EFFECT OF KETOCONAZOLE AND RIFAMPICIN ON THE PHARMACOKINETICS OF RANITIDINE IN HEALTHY HUMAN VOLUNTEERS: A POSSIBLE ROLE OF P-GLYCOPROTEIN

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SUMMARY

The aims of this study were to determine the effect of ketoconazole and rifampicin on the oral pharmacokinetics of ranitidine in human volunteers and to investigate the role of P-glycoprotein (P-gp) using *in vitro* systems. A randomized, placebo controlled crossover oral pharmacokinetic study was conducted in 12 healthy male human volunteers and *in vitro* (everted sac) and *in situ* (intestinal loop) studies were conducted in rats to study the role of P-gp. There was a statistically significant ($p < 0.05$) difference observed in the pharmacokinetic parameters C_{max}, AUC and MRT after pretreatment with rifampicin (600 mg orally once per day for 7 days). The C_{max}, $AUC_{0-\infty}$, and MRT were decreased by 53%, 52%, and 18%, respectively. Ketoconazole treatment (200 mg orally once per day for 5 days) increased the C_{max}, $AUC_{0-\infty}$ and $T_{½}$ by 78%, 74%, and 56%, respectively, whereas T_{max} was decreased by 31%. No statistically significant differences were observed in renal clearance (CL_R) of ranitidine after treatment with either ketoconazole or rifampicin. Presence of ketoconazole significantly reduced the mean cumulative

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efflux concentrations (serosal to mucosal) of ranitidine to 35%, 41% and 55% in the duodenal, jejunum and ileal regions of the everted sacs, respectively, whereas, the mean cumulative efflux concentrations of ranitidine were increased by 14%, 36% and 25% in duodenal, jejunal and ileal regions of the rat small intestine, respectively, after pretreatment with rifampicin. The presence of ketoconazole improved the absorption of ranitidine significantly by increasing the percentage of total dose disappearing from the loops of duodenum, jejunum and ileum of rat small intestine by 82%, 84% and 85%, respectively. In contrast, treatment with rifampicin decreased the absorption of ranitidine by decreasing the percentage of total dose disappearing in duodenal, jejunal and ileal regions of the intestinal loops by 40%, 39% and 25%, respectively. Ranitidine was shown to be a P-gp substrate *in vivo* in human volunteers and it was found that oral bioavailability of ranitidine was influenced at the intestinal absorption phase.

KEY WORDS

ranitidine, ketoconazole, rifampicin, oral pharmacokinetics, P-glycoprotein, human, rat

INTRODUCTION

The concept of poor absorption of drugs due to intestinal metabolism and efflux transporters was not previously considered to be clinically significant by most researchers /1,2/. Watkins and coworkers were the first to report that a major cytochrome P-450 enzyme (CYP), CYP3A, is relatively abundant in the intestinal mucosa, and substrates of this enzyme may have poor oral bioavailability due to extensive first pass metabolism in the intestine /3/. The high level of CYP3A in the intestine becomes of even greater importance when it is recognized that more than 50% of human drugs may be substrates for this enzyme /4/.

The poor oral bioavailability of drugs due to intestinal metabolism is further supported by P-glycoprotein (P-gp), a transmembrane protein, an efflux pump, which is capable of the active transport of the drug from intestinal, renal and hepatic cells. P-gp has received

increasing attention as a significant factor in the elimination of a number of drugs /5,6/.

Recent reports have shown that the P-gp mediated transport of a substance is modified not only by other P-gp substrates but also by CYP3A related compounds, because there are some overlapping substrate specificities between CYP3A and P-gp /1/. Thus, the first pass effect in the gut may be affected by the inhibition or induction of CYP3A and/or P-gp caused by drug-drug interactions. Several pharmacokinetic studies using these enzyme systems illustrate the importance of these biological proteins in drug absorption and disposition.

Ranitidine blocks H_2 receptors in the stomach and prevents histamine-mediated gastric acid secretion. The oral bioavailability of ranitidine is 52 ± 11% and it is eliminated mainly through urine and biliary systems /7,8/.

