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Bioavailability and Interaction Potential of Atorvastatin and Losartan on Co-administration in Healthy Human Subjects

Tausif Ahmed[1], Sivacharan Kollipara[1], Anirudh Gautam[1], Radhika Gigras[1], Monica Kothari[2], Nilanjan Saha[2], Vijay Batra[1], Jyoti Paliwal[1,*]

[1]Metabolism and Pharmacokinetics Department, Ranbaxy Research Laboratories,Plot No. 20, Sector-18, Gurgaon, Haryana 122015, India
[2]Department of Medical Affairs and Clinical Research,Ranbaxy Research Laboratories, Plot no. 77B, Sector 18,Udyog Vihar Industrial Area,Gurgaon-122015, Haryana, India

*Corresponding author: Jyoti Paliwal,Metabolism and Pharmacokinetics Department, Ranbaxy Research Laboratories, Plot # 20, Sector-18, Udyog Vihar Industrial Area, Gurgaon-122015, Haryana, India, Tel : + 91-124-2342001 / 91-124-5195021; Fax: +91-124-2343454; E-mail: jyoti.paliwal@ranbaxy.com

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Abstract

A randomized, open-label, balanced, three-treatment, three-sequence, three-period single dose crossover pharmacokinetic interaction study was conducted to evaluate the potential of interaction between Atorvastatin (AT) and Losartan (LS). Subjects were administered either 40 mg AT or 100 mg LS or combination of both in either of the periods. Blood samples were collected at regular intervals to measure the plasma concentrations of AT, O-hydroxy atorvastatin (O-HAT), LS and its carboxylic acid metabolite (LS-CA) for pharmacokinetic analysis. Co-administration of AT and LS was well tolerated without any significant change in area under the curve (AUC) of either of the drugs or their respective metabolites. There was an increase in C_{max} (ng/mL) of AT, O-HAT, LS, and LS-CA by 29% (38.8(±20.9) to 47.8(±18.4)), 86% (15.7±(10.6) to 29.8(±19.1)), 51% (503.0(±246.0) to 793.0(±376.0)) and 21% (971.0(±245.0) to 1189.0(±323.0)), respectively in combination treatment. Both AT and LS are substrates of P-glycoprotein (P-gp) and CYP3A4, and reported to be completely absorbed from gastrointestinal tract. Hence, this change in the rate of absorption appears to be due to transient saturation of P-gp and/or CYP3A4 during initial absorption phase in the gut wall prior to reaching in portal vein circulation. The increase in C_{max} of both drugs may not be clinically significant to call for dosage adjustment.

Key words: Atorvastatin; Losartan; Pharmacokinetic interaction

Introduction

Coronary heart disease (CHD) is one of the major life threatening diseases all over the world after acquired immune deficiency syndrome (AIDS) and cancer. The main reason for CHD is atherosclerosis, which is characterized by a risk factor called hypercholesterolaemia. A major breakthrough in the pharmacological treatment of hypercholesterolaemia has been the introduction of inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, popularly known as statins. The progression of atherosclerosis can be efficiently delayed by treatment with statins (Shepherd et al., 1995). Also members of this class have been shown to reduce the risk of cardiovascular morbidity and mortality in patients with or at risk for CHD. Based on clinical evidence, the United States National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III report extended the use of lipid-lowering treatments to a larger number of high-risk CHD patients who often receive more than one medication (NECP,

2002). Atorvastatin (AT), a synthetic reversible inhibitor of the microsomal enzyme HMG-CoA reductase belongs to the second generation of statins. AT is given orally as calcium salt over wide range of clinical dosages ranging from 10 mg to 80 mg/day. *In vivo,* AT is initially converted into its lactone form and further to two pharmacologically active metabolites, O-hydroxy atorvastatin (2-hydroxy atorvastatin, O-HAT) and P-hydroxy atorvastatin (4-hydroxy atorvastatin) by action of CYP3A4 (Jacobsen et al., 2000). AT and its metabolites are substrates for CYP3A4, uridine diphospho glucuronosyltransferases (UGT1A1, UGT1A3), P-glycoprotein (P-gp), organic anion-transporting polypeptide (OATP-C) and proton-monocarboxylic acid co-transporter (MCT) and subsequently eliminated by biliary secretion and direct secretion to intestine from blood (Clarke et al., 2003; Wu et al., 2000; Prueksaritanont et al., 2002; Hsiang et al., 1999; Le Couteur et al., 1996). Consequently, their pharmacokinetics may be subjected to various interactions with other drugs that interact with above systems. Like any other statin, asymptomatic increase in liver transaminases and myopathy are the most important and common adverse effects observed with AT treatment (Croom et al., 2005).

