EFFECT OF SILYMARIN ON THE ORAL BIOAVAILABILITY OF RANITIDINE IN HEALTHY HUMAN VOLUNTEERS

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SUMMARY

The effect of silymarin pretreatment on the pharmacokinetics of ranitidine was investigated in 12 healthy male human volunteers aged 19-26 years. After an overnight fast, ranitidine 150 mg was administered to the volunteers either alone or after 7 days pretreatment with thrice daily dose of 140 mg silymarin. The wash-out period between each treatment was 7 days. Serum levels of ranitidine were determined by HPLC. Pharmacokinetic parameters were determined based on non-compartmental model analysis using the computer program KINETICA. There was no influence of silymarin on the pharmacokinetics of ranitidine. Concomitant administration of silymarin at this dosage did not alter ranitidine C_{max} and $AUC_{0-\infty}$. There was a significant difference in area under the first moment curve (AUMC) and mean residence time. This result is useful in predicting the interaction of silymarin with other cytochrome 3A4 and P-glycoprotein substrates at normal dosage.

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KEY WORDS

silymarin, ranitidine, pharmacokinetics, human

INTRODUCTION

Epidemiological studies have shown the frequent consumption of fruits and vegetables is associated with a lowered risk of pathological conditions /1,2/. The protective effect has been attributed in part to the flavonoids, which are ubiquitously present in plant-derived foods and are important constituents of the human diet. These flavonoids may thus be able to alter the pharmacokinetics of dihydropyridines in humans. Several flavonoids have been shown to inhibit the cytochrome (CYP) 3A catalyzed metabolism of dihydropyridines in human liver microsomes /3/.

The use of herbal extracts has increased enormously because of their efficacy coupled with decreased risk of side effects. Silymarin is a flavonoid obtained from the plant *Silybum marianum* belonging to the family Asteraceae /4/. It is used in the supportive treatment of acute or chronic hepatitis and cirrhosis induced by drugs or toxins /5/. It is a herbal drug that has the potential to influence drug metabolism by decreasing the metabolic activity of CYP3A4, a ubiquitous enzyme responsible for hepatic and intestinal metabolism /6/. It may also alter absorption, distribution and elimination through inhibition of P-glycoprotein (P-gp) /7/.

Clinical data are sparse with regard to sylimarin's effect on drugs that are metabolized by the CYP450 system and effluxed by P-gp /8/. Because of the synergistic role of CYP3A4 and P-gp, the rate of elimination of drugs is very fast. Ranitidine is a histamine (H_2) receptor antagonist which mainly blocks H_2 receptors in the stomach. It prevents histamine mediated gastric acid secretion and is useful in the treatment of duodenal ulcers, benign gastric ulcers, reflux oesophaghitis, etc. Metabolic studies of ranitidine have revealed that it is mainly metabolized by oxidation to give *N*-oxide-, *S*-oxide- and desmethyl-ranitidine /9,10/. It is eliminated mainly through the urine and the biliary system /11/.

Because of the widespread use of silymarin, we wanted to evaluate its effect on the oral bioavailability of ranitidine which is a substrate of CYP3A4 and P-gp. As silymarin is usually taken for months at a time

in the treatment of hepatitis and cirrhosis, there is every chance of taking ranitidine at the same time. This study was conducted in healthy male volunteers.

MATERIALS AND METHODS

Subjects

This study was conducted in 12 healthy volunteers (age range 19-26 years, weight 50-68 kg). The participants were enrolled in the study after a thorough medical examination and standard laboratory tests. They were not allowed to take any other drug just before or during the study. They were all non-smokers. Volunteers had no history of ill-health during the preceding 6 months. Volunteers were excluded from the study if they had food allergies or were allergic to ranitidine or silymarin. All participants were briefed about the study and the study was approved by Institutional Ethics Committee.

Materials

Ranitidine tablets 150 mg (Zinetac®, Glaxo India Ltd.), silymarin capsules 140 mg (Sivylar®, Ranbaxy Laboratories India Ltd.), methanol HPLC (E. Merck Ltd., India), acetonitrile HPLC (E. Merck Ltd.) were used. All other chemicals used were of AR grade.