Ranitidine is a small, relatively hydrophilic drug believed to cross the intestinal epithelium passively via a predominantly paracellular route /9/, and as such is not thought to be a typical substrate for P-gp. Preliminary observations in the human colonic cell line Caco-2 /10/ suggested ranitidine may be a substrate for this transporter, but no detailed information is available on the role of P-gp in modulating the permeability of this drug in normal intestine. For the first time, Collett *et al.* /11/ and Lentz *et al.* /12/ have reported the prominent role of P-gp, an efflux pump, on intestinal permeability of ranitidine in Caco-2 cell lines and rat intestines. According to these studies, serosal to mucosal transport of ranitidine and cimetidine was significantly reduced by known P-gp substrates, verapamil and cyclosporine, but not by probenecid, an inhibitor of multidrug related protein, in rat ileum and Caco-2 cell monolayers /11/. However, so far, no clinically significant P-gp mediated *in vivo* drug-drug interactions of ranitidine or cimetidine with any other P-gp related compounds have been reported. Although a significant drug interaction was reported recently with ranitidine and fosamprenavir (FPV), a phosphate ester prodrug of amprenavir (APV), in human volunteers, in which ranitidine significantly decreased the plasma exposure of APV by possibly preventing the conversion of the prodrug, FPV, to APV by elevating the gastric pH /13/. All these *in vitro* studies prompted us to investigate whether these observations have any significance *in vivo*, especially in human volunteers. The known P-gp inducer, rifampicin /14,15/, and P-gp

inhibitor, ketoconazole /2,16/, were used in our investigation to study the oral bioavailability of ranitidine under induced and inhibitory conditions of P-gp in human volunteers. These *in vivo* observations were further investigated using everted gut sac and intestinal loop studies in rats.

MATERIALS AND METHODS

Materials

Ranitidine 150 mg tablets (Zenetac®150) were obtained from Glaxo India Ltd., Mumbai, India. Rifampicin capsule 600 mg (R-cin® 600) and ketoconazole tablets 200 mg (Nizral®) were purchased from Lupin Limited, Aurangabad, India, and Johnson and Johnson India Limited, Mumbai, India, respectively. Pure samples of ranitidine, rifampicin and ketoconazole were received as a gift from Glaxo India Ltd., India and Astra-IDL, India, Lupin Limited, Aurangabad, India and Sun Pharma, Baroda, India, respectively. Acetonitrile (HPLC grade) was obtained from Qualigens Chemicals, Mumbai, India. Ammonium acetate (AR grade), sodium lauryl sulfate (AR grade) and Dichloromethane (AR grade) were purchased from S.D. Fine Chemicals, Mumbai, India, Loba Chemie Pvt. Ltd., Mumbai, India, and E. Merck (India) Ltd., Mumbai, India, respectively.

Subjects

A randomized, placebo controlled crossover study was conducted in 12 healthy non-smoking male subjects (mean age ± SD, 28 ± 2 years, mean height 163.4 ± 5 cm, mean weight 64.5 ± 5 kg). The subjects were randomized using a simple randomized procedure after screening tests were completed. Each subject gave informed consent before taking part in the study, which was approved by the Ethics Committee of the University College of Pharmaceutical Sciences, Kakatiya University, Warangal. The study was conducted in agreement with the declaration of Helsinki (Somerset West Amendment 1996). No concomitant drug therapy, including over-the-counter drugs, was allowed for 2 weeks before and during the study period.

Study design

The study consisted of three periods (ranitidine with placebo, ranitidine with ketoconazole, ranitidine with rifampicin). A single oral dose of 150 mg of ranitidine administration on the study day was preceded by the following treatment regimens: (1) ketoconazole 200 mg orally once per day for 5 days (including concomitant administration on the study day); (2) rifampicin 600 mg orally once per day for 7 days; (3) placebo once per day orally for 5 days. Ketoconazole was taken 0.5 hour prior to ranitidine administration and continued in the dosage regimen described above until the end of the study period. A drug free interval of at least 2 weeks was kept between study periods.

Blood and urine sampling

Blood samples of 4-5 ml were collected manually through the antecubital vein before and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after ranitidine administration. Subjects were instructed to empty the bladder just prior to ranitidine administration and a voided urine sample was collected. Total urine was collected at 0, 2, 4, 8, 12 and 24 hours after dosing.

Drug analysis

An HPLC method was used to determine the drug in serum, urine, intestinal everted sac and loop samples (see below) as previously described /17/. The HPLC system (Shimadzu, Japan) consisted of LC-10AT solvent delivery module, SPD-10A, UV-visible spectrophotometric detector with LC10 software. The column used was Tracer Analytica, Nucleosil, C8 (stainless steel column of length 25 cm and internal diameter of 0.4 cm packed with porous silica spheres of 5 μ diameter). The mobile phase consisting of 50 mM ammonium acetate containing 25 mM sodium lauryl sulfate (pH 3.8, adjusted with glacial acetic acid), acetonitrile and methanol mixture (40:40:20, v/v) was used at a flow rate of 1.0 ml/min. The eluate was monitored at 330 nm.