Losartan (LS) is an orally active, highly selective antagonist that blocks the binding of angiotensin II to the angiotensin II type 1 (AT1) receptor subtype without agonist properties (Chiu et al., 1991; Wong et al., 1996). LS is very effective and safe in the treatment of hypertension with or without left ventricular hypertrophy. It is currently indicated for the treatment of hypertension and is well tolerated in patients with concurrent conditions. After oral administration approximately 14% of LS dose is converted to the pharmacologically active carboxylic acid metabolite (E-3174, LS-CA) by action of CYP2C9, CYP3A4 and CYP2C10 (Lo et al., 1995). Various studies showed that E-3174 is 10- to 40-fold more potent than its parent compound (Sachinidis et al., 1993). The terminal half-life of LS is found to be significantly lesser (1.5-2 h) compared to E-3174 (6-9 h). Like any other angiotensin receptor antagonist, LS is devoid of any significant adverse effects (Mann et al., 1999).

Even though hypertension and hyperlipidaemia are independent factors in cardiovascular diseases, they commonly co-exist in large number of patients indicating the necessity of combination therapy of both the drugs (Goode et al., 1995). A concomitant management of these two risk factors has been suggested to reduce the extent of cardiovascular complications. Although these drugs are well tolerated, their pharmacokinetic drug-drug interactions can lead to some adverse clinical consequences. The worldwide withdrawal of Cerivastatin in August 2001 because of its association with fatal rhabdomyolysis further underscores the importance of considering the drug-drug interaction of the available statins (Davidson, 2002). Drug-drug interactions may occur by either inhibition or induction of relevant enzyme(s) and/or transport protein(s). In the present study possible pharmacokinetic drug interaction of AT with LS was explored since: (1) both are prescribed for extended use therapy (2) both the drugs are metabolized by common enzymes and (3) metabolic products of AT and LS are active.

Methods

Study Participants

The study was conducted in 18 healthy male Asian subjects between 18 to 45 years of age and weight from 47 to 75 kg. The subjects were considered to be eligible if they satisfied the following inclusion criteria: a body mass index between 19 and 28 kg/m^2, were healthy as determined by medical history, physical examination of subjects performed with in 21 days prior to commencement of study and had normal renal and hepatic function based on clinical laboratory measurements.

Subjects were excluded if they had participated in any other investigational drug study or blood donation 3 months prior to the study commencement. The exclusion criteria also included development of hypersensitivity reactions to AT and LS group of drugs, presence of disease markers of HIV, Hepatitis B or syphilis infection, positive for urinary testing of drugs of abuse (opiates or cannabinoids), abnormality towards clinical examinations (12-lead electrocardiogram, chest X-ray, serum creatinine, blood urea nitrogen, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, serum bilirubin, plasma glucose or serum cholesterol), and microscopic examination of urine (presence of RBC, WBC (>4/HPF), positive glucose or protein). Subjects were also excluded if they were on any other planned treatment during its course until the study ends, including vitamins, mineral supplements, over-the-counter products. If the subjects were taking alcohol (>2 units/day), caffeine (> 5 cups of coffee or tea/day), or smoking (>10 cigarettes/day), they were also excluded as per US Food and Drug Administration (FDA) dietary guidelines. The consumption of alcohol, beverages and food, containing methylxanthines was not permitted for the volunteers 48 h prior to the study and after drug administration until the last blood sample was collected in the respective study period.

All the subjects provided written informed consent to participate after explaining the nature and purpose of the study. The study protocol was approved by the Fortis Hospital

Institutional Review Board (IRB) and was in accordance with the ethical principles described in the Declaration of Helsinki, guidelines for International Conference on Harmonization-Good clinical practices (ICH-GCP).

Study Design and Sampling Schedule

The study was conducted at Clinical Pharmacology Unit, Ranbaxy Laboratories Limited, Noida, India. The bioanalysis was carried out at Suven Life Sciences, Hyderabad, India. The study was designed as an open label, balanced, randomized, three-treatment, three-sequence, three-period, single-dose, crossover, pharmacokinetic drug-drug interaction study. The subjects were randomized in such a way that they received either of the following treatments:

Treatment-A: Two 20 mg tablets each containing 20 mg of Atorvastatin calcium, manufactured by Ranbaxy Laboratories Ltd, India.

Treatment-B: Two 50 mg film coated (FC) tablets each containing 50 mg Losartan potassium, manufactured by Ranbaxy Laboratories Ltd, India.

Treatment-C: Two 20 mg tablets each containing 20 mg of Atorvastatin calcium + Two 50 mg FC tablets each containing 50 mg Losartan potassium.