Methods

The first part of the study consisted of oral administration of one tablet of 150 mg of ranitidine alone and second part of the study was conducted after a wash-out period of one week. Silymarin 140 mg capsules were administered thrice daily for 7 days. On day 8, a tablet of ranitidine 150 mg and a capsule of silymarin 140 mg were administered simultaneously.

Collection of blood and urine samples was made at the time of administration of ranitidine alone and on day 8 of silymarin co-administration with ranitidine. Blood samples were collected from the median cubital vein at intervals of 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours and urine voided during 0-2, 2-4, 4-8, 8-12 and 12-24 hours after administration was collected. Blood was allowed to clot,

centrifuged, and serum was separated. Serum and urine samples were stored at -20°C until analysis.

Assay

Ranitidine in serum and urine was estimated by reversed phase high performance liquid chromatography (RP HPLC) using the method of Krishna Kumar *et al.* /12/. To 1 ml of serum in a stoppered test tube of metaclopropamide (internal standard) solution (0.1 mg/ml) was added followed by 0.6 ml of 2 M NaOH solution. The mixture was vortexed for 1 min. Then 500 mg of sodium chloride and 8 ml of dichloromethane were added and vortexed for 5 min. The test tubes were centrifuged at 5,000 rpm for 30 min. The organic layer was separated and collected in a clean test tube. The dichloromethane was evaporated to dryness and the residue was reconstituted in 0.1 ml of the mobile phase. About 20 μl was injected on to the HPLC column.

The HPLC unit (Shimadzu Corporation, Kyoto, Japan) was equipped with a 25 cm x 0.4 cm nucleosil C_8 reversed phase column and UV spectrophotometric detector. The mobile phase was 50 mM ammonium acetate containing 25 mM sodium lauryl sulphate (pH 3.8 adjusted with glacial acetic acid) and acetonitrile mixture (54:46). Flow rate was 1 ml/min, the UV detector was set at 330 nm, and the detector sensitivity was 0.0005 AUFS. For urine analysis, a similar mobile phase but with different composition, i.e., 70:30, was used.

The calibration curve was prepared by adding 25-1,000 ng of ranitidine to 1 ml of serum obtained from untreated volunteers. The samples were treated in the same manner as the test samples. The peak height ratios obtained at different concentrations of drug were plotted against drug concentrations. The slope of this plot determined by the method of least square regression analysis was used to calculate ranitidine concentration in the unknown samples; the reproducibility of the assay method was tested by analyzing the serum sample spiked with four different concentrations and estimating their drug content on the same day as well as on different days. The coefficient of variation of each concentration was lower than 5%. The lower limit of quantification of ranitidine using this method was 25 ng/ml.

Treatment of bioavailability data

The peak serum concentration (C_{max}) and time to reach peak levels (T_{max}) were obtained from the experimental data. The other pharmacokinetic parameters, elimination half-life ($t_{½}$), overall elimination rate constant (K_e), area under serum concentration time curve (AUC), area under the first moment curve (AUMC), mean residence time (MRT), apparent volume of distribution for fraction of dose absorbed (Vd/f), clearance (Cls/f), and total renal clearance were obtained from a non-compartmental model using the KINETICA computer programme.

Statistical analysis

The differences in the pharmacokinetic parameters obtained were statistically tested using Student's paired t-test. Differences in the sample means were considered significant at $p < 0.05$.

RESULTS

None of the volunteers experienced any toxic effect related to either silymarin or rantidine. The plots of mean serum concentration of ranitidine before and after treatment with silymarin in healthy human volunteers are shown in Figure 1. The pharmacokinetic parameters of ranitidine before and after treatment are shown in Table 1. Cumulative urinary excretory patterns of ranitidine before and after treatment with silymarin are shown in Figure 2. Cumulative urinary excretory data of ranitidine before and after treatment in healthy human volunteers are shown in Table 2. C_{max} increased slightly but did not reach statistical significance. The mean C_{max} for ranitidine alone was 313.495 ± 110.503 and after pretreatment with silymarin was 337.216 ± 147.9526.