Pharmacokinetic data analysis

Ranitidine serum concentration-time data were analyzed for each subject using non-compartmental methods. Basic pharmacokinetic parameters, including peak serum concentration (C_{max}), time to reach peak serum concentration (T_{max}), elimination half-life ($T_{½}$), area under the serum concentration-time curve (AUC), mean residence time (MRT), total renal clearance (CL_R) for the drug under consideration, were obtained in each subject from the serum concentration versus time profile on an IBM compatible personal computer using KINETICA™ software (Inna Phase Corp. 2000).

Animal studies

All experiments on rats were conducted after approval from the institutional animal ethics committee of University College of Pharmaceutical Sciences, Kakatiya University. Male albino Wistar rats weighing 200-300 g were purchased from National Institute of Nutrition (NIN), Hyderabad, India, and acclimatized to laboratory conditions for one week prior to experiments. Each group of rats was housed in a cage and maintained at about 25°C and 60% relative humidity with a 12 h light/dark cycle. Rats were fed with controlled diet and purified water *ad libitum* and divided into groups, each group consisting of six rats.

Everted sac study

The general experimental procedure and surgery was described previously /18/. 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. The test compound (10 mg/ml of ranitidine) was dissolved in pH 7.4 isotonic Dulfecco's PBS (D-PBS) containing 25 mM glucose and 4% DMSO. The drug solution (1 ml) was introduced into the everted sac (serosal side) and both ends of the sac were ligated tightly and immersed into 40 ml of D-PBS. 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 gas mixture, transport of the ranitidine from serosal to mucosal surfaces across the intestine was measured by sampling the mucosal medium periodically for 90 minutes. Transport

of ranitidine was studied in the absence or presence of ketoconazole (200 μM in 100% DMSO) and under induced conditions after pre-treatment with rifampicin (60 mg/kg/p.o. for 7 days).

In situ *loop technique*

The experimental procedure used was described by Nakayama *et al.* /19/. Three loops (each 10 cm) were prepared in the duodenal, jejunal, and ileal regions of male Wistar rats under sodium pento-barbital anesthesia. One milliliter of ranitidine solution (0.2 mM) was injected into each loop with a syringe. Three loops were cut off after one hour and the contents were collected for determining the amount of ranitidine in each loop. In the inhibition study, the disappearance of ranitidine alone or in the presence of ketoconazole (200 μM) was determined after 1 h by taking out the contents of the various parts of the intestinal loops. In induction studies, each group of animals was treated with 60 mg/kg of rifampicin orally once daily for 7 days and the disappearance of ranitidine was measured in each loop. Rifampicin and ketoconazole were dispersed in sodium carboxymethyl cellulose (0.25%, w/v) for treating the rats.

Statistical analysis

Data were analyzed using a personal computer with Sigma Stat software package (Jandel Corp. CA, USA). One way ANOVA was used to calculate the pharmacokinetic data of ranitidine. A value of $p < 0.05$ was considered to be significant, and results are expressed as means ± SD.

RESULTS

Human volunteer studies

The mean ± SD (n = 12) serum concentration verses time profiles and pharmacokinetic parameters of ranitidine after treatment with placebo, ketoconazole or rifampicin are shown in Figure 1 and Table 1, respectively. The observed pharmacokinetic parameters of ranitidine upon oral administration of 150 mg in human volunteers were comparable with those in previous reports /19,20/. There was a statistically significant difference observed in pharmacokinetic