A washout period of 14 days was enforced between administrations of each treatment/period. After an overnight fasting (12 h), volunteers were given a single dose of either of the treatments with 240 ml of drinking water. No food was allowed until 4 h after dose administration. Water intake was allowed 2 h after the dose. Standard meals lunch, snacks and dinner were served to the subjects at 4, 9 and 13 h, respectively after administration. All subjects were dosed while seated and were asked to remain seated or ambulatory for the first two hours following drug administration in each period. Thereafter, the subjects were allowed to engage in normal activities avoiding severe physical exertion until discharge in each period. Blood samples were collected at pre-dose and at 0.25, 0.5, 0.75, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 3.33, 3.67, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 36, 48 and 72 h post-dose in each period to measure AT, O-HAT, LS and LS-CA in plasma. A total of seventy five 5-ml blood samples were collected form each subject, during the course of the study through indwelling cannulae placed in forearm veins. The pre-dose blood sample in each period was collected within a period of 1.5 h before dosing and the post-dose samples were collected within 2 min of the scheduled time. After collection, the blood samples were centrifuged under refrigeration as soon as possible to separate plasma. The plasma samples were stored at -50 °C or lower until analysis.

Safety Assessments

Vital signs of oral temperature, sitting blood pressure and radial pulse were measured during subject admission, prior to each dosing and 2, 4, 8, 12, 24, 36, 48 and 72 h after administration of study drug in each study period. Vital signs measured prior to administration of the dose were taken within 1.5 h of the scheduled dosing time. At all other times, vital signs were taken within 45 min of the scheduled time. Adverse effects, defined as unwanted medical occurrences developing after administration of medication regardless of any special cause were monitored for the study subjects. Subjects were asked about any adverse effects in each period before and after study drug(s) administration and approximately every 4 to 5 h thereafter until the discharge.

Quantification of Atorvastatin and O-Hydroxy Atorvastatin

Concentrations of AT and O-HAT were quantified using validated liquid chromatography tandem mass spectrometry (LC-MS/MS) (Applied Biosystems, MDS SCIEX, Canada) assay using Rosuvastatin as internal standard (IS). Both the compounds were tuned in positive mode for multiple reaction monitoring (MRM). In MRM mode, 559.20 → 440.20, 575.20 → 466.40 and 482.10 → 258.30 were selected as transition ions for AT, O-HAT and IS, respectively. Both the compounds were extracted from 1.0 ml human plasma by simple and single step liquid-liquid extraction with diethyl ether and dichloromethane (70:30, v/v). After reconstitution, 10 µl was injected on HPLC system comprising of Agilent 1100 series (Agilent, USA) pump, autosampler, and column oven. Analytes were eluted using 0.03 % (v/v) formic acid and acetonitrile (30:70, v/v) as mobile phase at a flow rate of 1.0 ml/min. Data acquisition and integration of chromatographic peaks were carried out using Analyst version 1.4.1 (Applied Biosystems, MDS SCIEX, Canada). The bioanalytical method was specific with no interfering impurities at retention time of analyte or IS. The method exhibited linearity of response over a range of 250 – 25000 pg/ml for AT (R^2=0.9998) and O-HAT (R^2=0.9995). Quality control (QC) samples at three levels low (750 pg/ml), middle (7500 pg/ml) and high (20000 pg/ml) for AT and O-HAT were used during routine analysis. The within batch precision of assay (%CV) ranged from 4.38 to 7.32 for AT and 2.98 to 5.60 for O-HAT. The within batch accuracy of AT ranged from 101.63 to 104.93% while for O-HAT it ranged from 98.69 to 103.43%. The between batch precision of assay (%CV) ranged from 3.72 to 6.02 for AT and 2.33 to 4.65 for O-HAT, the between batch accuracy of AT ranged from 100.11 to 103.56% while

for O-HAT it ranged from 98.07 to 101.21%.

Quantification of Losartan

Quantification of LS was performed by a validated LC-MS/MS (Applied Biosystems, MDS SCIEX, Canada) method using Valsartan as IS. After tuning the compounds in positive mode, the transition ions 423.10 → 207.00, 436.20 → 306.10 were selected for estimation of LS and IS in MRM. The plasma samples (200 µL) were extracted using a single step liquid-liquid extraction with tetra-butyl methyl ether (TBME). A HPLC system (Shimadu, Japan) consisting of degasser (Shimadzu DGU-20A5), pump (Shimadzu LC-10AD*VP*), autosampler (Shimadzu SIL-HTC) and column oven (Shimadzu CTO-10AS *VP*) was used for chromatographic separation. After 5 µL of injection, analyte was eluted using mobile phase consisting of 0.03 % (v/v) formic acid and acetonitrile (25:75, v/v) delivered at flow rate of 1.0 ml/min. Analyst Version 1.4.1 (Applied Biosystems, MDS SCIEX, Canada) was used for acquisition of data and chromatographic peak integration. The bioanalytical method was specific with no interfering peaks at retention time of analyte or IS. The developed method exhibited linearity over dynamic range 20 – 1000 ng/ml for LS (R^2= 0.9978). QC samples at three levels low (60 ng/ml), middle (300 ng/ml) and high (800 ng/ml) were used for routine sample analysis. The within batch precision ranged from 3.17 to 5.16 and accuracy ranged from 95.55 to 105.65%. The between batch precision and accuracy ranged from 1.65 to 4.82 and 97.02 to 104.97%, respectively.

