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Effect of silymarin supplement on the pharmacokinetics of ornidazole in healthy volunteers

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Abstract

The objective of investigation was to study the influence of silymarin pretreatment on the pharmacokinetics of ornidazole in healthy human volunteers. A single oral dose of ornidazole (500 mg) tablet was administered to nine volunteers, either alone or after 9 days pretreatment with a daily dose of silymarin (140 mg) capsule after an overnight fasting. Blood samples were collected from antecubital vein for 48 h, and the serum concentration of ornidazole was determined using HPLC. Silymarin treatment resulted in the significant increase in $AUC_{(0-\infty)}$, $C_{max.}$ and $t_{1/2}$ by 30.25 %, 16.25 %, and 7.697 % respectively. Silymarin, a mixture of flavonolignans may alter the pharmacokinetics of ornidazole, probably by inhibiting the cytochrome P450 enzymes and p-glycoprotein.

Keywords: Silymarin, ornidazole, healthy human volunteers, pharmacokinetics.

Introduction

Flavonoids are present in fruits, vegetables, plant-derived beverages, and many herbal products. The average daily intake of total flavonoids in the U.S. diet is estimated to 0.2–1 g (Kuhnau 1976). Given the increasing availability of flavonoid-containing dietary supplements and herbal products, possible flavonoid–drug and flavonoid–flavonoid interactions may become more likely. Silymarin is the polyphenolic fraction from the seeds of milk thistle (Silybum marianum) and is composed of various flavonoids, including silybin (major component), silydianin, and silychristine (Lorenz et al. 1984, Mayer et al. 2005). Silymarin has been used as a supplementary treatment to protect the liver and to treat liver diseases such as gallbladder disorders, hepatitis, cirrhosis, and jaundice (Mayer et al. 2005, Ball and Kowdley 2005). *In vitro* reports suggest that silymarin has the potential to influence the drug metabolism by decreasing the metabolic activity of cytochrome P450 3A4, a ubiquitous enzyme responsible for hepatic and intestinal metabolism (Beckmann-knopp et al. 2000, Zuber et al. 2002). It may also alter drug absorption, distribution, and elimination through inhibition of P-glycoprotein (Zhang et al. 2002).

Ornidazole (ORN), a nitroimidazole derivative is used in the treatment of hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of urogenital tract and bacterial vaginosis. It is also used in the treatment and prophylaxis of susceptible anaerobic infections. Ornidazole is mainly metabolized in the liver to α–(chloromethyl)-2-hydroxymethyl-5-nitroimidazole-1-ethanol (M1) and 3-(2-methyl -5-nitroimidazole 1-yl)-1,2-propane-diol (M4) (Schwartz et al. 1979).

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In our previous investigation, ORN was found to be a substrate for both P-glycoprotein (P-gp) and cytochrome P4503A (CYP3A) (Kumar et al. 2007, Ramesh et al. 2006).

P-gp, a 170 KDa plasma membrane glycoprotein encoded by human MDR1 gene and murine mdr 1a, mdr 1b, and mdr 2 genes, belongs to the super family of ATP binding cassette transporters (Gottesman et al. 2000). P-gp functions as energy dependent efflux pump that lowers intracellular drug concentrations and when expressed in tumor cells causes the MDR phenotype by active extrusion of a wide range cancer chemotherapeutic drugs. In addition to being expressed in tumor cells, P-gp is also expressed in healthy tissues like intestine, liver, kidney, pancreas, adrenals, and brain (Cordon-Cordo et al. 1990, Sugawara et al. 1988). It transports structurally and functionally diverse compounds and several lines of evidence indicate that most of the substrates of P-gp are hydrophobic, and/or organic cations at physiological pH. It has been postulated that the drug transporting P-gp and CYP3A are functionally linked components of the xenobiotic detoxification cascade that limits the bioavailability of several drugs. There is a strong overlap between substrate specificities and tissue distribution of P-gp and CYP 3A (Wacher et al. 1995).

The objective of present investigation is to study the influence of silymarin pretreatment on the pharmacokinetics of ornidazole in healthy human volunteers.

Materials and Methods

Materials

Ornidazole and tinidazole pure drugs were kindly gifted by Zydus Cadila Health Care Ltd, Ahmedabad, India and Aristo Pharmaceuticals Ltd., Mumbai, India., respectively; Ornidazole (500 mg), ORNI® of Zydus Cadila Health Care Ltd and Silymarin (140mg) Sivylar®, of Ranbaxy laboratories, Delhi (India) were purchased from local market. All solvents used were of HPLC grade and the other chemicals used were of analytical grade and were purchased from Merck, Mumbai, India.

