifampicin 600mg (2 × 300mg capsules) under direct observation. Serum concentrations of tinidazole were measured by reverse-phase high performance liquid chromatography. Pharmacokinetic parameters were determined from non-compartmental model analysis using the computer program RAMKIN.

Results: A significant difference was observed in area under the concentration-time curve (AUC) from 0 to 48 hours (254.77 ± 31.46 *vs* 208.07 ± 25.57 mg·h/L, $p < 0.0001$), AUC from 0 to infinity (299.86 ± 47.70 *vs* 231.54 ± 36.19 mg·h/L, $p < 0.0001$], elimination half-life (16.98 ± 2.73 *vs* 13.93 ± 3.45h, $p < 0.0018$) and clearance (27.62 ± 3.61 *vs* 35.82 ± 4.95 ml/h/kg, $p < 0.0001$) of tinidazole administered before and after rifampicin pretreatment. However, peak concentration (C_{max}), time to reach C_{max} and apparent volume of distribution were not affected significantly.

Conclusions: Rifampicin pretreatment reduced the AUC of tinidazole by 23% and increased the clearance by 29%. This may be due to increased metabolism of tinidazole as a result of the induction of cytochrome P450 2C9 and 3A4 in liver/intestine and/or P-glycoprotein–mediated exsorption into the intestines. This interaction, however, may not have significant clinical relevance and does not warrant dosage adjustment because the extent of alteration in bioavailability of tinidazole is less than 25%.

Tinidazole is a 5-nitroimidazole with selective activity against anaerobic bacteria and protozoa. It is bactericidal at low concentrations and its spectrum covers most anaerobic bacteria and some capnophilic micro-organisms. Clinical studies have shown that tinidazole is efficacious in the treatment of anaerobic infections, including respiratory tract infections, intra-abdominal sepsis and obstetric and gynaecological infections. Since tinidazole has no activity against aerobic bacteria, it must be combined with other antibacterial agents in the treatment of mixed infections involving aerobic and anaerobic bacteria. Tinidazole has also been used successfully alone or in combination with other antimicrobial agents for prophylaxis in patients undergoing elective colonic and abdominal surgery, emergency appendectomy and gynaecological surgery.[1]

Rifampicin (rifampin) is known as an effective treatment choice in mycobacterial infections (tuberculosis and leprosy), and its usefulness in non-mycobacterial infections has recently been reviewed by Vesley et al.[2] They pointed out that it might be effective in combination with other antibacterials when conventional therapies are not effective. It is clinically effective against a wide variety of micro-organisms including *Staphylococcus aureus*, *Legionella pneumophila*, group A *Streptococcus*, *Brucella* spp., *Haemophilus influenzae* and *Neisseria meningitidis*, and also has *in vitro* activity against penicillin-resistant *Streptococcus pneumoniae*, *N. gonorrhoeae*, *Chlamydia trachomatis*, *H. ducreyi* and many Gram-negative rods. Rifampicin is a useful drug for several types of bacterial infections because of its broad spectrum of activity and excellent tissue penetration.

There is a possibility of the occurrence of diarrhoea/amoebiasis or other protozoal infections in patients with mycobacterial infections receiving long-term therapy with rifampicin. In these cases tinidazole can be prescribed, and it is thus important to consider the effect of rifampicin-mediated enzyme induction on the pharmacokinetics of tinidazole.

Tinidazole is metabolised by the cytochrome P450 2C9 (CYP2C9) and CYP3A4 isozymes of the liver microsomal enzymes.[3,4] Rifampicin is a well known inducer of both isozymes, but there is no report available on the effect of rifampicin-mediated enzyme induction on the pharmacokinetics of tinidazole.

A recent study investigated the effect of phenobarbital-mediated enzyme induction on tinidazole metabolism in rats. The authors showed that pretreatment with phenobarbital increased the metabolism of tinidazole by about 62%.[5] If a similar interaction occurs between rifampicin and tinidazole in humans, there would need to be a drastic dosage adjustment for tinidazole. As there is an overlap in the mechanism of enzyme induction by phenobarbital and rifampicin,[6] and a recent increase in the frequency of co-prescription of rifampicin and tinidazole, a study of the effect of rifampicin pretreatment on the pharmacokinetics of tinidazole in humans was considered important.

The pharmacokinetic parameters of tinidazole would be expected to be most influenced at steady state. It would have been more meaningful to observe the effect of rifampicin pretreatment on the steady-state pharmacokinetics of tinidazole, which would reflect the real clinical situation, but due to practical limitations, the present pharmacokinetic study was conducted following single-dose administration of tinidazole, before and after rifampicin pretreatment.