Quantification of Carboxylic Acid Metabolite of Losartan

Plasma samples containing LS-CA were analyzed by validated LC-MS/MS (Applied Biosystems, MDS SCIEX, Canada) assay using Valsartan as IS. Mass transitions 435.10 → 156.90 and 434.30 → 350.20 were selected for MRM for LS-CA and IS respectively, after tuning in negative mode. The plasma samples (200 µL) were extracted by one step liquid-liquid extraction with TBME. The HPLC and data acquisition systems were same as described in section 'Quantification of Losartan'. After reconstitution, 10 µL of injection were made and analytes were eluted using 10 mM ammonium acetate and methanol (40:60, v/v) as mobile phase at a flow rate of 1.0 ml/min. The developed method was specific and presented linearity over dynamic range of 20 – 1000 ng/ml (R^2=0.9998). QC samples at three levels low (60 ng/ml), middle (300 ng/ml) and high (800 ng/ml) were used during routine sample analysis. The within bath precision was ranged from 1.55 to 2.31, while between batch precision ranged from 0.87 to 2.26. The within batch and between batch accuracies ranged from 98.13 to 102.49% and 98.15 to 101.49%, respectively for LS-CA.

The accuracy and precision for calibration curve standards and QC samples in all bioanalytical methods met the acceptance criteria as per USFDA guidelines (USFDA, 2001).

Pharmacokinetic and Statistical Analysis

Pharmacokinetic analysis was performed by means of model independent method (non-compartmental approach) using validated WinNonlin Professional software (Version 4.1, Pharsight Corporation, Cary, North Carolina). Actual sampling times were used to estimate the pharmacokinetic parameters of studied drugs and respective metabolites. The elimination rate constant (λ_z) was obtained as the slope of the linear regression of the log transformed concentration values versus time data in the terminal phase. The elimination half life ($T_{1/2}$) was calculated as 0.693/ λ_z. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the log-linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t}+C_t/\lambda_z$, where C_t is the last measurable concentration. Pharmacokinetic drug-drug interaction between AT, LS and their metabolites was assessed by comparing $AUC_{0-\infty}$, AUC_{0-t} and C_{max} from single agent with respective parameters obtained after coadministration. A student t-test was applied for the statistical hypothesis testing of median T_{max} values obtained in both treatments. p-value at 5% significance level was determined in each case. A parametric (normal-theory) general linear model (GLM) was applied to the natural log transformed AUC and C_{max}. An analysis of variance (ANOVA) with subject, treatment, and period as factors was performed by using the GLM procedure in SAS (Version 8.1, SAS Institute, Cary, North Carolina). The results for AUC and C_{max} were reported as 90% confidence interval (CI) surrounding the ratio (percentage) of the geometric means of the pharmacokinetic measures with or without the second drug. The 90% CI for AUC_{0-t}, $AUC_{0-\infty}$, C_{max} were calculated for the following natural log transformed least square mean ratio:

$$\frac{Atorvastatin + Losar\ tan\ (Treatment - C)}{Atorvastatin\ (Treatment - A)\ or\ Losar\ tan\ (Treatment - B)}$$

A statistically significant drug-drug interaction would be concluded, if the 90% CI of the ratio of geometric means lie outside the limits of 80 to 125% for natural log transformed AUC and C_{max}.

Results

Demographics

Eighteen male Asian subjects satisfying the inclusion/exclusion criteria participated in the study and thirteen of them completed the study. Three subjects were withdrawn from study due to adverse events such as dizziness and vomit-

ing. One subject was withdrawn due to pain in the left ear and other dropped out from the study after period I due to personal reason. The data obtained from dropped out and withdrawn subjects were not included in pharmacokinetic and statistical analysis. The eighteen subjects who participated in the study had age of 24.17±6.53 years, height of 167.72±6.55 cm and weight of 59.96±7.60 Kg.

Pharmacokinetics

Atorvastatin and O-Hydroxy Atorvastatin

The pharmacokinetic parameters of AT and O-HAT after administering AT alone (Treatment-A) and in combination with LS (Treatment-C) are listed in Table 1. The mean plasma concentration versus time profiles of AT and O-HAT for both the treatments are given in Figure 1. Pharmacokinetic parameters of the parent and metabolite were in agreement with reported values (Lennernas, 2003). The student t-test revealed statistically insignificant difference between median T_{max} obtained in Treatments A and C for both AT (p=0.5015) and O-HAT (p=0.6438). Also, there was no significant difference in AUC_{0-t}, $AUC_{0-\infty}$, and $T_{1/2}$ values, when administered alone and in combination with LS. However, higher C_{max} values of AT and O-HAT were observed when AT and LS were administered simultaneously to healthy male volunteers compared to administration alone. The C_{max} of AT increased by 29%, while that of metabolite increased by 86%. The summary statistics of pharmacokinetic parameters for AT and its metabolite is listed in Table 3. It can be seen from Table 3 that 90% CI of the ratio of least square means of log transformed AUC_{0-t} and $AUC_{0-\infty}$ for AT and O-HAT were found to be very well with in limits of 80-125%, as per regulatory requirements. However, the 90% CI for log transformed C_{max} fell out side the regulatory limits of 80-125%.