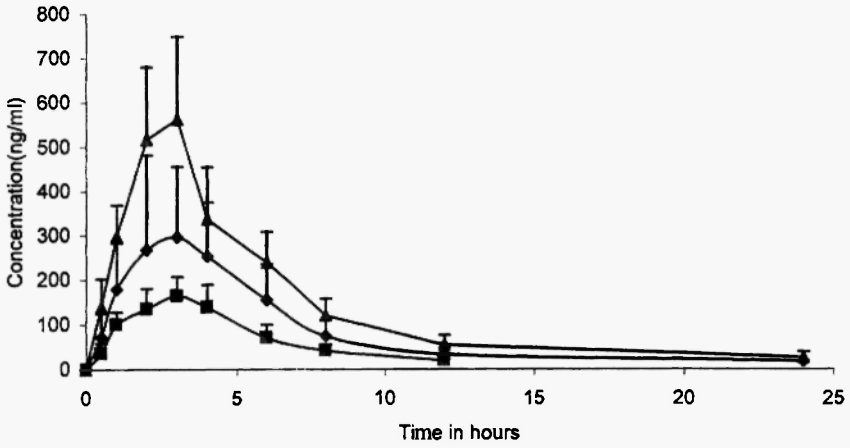

Fig. 1: Mean ± SD serum concentration-time curves of ranitidine in healthy human volunteers (n = 12) after oral administration of ranitidine (150 mg /once per day) with placebo, after pretreatment with rifampicin (600 mg/oral/once per day/7 days) and in combination with ketoconazole (200 mg/oral/once per day/5 days). ♦ ranitidine with placebo; ▲ ranitidine + ketoconazole; ■ ranitidine + rifampicin.

parameters C_{max}, AUC and MRT after pretreatment with rifampicin. No statistically significant difference was observed in pharmacokinetic parameters T_{max} and $T_{½}$ after rifampicin pretreatment. The serum concentrations of ranitidine were significantly lowered after pretreatment with rifampicin when compared to treatment with placebo. The C_{max}, $AUC_{0-\infty}$, and MRT were decreased by 53% ($p < 0.05$), 52% ($p < 0.05$) and 18% ($p < 0.05$), respectively. All the above statistically significant pharmacokinetic changes were observed at a power of more than 0.8 at $\alpha = 0.05$.

Treatment with ketoconazole produced statistically significant changes in the pharmacokinetic parameters C_{max}, T_{max}, AUC and $T_{½}$ of ranitidine in healthy human volunteers. There was a change observed in the pharmacokinetic parameter MRT, which was not statistically significant. Ketoconazole treatment increased the C_{max}, $AUC_{0-\infty}$ and $T_{½}$ by 78% ($p < 0.001$), 74% ($p < 0.05$), and 56% ($p < 0.05$), respectively. However, T_{max} was decreased by 31% ($p < 0.05$). In urinary data, there was no statistically significant difference observed

in CL_R between ranitidine with placebo, with rifampicin, and with ketoconazole in human volunteers. The CL_R of ranitidine after treatment with placebo, ketoconazole and rifampicin was 774.3 ± 469.54, 924.4 ± 539.3 and 617.4 ± 231.1 ml/min, respectively (Table 1).

Animal studies

In everted sac studies, the transport of ranitidine from serosal to mucosal surface across everted rat intestine in the absence or presence of ketoconazole, a potent CYP3A4 and P-gp inhibitor /2,15/, and also under induced conditions of P-gp with rifampicin, a known CYP3A and P-gp inducer /13,14/, was determined at duodenum, jejunum and ileal regions of rat small intestine. Mean ± SD (n = 6) cumulative efflux patterns of ranitidine across the everted sacs of the duodenum, jejunum and ileum are shown in Figures 2-4 and Table 2. The presence of ketoconazole significantly ($p < 0.05$) decreased the transport of ranitidine in all three regions of the small intestine. Treatment with

TABLE 1

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

	+ Placebo	+ Ketoconazole	+ Rifampicin
C_{max} (ng/ml)	365.4 ± 197.1	649.2 ± 45.3*	171.0 ± 45.3*
T_{max} (h)	3.0 ± 0.8	2.1 ± 0.7*	3.2 ± 0.6
AUC_{0-24} (ng/ml.h)	1817.6 ± 858.3	3186.0 ± 609.5*	855.7 ± 206.1*
$AUC_{0-\infty}$ (ng/ml.h)	1988.5 ± 923.6	3465.1 ± 761.8*	962.5 ± 216.5*
$T_{½}$ (h)	4.5 ± 1.7	7.1 ± 2.0*	3.2 ± 1.9
MRT (h)	6.8 ± 1.9	8.2 ± 2.3	5.6 ± 1.4*
CL_R (ml/min)	774.7 ± 469.5	924.4 ± 539.6	617.4 ± 231.1

* $p < 0.05$, one way ANOVA.

Doses: ranitidine 150 mg once on the study day; rifampicin pretreatment 600 mg/oral/once per day/7 days; ketoconazole pretreatment 200 mg/oral/once per day/5 days.

C_{max} = peak serum concentration; T_{max} = time to reach peak serum concentration; $T_{½}$ = elimination half-life; AUC = area under serum concentration-time curve; MRT = mean residence time; CL_R = total renal clearance.