Human Volunteer Study

The pharmacokinetic study was conducted in healthy human volunteers with the permission from institutional ethical committee of University College of Pharmaceutical Sciences, Kakatiya University, India. Nine healthy male volunteers with an age of 24.89 ± 2.67 years (range 21 to 29 years), a mean height of 167.1 ± 5.5 cm (range 160.0 to 175.3 cm) and mean body weight of 60.0 ± 4.98 kg (52 to 65 kg) were participated in the study. The volunteers were briefed about the study and written informed consent was obtained from all the volunteers. The volunteers participated were non-smokers and non-alcoholics and avoided citrus fruit products for one week before and throughout the study period.

In the first phase, all the volunteers were given an oral administration of ornidazole (500 mg) tablet alone. The blood samples (3 mL) were collected from antecubital vein at pre-dose, 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 h, after the administration of ornidazole. The second part of the study was conducted after washout period of two weeks. Silymarin (140 mg) capsule was administered once daily for 9 days and on day 10, single tablet of 500 mg of ornidazole was administered. Blood samples were collected as described above and centrifuged at 3000 rpm for 15 min and the supernatant serum was separated and stored at -80 ° C (Polar 340V, Angelantoni Industrie, S.P.A) until analysis.

Quantification of Ornidazole

Ornidazole in serum samples was estimated by reversed phase high performance liquid chromatography (HPLC) method (Soma et al. 2005) The HPLC system (Shimadzu, Japan) consisted of an LC10AT solvent delivery module, UV-visible spectrophotometer detector (SPD-10AVP). The mobile phase consisted of acetonitrile: 2 µM ammonium acetate buffer, pH 4.8: methanol (70:20:10) with a flow rate of 1ml/min. The column used was Phenomenex, Gemini™, U.S.A (RP C18 column; 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5 µm diameter) and the eluent was monitored at 318 nm. Sensitivity was set at 0.001 AUFS.

To 250 µL of serum, 20 µL of tinidazole (100 µg/mL) were added as internal standard and vortexed for 2 min. Methanol (250 µl) was added to serum samples for protein precipitation, vortexed for one minute and centrifuged at 13,000 rpm for 8 min using Biofuge Fresco Centrifuge (Heraeus, Germany). Supernatant (20 µL) was injected into column. A linear calibration curve in the range of 0.2- 8.0 µgwas established (r^2=0.9949) in serum.

Pharmacokinetic Analysis and Statistical Analysis

The pharmacokinetic parameters, peak serum concentrations (C_{max}) and time to reach peak concentration (T_{max}) were directly obtained from concentration-time data. Various pharmacokinetic parameters like area under the curve (AUC), elimination half life ($T_{1/2}$), volume of distribution (Vd), and total body clearance (CL), for each subject were calculated using a non compartmental pharmacokinetic program Win Nonlin (Pharsight, Palo Alto, CA). The mean pharmacokinetic parameters of ornidazole obtained before and after pretreatment with silymarin were compared by student's paired t-test (paired data) using SPSS® 7.5 version. A value of $p<0.05$ was considered to be statistically significant.

Results

A human volunteer study was conducted to investigate the influence of 9 days pretreatment of silymarin on the pharmacokinetics of ornidazole on healthy individuals. All treatments were well tolerated by the human volunteers and they did not suffer from any adverse effects during the study period. Mean serum concentrations of ornidazole before and after pretreatment with silymarin were shown in Fig.1 and the pharmacokinetic parameters of ornidazole were presented in Table 1. An average silymarin pretreatment resulted in 27.20 % and 30.25 % increase in AUC $_{(0-48)}$ and AUC $_{(0-\infty)}$ respectively. The mean AUC $_{(0-48)}$ of ornidazole increased from 61.5 ± 17.43 to 78.23 ± 14.15 µg/ml/h, and the mean AUC $_{(0-\infty)}$ of ornidazole increased from 69.13 ± 19.44 to 90.04 ±16.44 µg/ml/h upon pretreatment with silymarin and these results were found to be statistically significant ($p<0.05$). There is no significant change in the time required to reach the maximum concentration (T_{max}) in the volunteers before and after the silymarin pretreatment.

Silymarin pretreatment showed an increase ($p<0.05$) in the C_{max} of ornidazole by 16.25 %. The C_{max} values were found to be 4.0 ± 0.72 µg/ml and 4.65 ± 0.54 µg/ml before and after pretreatment with silymarin respectively. There was a significant ($p>0.05$) decrease in clearance of ornidazole by 25.71 % with an increase ($p<0.05$) in elimination half-life ($T_{1/2}$) by 7.70 %.

Discussion

Transporters play key roles in the absorption, disposition, toxicity, and efficacy of drugs, and thus transporter based drug interactions can lead to pharmacokinetic changes, including increased toxicity and lack of efficacy, in co administered substrates (Shitara and Sugiyama 2006). Silymarin is a polyphenolic flavonoid mixture, composed mainly of silybinin (or silybin), which is thought to be the primary bioactive component, and lesser amounts if silydianin and silychristin (all silychristin stereoisomers). Silymarin is moderately absorbed from gastrointestinal tract with an oral absorption of 23-47 %.