PK Parameter	Alone (A)	Combination (C)
AT		
T_{max} (h)*	0.50 (0.50-2.67)	0.75 (0.50-4.00)
C_{max} (ng/ml)	38.8±20.9	47.8±18.4
AUC_{0-t} (h·ng/ml)	102±50.9	97±29.7
$AUC_{0-\infty}$ (h·ng/ml)	130±54.2	112±30.5
$T_{1/2}$ (h)	6.89±2.92	4.10±1.70
O-HAT		
T_{max} (h)*	0.75 (0.50-4.50)	1.00 (0.50-4.50)
C_{max} (ng/ml)	15.7±10.6	29.8±19.1
AUC_{0-t} (h·ng/ml)	109±49.1	109±54.2
$AUC_{0-\infty}$ (h·ng/ml)	116±49.6	117±52.0
$T_{1/2}$ (h)	7.17±4.01	8.92±5.93

* For T_{max}, median (range) are reported

Table 1: Pharmacokinetic parameters (Mean±SD) for Atorvastatin (AT) and O-Hydroxy Atorvastatin (O-HAT) (n=13).

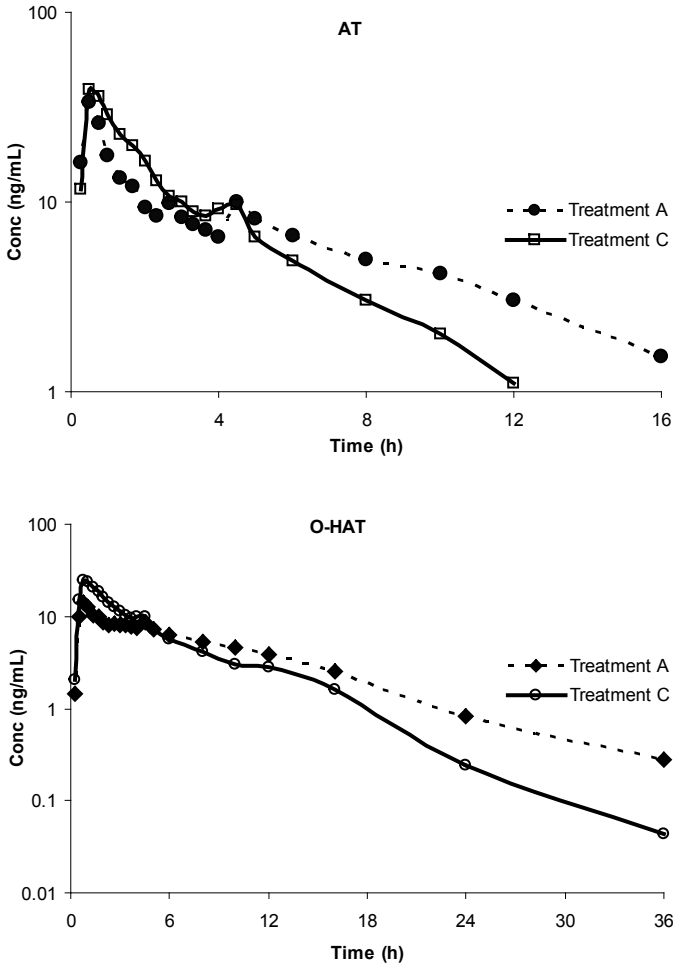

Figure 1: Mean plasma concentration vs time profiles of Atorvastatin and O-hydroxy Atorvastatin following administration of Atorvastatin alone (Treatment-A) or in combination with Losartan (Treatment-C) (n=13).

Losartan and Carboxylic Acid Metabolite of Losartan

The mean plasma concentration versus time profiles for both treatments is given in Figure 2. The pharmacokinetic parameters of LS and LS-CA were outlined in Table 2 and were in accordance with reported values in literature (Sica et al., 2005). There was no statistically significant difference in median T_{max} values between Treatment B and C for LS (p=0.1507). However, a significant difference in T_{max} was observed for LS-CA (p=0.0005). Compared with the administration of LS alone, the concomitant administration of AT did not significantly alter the AUC_{0-t}, $AUC_{0-\infty}$, and $T_{1/2}$ of LS and its carboxylic acid metabolite LS-CA. In contrast, a significant increase in C_{max} by 51% and 21% was observed for LS and LS-CA respectively in combination treatment. The summary statistics of pharmacokinetic parameters in Table 3 indicates that 90% CI for the ratio of least square means of log transformed AUC_{0-t}, $AUC_{0-\infty}$ were well within the regulatory limits of 80-125%. However as observed in case of AT, the 90% CI for ratio of least square means of log transformed C_{max} values of both LS and LS-CA were out of the acceptance criteria of 80-125%.