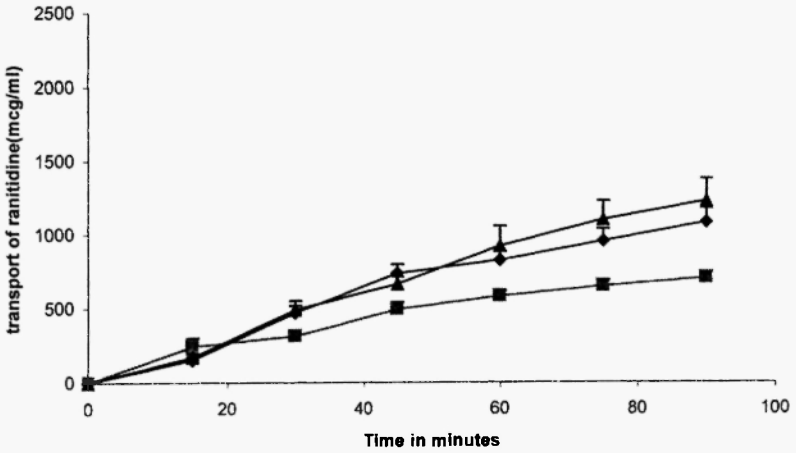

Fig. 2: Mean ± SD cumulative transport (serosal to mucosal) patterns of ranitidine (♦) in the presence of ketoconazole (■) and after pretreatment with rifampicin (▲) in duodenal everted sacs in Wistar rats (n = 6).

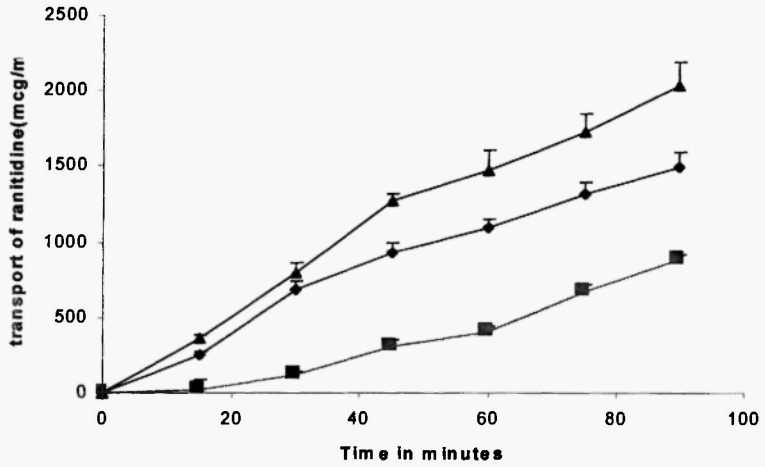

Fig. 3: Mean ± SD cumulative transport (serosal to mucosal) patterns of ranitidine (♦) in the presence of ketoconazole (■) and after pretreatment with rifampicin (▲) in jejunal everted sacs in Wistar rats (n = 6).

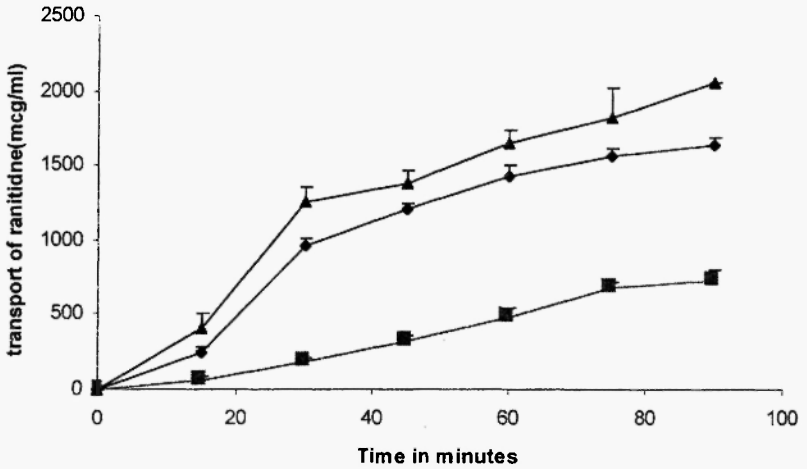

Fig. 4: Mean ± SD cumulative transport (serosal to mucosal) patterns of ranitidine (♦) in the presence of ketoconazole (■) and after pretreatment with rifampicin (▲) in ileal everted sacs in Wistar rats (n = 6).

ketoconazole reduced the mean cumulative transport of ranitidine to 35% ($p < 0.05$), 41% ($p < 0.05$) and 55% ($p < 0.05$) in the duodenal, jejunum and ileal regions of the everted sacs, respectively. The mean cumulative transport of ranitidine was increased by 14% ($p < 0.05$), 36% ($p < 0.05$), and 25% ($p < 0.05$) in duodenal, jejunal and ileal regions of the rat small intestine, respectively, after pretreatment with rifampicin.