C_{max}, AUC_{0-12}, $AUC_{0-\infty}$, K_e, $t_{½}$ and T_{max} were not significantly altered after pretreatment with silymarin. AUMC showed a significant increase of approximately 11% from 13,795.47 ± 8,280.14 to 15,341.16 ± 9,280.353 ng/ml/h after pretreatment with silymarin. MRT showed ~13% rise from 6.87 ± 1.70 to 7.62 ± 1.56 h after pretreatment with silymarin which was statistically significant ($p < 0.05$).

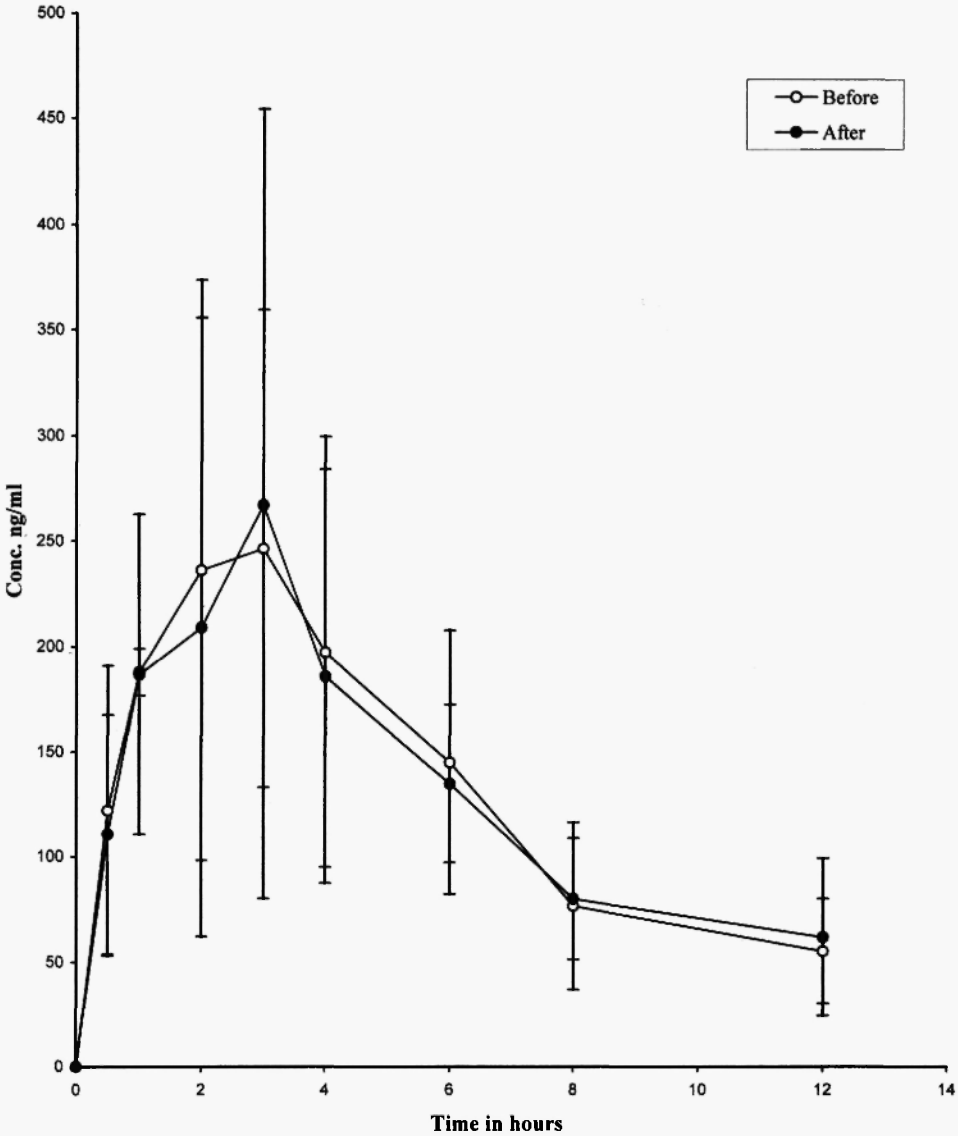

Fig. 1: Serum concentration profiles (means ± SD) of ranitidine before and after treatment with silymarin in healthy human volunteers (n = 12).