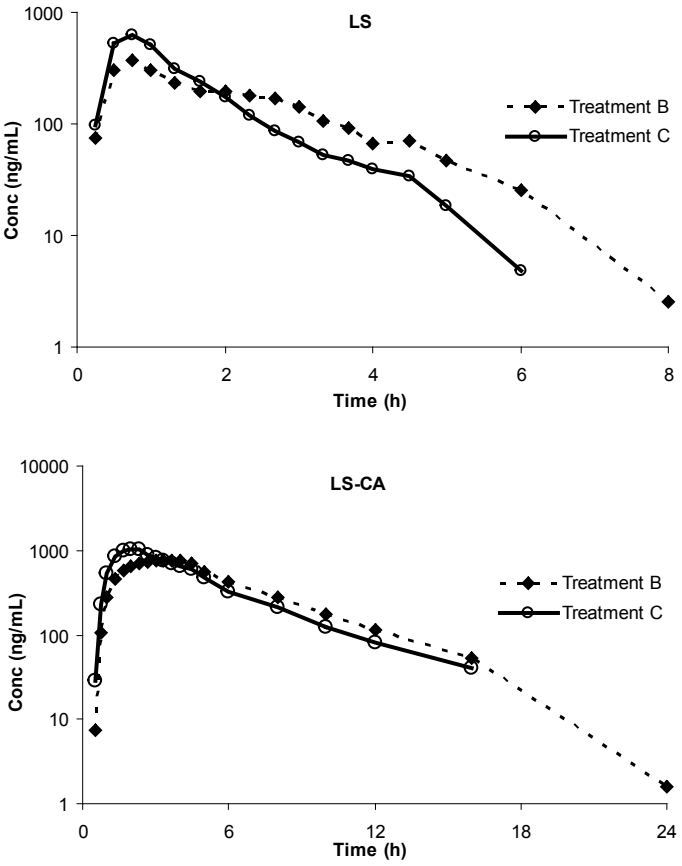

Figure 2: Mean plasma concentration vs time profiles of Losartan and Carboxylic acid metabolite of Losartan following administration of Losartan alone (Treatment-B) or in combination with Atorvastatin (Treatment-C) (n=13).

PK Parameter	Alone (B)	Combination (C)
LS		
T_{max} (h) *	1.33 (0.50-3.67)	0.75 (0.50-4.00)
C_{max} (ng/ml)	503.0±246.0	793.0±376.0
AUC_{0-t} (h·ng/ml)	828±373.0	869±264.0
$AUC_{0-\infty}$ (h·ng/ml)	879±382.0	903±274.0
$T_{1/2}$ (h)	1.23±0.30	0.93±0.39
LS-CA		
T_{max} (h) *	3.00 (1.67-4.00)	1.67 (1.33-4.50)
C_{max} (ng/ml)	971.0±245.0	1189.0±323.0
AUC_{0-t} (h·ng/ml)	5007±1231.0	4964±1385.0
$AUC_{0-\infty}$ (h·ng/ml)	5256±1213.0	5173±1399.0
$T_{1/2}$ (h)	3.51±0.53	3.61±0.65

*For T_{max}, median (range) are reported

Table 2: Pharmacokinetic parameters (Mean±SD) for Losartan (LS) and carboxylic acid metabolite of Losartan (LS-CA) (n=13).

Adverse Events

All the subjects were monitored during three periods and no clinically significant adverse effects were reported even during Treatment-C. Three subjects were withdrawn from the study due to vomiting and dizziness. Out of total 11 adverse events observed during the study, 2 were dizziness during pre-dose and remaining 9 post-dose adverse events as listed in Table 4.

Discussion

A number of drug interactions of AT have been reported in literature. Boyd *et al.* reported that concomitant administration of 80 mg AT increased the steady-state digoxin

Compound	ln $AUC_{0-\infty}$		ln AUC_{0-t}		ln C_{max}	
	% Ratio	90% CI	% Ratio	90% CI	% Ratio	90% CI
AT	90.96	(80.20, 102.56)	100.15	(89.58, 111.95)	128.53	(105.62, 156.44)
O-HAT	99.41	(85.45, 115.65)	97.15	(82.28, 114.68)	185.47	(157.96, 217.71)
LS	105.44	(93.28, 119.20)	105.40	(93.16, 119.23)	151.01	(116.88, 195.09)
LS-CA	96.61	(91.54, 101.97)	97.36	(92.18, 102.82)	121.30	(109.61, 134.26)

Table 3: Summary statistics of pharmacokinetic parameters for Atorvastatin (AT), O-Hydroxy Atorvastatin (O-HAT), Losartan (LS) and carboxylic acid metabolite of Losartan (LS-CA) (n=13).