Ornidazole is a 5-nitroimidazole with antiprotozoal and antibacterial properties against anaerobic bacteria. It is extensively metabolized before excretion, with 2-hydroxy methyl ornidazole being the major metabolite, which is similar to that of metronidazole and the formation of this metabolite is catalyzed by CYP 3A in case of metronidazole. All of the information available indicated that the elimination of ornidazole from plasma depends primarily on the rate of metabolism (Wacher et al. 1995). Jian et al. (2007) reported that silymarin does not have any effect on CYP3A or P-gp efflux transporter effect at recommended supplementation regimen (Jian et al. 2007). Similar findings were observed in our previous

investigation (Nageshwar Rao et al. 2007). Results of our study revealed that silymarin pretreatment alters the mean serum concentrations of ORN. After pretreatment with silymarin, there was a significant decrease in clearance ORN with a resultant increase in C_{max}, $T_{1/2}$ and $AUC_{(0-\infty)}$. In primary cultures of human hepatocytes, silymarin treatment significantly reduced the activity of CYP 3A enzyme as determined by the formation of 6- α-hydroxy testosterone (Ramachandran et al. 2000) and in a similar type of study by Chitra Sridhar et al. (2004), silybin has inactivated purified recombinant cytochrome P450 3A4 and 2C9 in a mechanism based manner, as measured by the inactivation of 7-benzyloxy-4-(triflouromethyl)-coumarin–O-debenzylation activity (CYP3A), testosterone metabolism to 6-α-hydroxy testosterone and the 7-ethoxy–4-(triflouromethyl)-coumarin-O-deethylation activity of purified human P450 2C9.

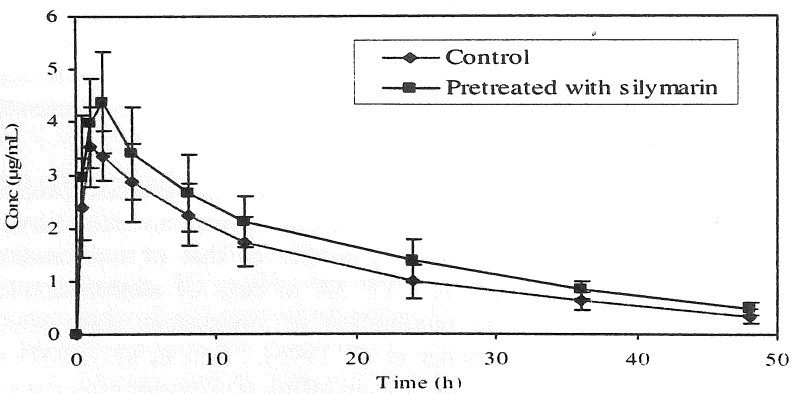
From the above reports and the results of our study, it is presumed that, like metronidazole, ornidazole is metabolized to a major extent by cytochrome P 450 3A4 and silymarin inhibited the CYP 3A mediated metabolism of ornidazole resulting in the decreased clearance with a concomitant increase in elimination half-life and $AUC_{(0-\infty)}$. Zhang and Morris (2003) have reported the P-glycoprotein inhibitory action of silymarin, by direct interaction with P-gp substrate binding, from their *in vitro* studies using human hepatocyte cultures. Hence the role of silymarin to inhibit P-glycoprotein should also be considered.

Table 1. Pharmacokinetic Parameters of ornidazole before and after pretreatment with silymarin followed by single dose of ornidazole (500 mg) on tenth day, values represented are mean ± SD (n=9)

Parameter	Control	Pretreatment with sylimarin
C_{max}(μg/ml)	4.00 ± 0.72	4.65 ± 0.54
T_{max}(hrs)	1.67 ± 1.0	2.00 ± 0.866
$AUC_{(0-48)}$(μg/ml/hr)	61.51 ± 17.43	78.23 ± 14.15
$AUC_{(0-\infty)}$(μg/ml/hr)	69.13 ± 19.44	90.04 ± 16.44
$T_{1/2}$(hr)	15.46 ±1.85	16.65 ± 1.90
CL(ml/hr/kg)	7.70 ±1.93	5.72 ± 1.02
Vd (ml/kg)	173.09±52.25	137.57 ± 30.26

AUC area under the concentration-time curve; C_{max} peak concentration;
T_{max} time to reach C_{max}; $T_{1/2}$ elimination half-life;
CL total body clearance; Vd Apparent volume of distribution

Figure 1. Serum ornidazole concentration versus time profile in healthy human volunteers before and after pretreatment with silymarin, values represented are Mean ± S.D (n=9)



Conclusion

Based on these results, it is proposed that silymarin pretreatment may alter the pharmacokinetics of ornidazole by the inhibition of cytochrome P450 (CYP) enzymes and/or inhibition of P-glycoprotein.

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