TABLE 1

Pharmacokinetic parameters of ranitidine in healthy human volunteers (n = 12)

	Before treatment	After treatment
C_{max} (ng/ml)	313.50 ± 110.50	337.22 ± 147.95
T_{max} (h)	1.96 ± 877	2.38 ± 1.10
AUC_{0-12} (ng/ml/h)	1,577.17 ± 572.13	1,511.23 ± 557.43
$AUC_{0-\infty}$ (ng/ml/h)	920.52 ± 767.65	1,928.23 ± 784.73
K_e (h^{-1})	0.1865 ± 0.06951	0.1698 ± 0.0641
$t_{½}$ (h)	4.20 ± 1.38	4.78 ± 1.44
AUMC (ng/ml/h)	13,795.47 ± 1.38	15,341.16 ± 9,280.35
MRT (h)	6.87 ± 1.70	7.62 ± 1.55
V_d/f (l/kg)	8.48 ± 2.24	9.75 ± 4.55
Cl/f (l/h)	1.55 ± 0.73	1.59 ± 0.66

C_{max} = peak serum concentration; T_{max} = time to reach peak serum level; AUC_{0-t} = area under the serum concentration time curve from 0 to t h; $AUC_{0-\infty}$ = total area under the serum concentration time curve; K_e = elimination rate constant; $t_{½}$ = elimination half-life; AUMC = area under the first moment curve; MRT = mean residence time; V_d/f = apparent volume of distribution; Cl/f = oral clearance.

TABLE 2

Urinary excretory pattern of ranitidine (mg) before and after treatment in healthy human volunteers (n = 12)

Time (h)	Before treatment	After treatment
0-2	6.25 ± 2.84	6.39 ± 3.81
2-4	16.62 ± 4.34	17.20 ± 5.88
4-8	25.80 ± 5.58	26.58 ± 5.71
8-12	29.95 ± 6.46	30.07 ± 6.72
12-24	31.86 ± 7.22	30.89 ± 5.71