Adverse Events	Treatment-A	Treatment-B	Treatment-C
Fever	01	01	00
Swelling at vein puncture site	00	01	00
Vomiting	01	01	01
Dizziness	00	01	00
Pain in left ear	01	00	00
Blister on lower lip	01	00	00
Total	**04**	**04**	**01**

Table 4: Summary of most frequently reported adverse effects post-dose during various Treatments (A, B or C).

concentrations by 20% (Boyd et al., 2000). The possible mechanism explained for this increase in concentration was inhibition of P-gp mediated secretion of digoxin in the intestine by AT and its metabolite. It was also shown that this inhibition occurred at high concentration of AT, achieved during the absorption phase. In an interaction study of AT and verapamil it was found that AT increased verapamil AUC by 43% via inhibition of P-gp and/or metabolism (Choi et al., 2008). Protease inhibitors, such as ritonavir 300 mg or saquinavir 400 mg BID along with nelfinavir 1250 mg BID, have been shown to moderately increase the plasma AUC and C_{max} (75-120%) of AT equivalents, when both the inhibitors and AT were administered repeatedly. In this study, the AUC of AT acid (parent) increased by 350% (Hsyu et al., 2001; Fichtenbaum et al., 2002). These drug interactions were also related to interaction between inhibition of both CYP3A4 and P-gp (Lennernas, 2003). AT is often used in combination with troglitazone for diabetic patients with dyslipidaemia (DiTusa et al., 2000). In a study with 31 patients receiving troglitazone 400 mg a clinically significant decrease in the lipid-lowering effect of AT was observed (DiTusa et al., 2000). On the other hand itraconazole, a potent CYP3A4 inhibitor increased the AUC's of AT and its lactone by 3-and 4 fold, respectively (Kantola et al., 1998). Erythromycin (500 mg QID) increased the C_{max} and plasma AUC of AT equivalents by 38% and 33%, respectively (Siedlik et al., 1999). This interaction may be due to the inhibition of both CYP3A4 and P-gp.

CYP3A4 is also the major enzyme responsible for the conversion of AT to its metabolites (Jacobsen et al., 2000). LS or its metabolite are not known to alter the activity of CYP3A4 and can be assumed that the concomitant administration did not alter the AUC of AT or its metabolite. AT and O-HAT, both are active and equally contribute to the efficacy of the statin. Hence, insignificant change in AUC indicates the retention of efficacy of AT and it's metabolite in combination therapy. Nevertheless, higher peak plasma concentrations (C_{max}) of AT and O-HAT were observed in combination therapy in comparison to when administered alone. The mean C_{max} and AUC were found to increase non-linearly with increase in dose for AT (Cilla et al., 1996). The possible reasons for non-linearity could be saturation of P-gp mediated efflux at the liver as it is the primary site of metabolism. However, C_{max} values higher than those observed in present study have been reported to be well tolerated for AT (at relatively higher doses) (Cilla et al., 1996; Malhotra et al., 2001). Hence it can be concluded that even though there is significant pharmacokinetic interaction, AT exhibits insignificant clinical interaction in combination therapy.

Few drug interaction studies have been conducted with LS. LS and hydrocholorothiazide did not affect each others pharmacokinetics (Sica et al., 2005). LS had no clinically important interaction with either of digoxin, warfarin or phenytoin (Smet et al., 1995; Kong et al., 1995; Fischer et al., 2002). LS is converted to its active metabolite by the action of metabolizing enzymes like CYP2C9 and CYP3A4 (Lo et al., 1995). *In vitro* studies with human liver microsomes revealed that sulphaphenazole, a specific CYP2C9 inhibitor blocked the conversion of LS to its metabolite by 81% and ketoconazole, a CYP3A4 inhibitor blocked by 12% at 10 μM concentration (Yun et al., 1995; Stearns et al., 1995). This data indicates that CYP2C9 contribution is seven times higher than that of CYP3A4 in the conversion of LS to its active carboxylic acid metabolite. AT, an inhibitor of CYP3A4 and P-gp did not alter the AUC of LS and its metabolite indicating the lack of pharmacokinetic interaction (Boyd et al., 2000; Mc Donnell et al., 2003; Renders et al., 2001). Moreover, LS-CA is responsible for majority of antihypertensive action of LS. This active metabolite is 10 to 40 times more potent than parent compound. As AUC of metabolite in treatment group receiving combination are comparable to those receiving LS alone and therefore the pharmacological effect is not expected to be affected by concomitant therapy. However, higher C_{max} values of LS and LS-CA were observed in combination treatment in comparison to administration of LS alone. The therapeutic response of LS correlates better to AUC than any single plasma concentration like C_{max} (McIntyera et al., 1997). Considering the excellent safety and tolerability profile of LS, higher C_{max}, a transient phenomenon, does not raise any safety issues. Even though there is significant increase in C_{max} for

LS and its metabolite, values more than 2-folds of observed C_{max} values in combination treatment were well tolerated by healthy subjects (Ohtawa et al., 1991).