In intestinal loop studies, Table 3 shows mean ± SD (n = 6) unabsorbed concentrations and the percentage disappearance of ranitidine from the duodenal, jejunal, and ileal loops in the presence of ketoconazole and after pretreatment with rifampicin. Figures 5-7 show the mean ± SD (n = 6) per cent dose of disappearance of ranitidine after incubation for one hour in the presence of ketoconazole and after pretreatment with rifampicin in rats. The percentage dose disappearance of ranitidine in the loop study in various regions of the small intestine was statistically significant ($p < 0.05$). The presence of ketoconazole improved the absorption of ranitidine by increasing the percentage of total dose disappearing from the loops of duodenum, jejunum and ileum of rat small intestine by 82% ($p < 0.05$), 84% ($p < 0.05$), and 85% ($p < 0.05$), respectively. In contrast, treatment with

TABLE 2

Cumulative efflux concentrations (μg/ml) and per cent decrease (-) or increase (+) in the transport (serosal to mucosal) of ranitidine over a period of 90 min in the three regions of intestinal everted sacs in male albino Wistar rats (n = 6) (mean ± SD)

	Ranitidine alone	With ketoconazole	With rifampicin
Duodenum	1078.2 ± 96.2	699.3 ± 42.2* (-35%)	1224.3 ± 151.2 (+14%)
Jejunum	1494.5 ± 153.3	880.8 ± 50.0* (-41%)	2036.8 ± 134.9* (+36%)
Ileum	1642.2 ± 40.3	732.3 ± 71.7* (-55%)	2054.7 ± 224.1* (+25%)

* $p < 0.05$, one way ANOVA.
For details see Methods.

TABLE 3

Luminal concentrations (μM) and per cent disappearance of administered dose of ranitidine after 1 hour in three regions of intestinal loops in male albino Wistar rats (n = 6) (mean ± SD)

	Ranitidine alone	With ketoconazole	With rifampicin
Duodenum	62.7 ± 10.4 (69%)	35.7 ± 5.5* (82%)	120.7 ± 6.3* (40%)
Jejunum	51.7 ± 6.9 (74%)	31.8 ± 5.2* (84%)	122.3 ± 6.5* (39%)
Ileum	64.0 ± 5.8 (68%)	30.4 ± 4.1* (85%)	150.5 ± 9.8* (25%)

* $p < 0.05$, one way ANOVA.
For details see Methods.

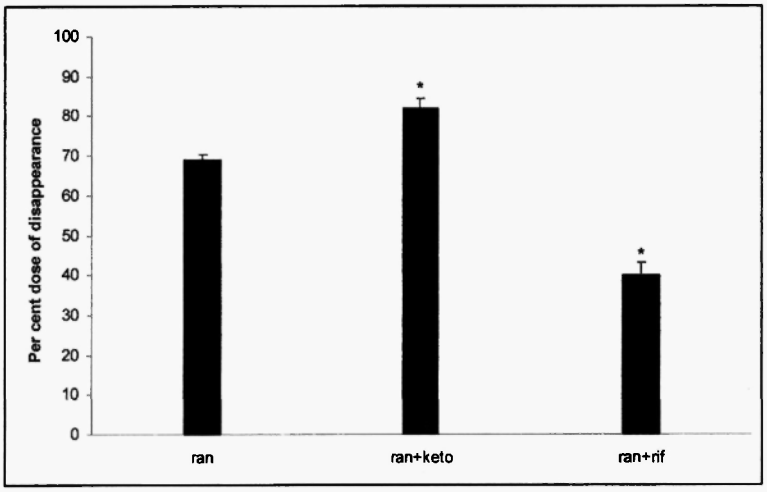

Fig. 5: Mean ± SD per cent dose of disappearance of ranitidine, when administered alone (ran) or in the presence of ketoconazole (ran+keto) or after pretreatment with rifampicin (ran+rif) in duodenal intestinal loops in Wistar rats (n = 6). * $p < 0.05$, one way ANOVA.