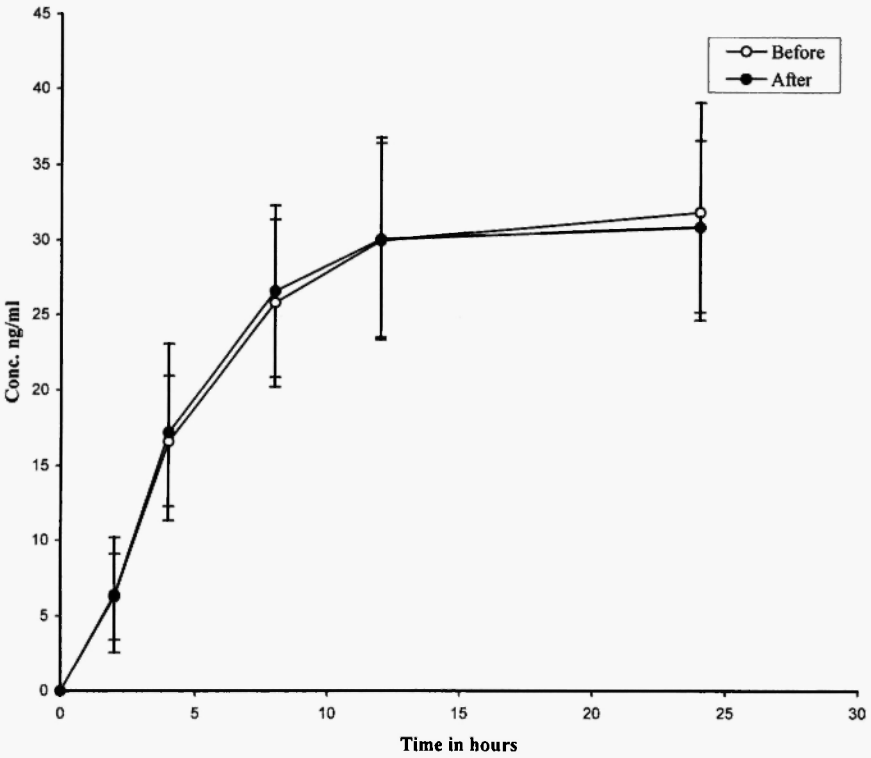

Fig. 2: Cumulative urinary excretory patterns of ranitidine before and after treatment with silymarin (means ± SD; n = 12).

Figure 2 shows the cumulative urinary excretory pattern of ranitidine before and after treatment with silymarin, and excretion data are given in Table 2. No statistically significant difference was observed in the cumulative amount of drug excreted (x^{α}_{μ}) in urine. The mean x^{α}_{μ} before and after treatment with silymarin were 31.86 ± 7.22 mg and 30.89 ± 5.71 mg, respectively.

DISCUSSION

From the present study, it appears that pretreatment with silymarin had no effect on the pharmacokinetics of ranitidine. Preliminary data suggest that silymarin may influence the metabolic capacity of CYP3A4, a CYP 450 isoenzyme responsible for the hepatic and intestinal metabolism of many important classes of drugs /6/. Silymarin also may alter drug absorption, distribution and elimination through inhibition of P-gp. A synergistic role of CYP3A4 and P-gp in limiting the oral bioavailability of many drugs has been proved /13,14/.

Administration of silymarin failed to influence either ranitidine $AUC_{0-\infty}$ or C_{max}, therefore at the dose given in this study it does not appear to have a significant influence on CYP3A4 mediated hepatic or intestinal metabolism of ranitidine (Fig. 1). Although there was a slight difference in the AUC and C_{max} with pretreatment, it was not statistically significant. The urinary excretion patterns before and after treatment with silymarin (Fig. 2) indicated that there was no statistically significant difference in urinary clearance and cumulative amount of drug excreted in the urine. This observation coincides with the blood data of ranitidine, which also showed no significant difference with the pretreatment.

Our study also demonstrates that *in vitro* studies of herbal products may give different results than those seen in clinical trials. Although two *in vitro* studies predicted that silymarin would increase ranitidine concentrations /6,8/, no such effect was seen in the present study. The possible reasons for the discordance are varied and include a) not studying the true active ingredient, b) using concentrations that are not relevant clinically, c) using an *in vitro* system that can only evaluate inhibition, and d) not being able to evaluate the effects of any metabolites that may be produced. In general, the results of *in vitro* studies of herbal products cannot be used as a basis for therapeutic decisions on drug interactions and require confirmation in a clinical trial.

The dosage of silymarin administered approximates the recommended hepatoprotectant dosage of silymarin (420 mg/day) and fall within the range of common silymarin (300-600 g/day) /5,15/. However, we cannot conclude that silymarin would not have influenced the pharmacokinetics of ranitidine at higher dosages.

Silymarin is poorly absorbed after oral administration (bioavailability 23-47%). Therefore, silymarin blood concentrations may have been too low to achieve an appreciable effect on either CYP3A4 or P-gp. Thus, in the absence of silymarin levels in blood we cannot rule out the possibility that inadequate silymarin absorption led to the observed absence of effect. Thus we can only conclude that silymarin 110 mg, 3 times/day failed to influence the pharmacokinetics of ranitidine in healthy subjects. Concomitant administration of silymarin at this dosage would not be expected to alter ranitidine C_{max} and $AUC_{0-\infty}$ to a clinically significant level. This result may be useful in predicting the interaction of silymarin with other CYP3A4 and P-gp substrates at the normal dosage.

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