Subjects and Methods

Study Participants

Twelve healthy male volunteers with a mean age of 24.3 ± 3.3 years (range 20 to 30 years), a mean height of 172.4 ± 5.0cm (range 165 to 180cm) and a mean bodyweight of 61.8 ± 6.6kg (range 54 to 70kg) participated in the study after undergoing a thorough physical examination. The volunteers were briefed about the study and written informed consent was obtained from all of them. The local ethics committee approved the study protocol.

The volunteers had no history of ill health during the preceding 6 months and none had taken

any medication for at least 15 days prior to the administration of tinidazole in the study. Volunteers were excluded from the study if they had food allergies or were allergic to tinidazole or rifampicin.

Methods

After an overnight fast (approximately 12 hours), each volunteer received a tinidazole 500mg tablet (Tiniba 500®; Cadila Healthcare Limited, Ahmedabad, India) with 200ml of water.

Venous blood samples of approximately 5ml were drawn from the antecubital vein at 0, 1, 2, 3, 4, 6, 8, 12, 24, 36 and 48 hours after drug administration. The blood was allowed to clot and centrifuged for 10 minutes at 3000 rpm (R8C; Remi Instruments, Mumbai, India). Serum was separated into amber-coloured vials and stored at −20°C until the analysis was performed. A once-daily dose of rifampicin 600mg (2 × 300mg capsules; R-cin 300®; Lupin Laboratories, Mumbai, India) was given for 5 consecutive days (from day 4 to 8) under direct observation. On day 9, tinidazole 500mg was given again and the sample collection was repeated.

Tinidazole in the serum samples was estimated by reverse-phase high performance liquid chromatography (HPLC) [Chaluvadi et al., unpublished work]. The HPLC system (Shimadzu, Japan) consisted of an LC-10AT solvent delivery module and a SPD-10A UV-Visible Spectrophotometric Detector. The mobile phase consisted of methanol : acetonitrile : 0.002 mol/L potassium dihydrogen orthophosphate buffer (7.5 : 7.5 : 85) with a flow rate of 1 ml/min. The column used was Altech C-18 (stainless steel column of length 25cm and internal diameter of 4.6mm packed with porous silica spheres of 5µm diameter, 100Å pore diameter) and the eluent was monitored at 320nm.

Metronidazole (6µl of 100 mg/L) was added to 0.3ml of serum as the internal standard and the mixture was shaken well on a vortex-mixer. An equal volume (0.3ml) of acetonitrile was added for protein precipitation, the mixture was shaken on a vortex-mixer for 1 minute and centrifuged at 3000 rpm for 10 minutes. 20µl of the supernatant was injected onto the column. A linearity calibration curve in the range of 0.08 to 100 mg/L was also established ($r^2 = 0.99$) in serum matrix. Interassay variability was determined at three different concentrations, 1, 10 and 30 mg/L, with coefficients of variance of 8.13, 5.92 and 3.18%, respectively.

The pharmacokinetic parameters peak plasma concentration (C_{max}), time to reach peak concentration (t_{max}), area under the plasma concentration-time curve (AUC), elimination half-life ($t_{½\ \beta}$), apparent volume of distribution (Vd/F) and apparent systemic clearance (CL/F) for tinidazole were obtained for each subject by using the computer program RAMKIN (DR Krishna, unpublished work) meant for calculation of model-independent parameters. In the present study, $AUC_{0\text{-}t}$ refers to the AUC from 0 to 48 hours and $AUC_{0\text{-}\infty}$ refers to the AUC from 0 to infinity. The $AUC_{0\text{-}t}$ value is more than 80% of the $AUC_{0\text{-}\infty}$ in the present study and hence the extrapolation to ∞ is valid. $AUC_{0\text{-}\infty}$ was calculated using the formula $AUC_{0\text{-}t} + (C_{l\text{-}ast}/K_{el})$, where C_{last} is the concentration in mg/L at the last timepoint and K_{el} is the elimination rate constant. Because tinidazole was administered orally, estimates of volume of distribution and clearance are uncorrected for the fraction of drug absorbed (F). However, F was assumed to be 1.0 on the basis of earlier reports on the pharmacokinetics of tinidazole.[7,8]

Statistical Analysis

The mean pharmacokinetic parameters obtained when tinidazole was given alone were compared with those obtained after rifampicin pretreatment using Student's t-test (paired data). A value of $p < 0.05$ was considered to be statistically significant.

Results

The mean ± standard deviation (SD) serum concentrations of tinidazole at different timepoints before and after rifampicin pretreatment are shown in figure 1. The pharmacokinetic parameters of tinidazole are presented in table I.