Wu et al., (2005) postulated that drug-drug interactions are not limited to enzymatic processes but are mediated by transporter-enzyme interplay especially for Biopharmaceutics Classification System (BCS) class 2 compounds such as AT (Wu et al., 2005). Following oral administration, major significant interactions would occur for BCS class 2 drugs that are substrates for both intestinal enzymes (e.g CYP3A, UGT's) and intestinal apical efflux transporters (e.g. P-gp, MRP2, BCRP). According to Wu et al. (2005) simultaneous inhibition of intestinal enzymes and apical efflux transporter would lead to less gut metabolism and this would synergistically increase systemic drug concentrations. The enzyme-efflux transporter interplay in the liver and kidney is not the same as at intestinal level due to the reverse order in which drug molecules encounter the two proteins (Wu et al., 2005). The increase in the C_{max} of both the drugs and their metabolites in the current study can be explained based on competitive co-inhibition of transporter proteins like P-gp present in gastro intestinal (GI) tract. Both AT and LS being substrates for P-gp and CYP3A4, it is reasonable to expect their interaction in GI tract at both these targets (Boyd et al., 2000; Soldner et al., 2000). Inhibition of counter transport by P-gp presumably saturates the CYP leading to increase in systemic exposure (increase in C_{max}) of both the drugs. Similar increase in exposure was observed when molar concentration of drug and respective metabolite were expressed in terms of parent drug (Figure 3, 4). Earlier studies have also hypothesized that non linear increase in AUC of AT may be due to saturation of P-gp and hence resulting in increased intestinal absorption (Lennernas, 2003). Since the absorption of AT and LS is nearly complete, their AUC's are not affected by this interaction in GI tract (Wu et al., 2000; Sica et al., 2005). Moreover, no change in the terminal elimination half-life suggests that systemic interaction in metabolism and/or excretion of these two co-administered drugs and their metabolites is unlikely. Hence it can be deduced that the interaction between the co-administered drugs is pre-systemic, possibly at the absorption site.

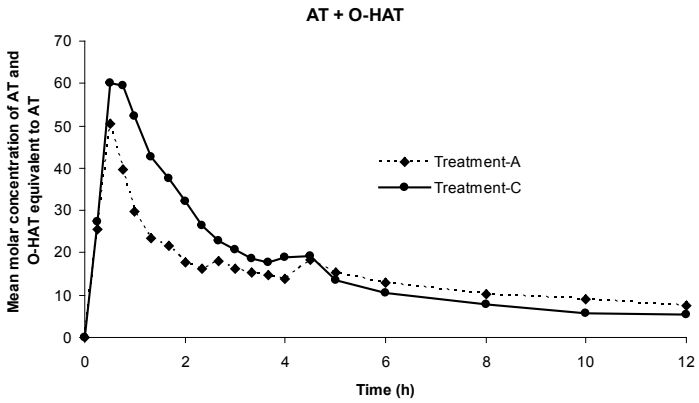

Figure 3: Mean molar concentration levels of AT and O-HAT plotted equivalent to AT following administration of Atorvastatin alone (Treatment-A) or in combination with Losartan (Treatment-C) (n=13).

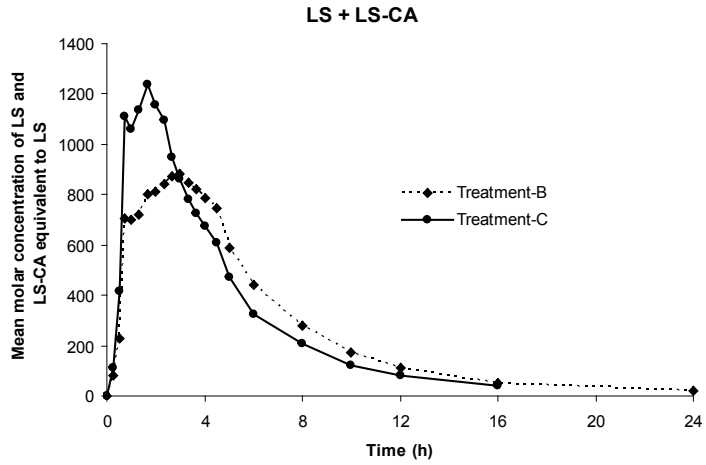

Figure 4: Mean molar concentration levels of LS and LS-CA plotted equivalent to LS following administration of Losartan alone (Treatment-B) or in combination with Atorvastatin (Treatment-C) (n=13).

In the present study, there were no clinically significant adverse events in either of the period and hence can be concluded that all the treatments were well tolerated by all the subjects.

Conclusion

The present study demonstrates pharmacokinetically significant but clinically insignificant interaction between AT and LS in healthy subject after administration of single oral doses simultaneously. The transient increase in the rate of absorption of both the drugs observed as increase in C_{max} of drugs and their active metabolites (total activity) was very well tolerated. Lack of adverse effect suggests that dose adjustment of both the drugs may not be necessary for their concomitant use in cardiovascular abnormalities. However, a clinical study in patients suffering from hypertension and hyperlipidaemia with similar design is recommended to validate the findings of this study in clinical practice.

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