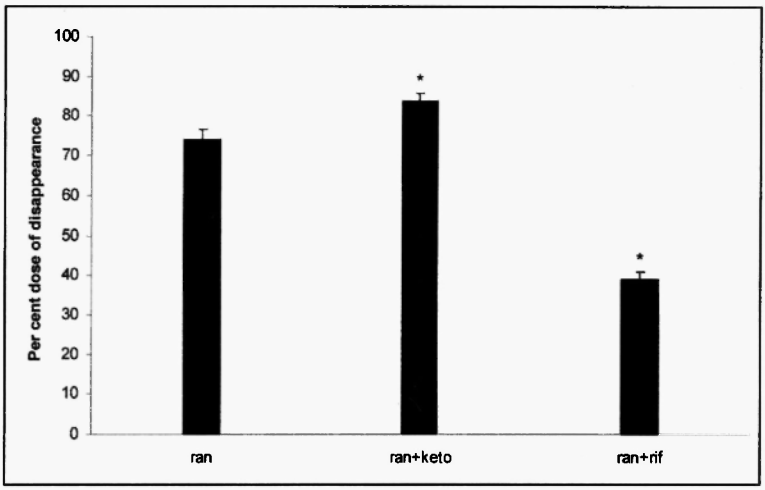

Fig. 6: Mean ± SD per cent dose of disappearance of ranitidine, when administered alone (ran) or in the presence of ketoconazole (ran+keto) or after pretreatment with rifampicin (ran+rif) in jejunal intestinal loops in Wistar rats (n = 6). * $p < 0.05$, one way ANOVA.

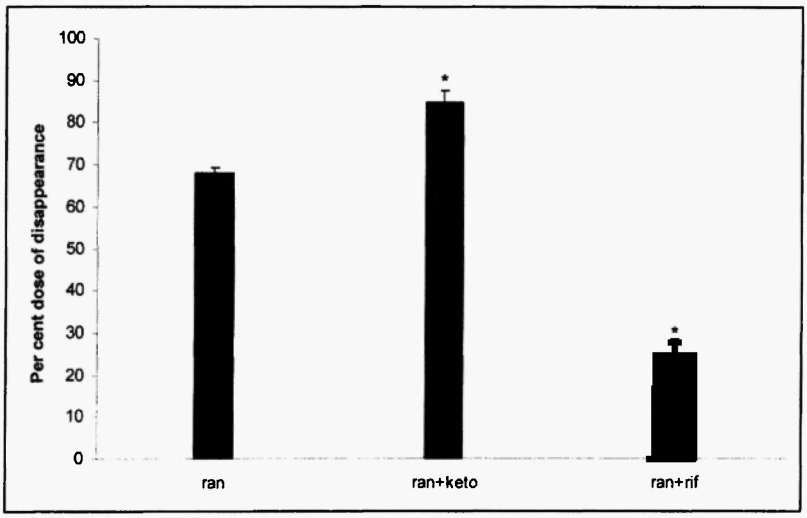

Fig. 7: Mean ± SD per cent dose of disappearance of ranitidine, when administered alone (ran) or in the presence of ketoconazole (ran+keto) or after pretreatment with rifampicin (ran+rif) in ileal intestinal loops in Wistar rats (n = 6). * $p < 0.05$, one way ANOVA.

rifampicin decreased the absorption of ranitidine by showing a decrease in the percentage of total dose disappearing in duodenal, jejunal and ileal regions of the intestinal loops by 40% ($p < 0.05$), 39% ($p < 0.05$) and 25% ($p < 0.05$), respectively (Table 3).

DISCUSSION

In the present study, we calculated the oral pharmacokinetic parameters of ranitidine after treatment with ketoconazole and rifampicin in healthy human volunteers, and the changes observed in pharmacokinetic parameters were further investigated using *in vitro* studies: everted sac and loop studies in rats. Treatment with ketoconazole significantly ($p < 0.05$) increased the C_{max}, AUC and $T_{½}$ of ranitidine by 78%, 74% and 106%, respectively, whereas T_{max} was decreased to 31%. The pharmacokinetic parameters C_{max}, AUC and MRT of ranitidine were decreased significantly, by 53%, 52% and 18%, respectively, after pretreatment with rifampicin in human