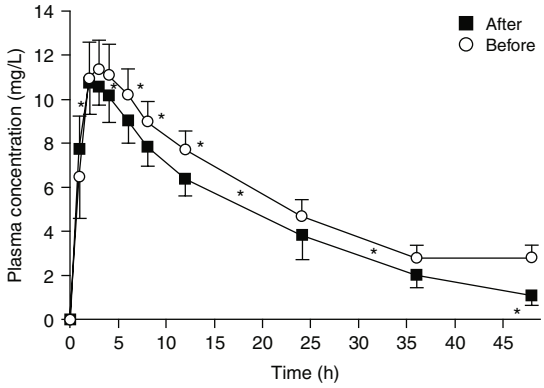

Fig. 1. Mean (± standard deviation) plasma concentration versus time profiles of tinidazole before and after pretreatment with rifampicin. * indicates significantly different ($p < 0.01$).

There was no statistically significant difference in the t_{max} and C_{max} of tinidazole in the volunteers before and after rifampicin pretreatment. This suggests that rifampicin pretreatment did not influence the rate of absorption of tinidazole. Although there was a 21% reduction in t_{max} after rifampicin pretreatment, this did not achieve statistical significance because of considerable variance.

Rifampicin pretreatment reduced the $AUC_{0-\infty}$ of tinidazole by 23%, increased the clearance by 29% and reduced $t_{½\beta}$ by 18%. These effects were statistically significant. The decreased bioavailability with unchanged C_{max} suggests that rifampicin pretreatment did not influence the rate of absorption but the extent of availability decreased due to significant induction of metabolism.

Discussion

The apparently increased metabolism and/or excretion of tinidazole after pretreatment with rifampicin may be due to increased expression of CYP2C9 and CYP3A4 in liver/intestine, and/or increased P-glycoprotein-mediated exsorption into the intestines.

From the literature it is evident that metronidazole, the prototype drug of the nitroimidazoles, is able to inhibit carbamazepine metabolism and elevate plasma carbamazepine concentrations to potentially toxic levels by inhibiting CYP3A4.[3] Metronidazole is also known to inhibit CYP2C9 and elevate phenytoin concentrations.[4] These reports suggest that under steady-state conditions, the induction of CYP2C9 and CYP3A4 by rifampicin and the consequent metabolism of tinidazole may be offset, to some extent, by the self-inhibition of these enzymes by tinidazole.

The assumption about the possible role of P-glycoprotein in the increased metabolism of tinidazole after rifampicin pretreatment was based on a report[9] where the bioavailabilities of digoxin were determined after its administration orally and intravenously to healthy volunteers before and after rifampicin pretreatment. The AUC of oral digoxin was significantly lower during rifampicin

Table I. Pharmacokinetic parameter values of tinidazole in human volunteers before and after pretreatment with rifampicin. Values are means ± SD (n = 12)

Parameter and units	Before	After	p-Value
C_{max} (mg/L)	11.71±1.43	11.39 ± 1.55	>0.05
t_{max} (h)	2.75 ±0.87	2.17 ± 0.94	>0.05
AUC_{0-t}(mg•h/L)	254.77 ± 31.46	208.07 ± 25.57	<0.0001
$AUC_{0-\infty}$ (mg•h/L)	299.86 ± 47.70	231.54 ± 36.19	<0.0001
$t_{½\beta}$ (h)	16.98 ± 2.73	13.93 ± 3.45	<0.0018
CL/F (ml/h/kg)	27.62 ± 3.61	35.82 ± 4.95	<0.0001
Vd/F (ml/kg)	667.98 ± 82.36	702.00 ± 115.23	>0.05

AUC_{0-t} = area under the concentration-time curve from 0 to 48h; **$AUC_{0-\infty}$** = area under the concentration-time curve from 0 to infinity; **C_{max}** = peak serum concentration; **CL/F** = apparent systemic clearance; **SD** = standard deviation; **$t_{½\beta}$** = elimination half-life; **t_{max}** = time to reach C_{max}; **Vd/F** = apparent volume of distribution.

pretreatment but the effect was less pronounced after intravenous administration of digoxin. Rifampicin pretreatment increased P-glycoprotein levels in duodenal biopsies by 3.5 ± 2.1-fold. The investigators concluded that a rifampicin-digoxin interaction appears to occur at the level of the intestine and that induction of intestinal P-glycoprotein could play a role in the interaction. This, however, requires further experimental evidence regarding the increased exorption of tinidazole in to the intestines.

Conclusions

Based on the results of the present investigation we suggest that, as well as increased metabolism of tinidazole because of induction of CYP2C9 and CYP3A4, there is an increased expression of P-glycoprotein, which might have resulted in decreased bioavailability of tinidazole. This, however, requires further experimental evidence.

The increase in metabolism of tinidazole after rifampicin pretreatment of healthy volunteers is 23%, as compared with 62% after phenobarbital pretreatment of rats.[5] The clinical significance of this interaction requires evaluation.

Metabolic drug interactions are most likely to be important if elimination is by a single process, and are less relevant if two or more routes are available unless the alternative pathways are saturable or give rise to toxic products.[10] This is an important reason for drug interactions due to induction or inhibition being less prominent clinically than might be expected.

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