volunteers. The observed pharmacokinetic data of ranitidine after both treatments indicate the influence of these P-gp modulators on the oral bioavailability of ranitidine, especially by acting through intestinal P-gp, apart from other regions, such as liver, biliary canaliculi, etc. Intestinal P-gp is known to be localized in the brush border membrane to pump molecules from the serosal side into the luminal side. Inhibition of intestinal P-gp may decrease drug exsorption and thus increase net drug absorption; in contrast, increasing the expression of P-gp in the intestine by a P-gp inducer decreases intestinal absorption. The significant pharmacokinetic changes observed in the C_{max} and T_{max} indicate that the absorption of ranitidine is influenced in the intestinal absorptive phase by ketoconazole and rifampicin. This implies that the improvement of oral bioavailability of ranitidine could be due to the inhibition of P-gp at the intestine/liver levels by ketoconazole; similarly, the decrease in oral bioavailability of ranitidine could be due to the induction of P-gp in the intestine/liver by rifampicin. Here, the possibility of influence of the CYP3A enzyme on the oral bioavailability of ranitidine could be ruled out because the metabolic pathway of ranitidine is not through this cytochrome system /21/. Ranitidine is mainly excreted as the *S*-oxide and *N*-oxide metabolites, or in unchanged form, through urine and/or bile /22/. Thus, whatever difference was observed in the oral pharmacokinetic parameters of ranitidine could be mainly due to the modulation of P-gp by ketoconazole and rifampicin. There have been several reports that the intestinal P-gp plays an important role in the oral bioavailability of P-gp substrates. Gomez *et al.* noted that treatment with ketoconazole resulted in a dramatic increase in the oral bioavailability of cyclosporine, of about 65% in healthy volunteers and 75% in kidney transplant recipients /23/. Greiner *et al.* /14/ 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. They have reported that treatment with rifampicin increased P-gp content by 3.5 ± 2.1 fold in duodenal biopsies of volunteers. All these above-mentioned data indicate that the modulation of P-gp (by induction/inhibition) by various inhibitors/substrates/inducers would influence the oral bioavailability of any drugs which is a substrate of P-gp.

The urinary excretion patterns of ranitidine after the treatments showed no statistically significant difference in CL_R of ranitidine after

pretreatment with ketoconazole or rifampicin in healthy human volunteers. This indicates that the pretreatments did not influence ranitidine's excretion through urine.

From the rat everted sac studies, based on these observed *in vivo* pharmacokinetic data of ranitidine in human volunteers, it was indicated that the alterations in oral bioavailability could be due to changes at the absorption site, that is in the intestine, because of the alterations in C_{max} and T_{max} in human volunteers. In this study, the mean ± SD cumulative exsorption concentrations of ranitidine in the presence of ketoconazole were decreased, whereas the concentrations were increased after pretreatment with rifampicin. Our data (Table 2) are in good agreement with those of Trezise *et al.*, who reported that the expression of multidrug resistance mdr1 mRNA varied in rat intestine, with a moderate expression in the duodenum and the jejunum, maximal expression in the ileum, and then a decrease in expression through the proximal and distal colon /24/. Yumoto *et al.* /17/ 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 vitro* (everted rat intestine) transport studies using rat ileum as comparable with the transport studies in Caco-2 cell monolayers.

In the intestinal loop experiments, the disappearance of ranitidine was shown to be region dependent, as depicted by the following order: duodenum>jejunum>ileum (Table 3). It was observed that the absorption of ranitidine was enhanced through the duodenal, jejunal and ileal loops of rat intestine in the presence of ketoconazole, and the absorption decreased after pretreatment with rifampicin. The percentage dose of ranitidine after one hour of incubation in all three regions of intestinal loops were significantly reduced in the presence of ketoconazole and increased with pretreatment by rifampicin. This indicates that ketoconazole improved the intestinal absorption of ranitidine by inhibiting exsorption, mediated by P-gp. Similarly, the absorption of ranitidine was reduced due to the enhanced exsorption through P-gp induction by rifampicin. The results of these studies suggest that the exsorption observed in everted sacs led to reduced absorption in intestinal loop preparations, which, in turn, may lead to the lowering of oral bioavailability of ranitidine in human volunteers.

Nakayama *et al.* /19/ studied the absorption behavior of various steroid hormones in rat intestine using the intestinal loop technique. They observed the absorption of these hormones in the presence of vinblastine, a known P-gp substrate, using the rat loop technique, and found that the disappearance of hydrocortisone, prednisolone, 6α-methylprednisolone, etc., was region-dependent.

Significant pharmacokinetic interactions have also been demonstrated between ranitidine and some P-gp modulators, nifedipine and theophylline /25/. Recognition of the much broader specificity of P-gp and its functional effects on intestinal drug transport could lead to strategies for improving absorption.

CONCLUSIONS

Our investigation has shown that ranitidine is a P-gp substrate *in vivo* in human volunteers. The observed changes in pharmacokinetic parameters indicate that the oral bioavailability of ranitidine is influenced at the intestinal absorptive phase.

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