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Effect of Efavirenz and Ritonavir on the pharmacokinetics of Losartan

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ABSTRACT

Drug interactions have become an increasingly complex challenge for treating patients with HIV infection. The present study was to evaluate the effect of efavirenz and ritonavir have any influence on the pharmacokinetics of losartan. Oral treatments of rat with efavirenz [100mg/kg] from days 1 to 13 days. On 14th day administration of efavirenz followed by Losartan [10mg/Kg] after 30 minutes. In addition ritonavir [100mg/Kg] followed similarly as efavirenz on 14th day administration of ritonavir + losartan followed after 30 minutes. Samples were estimated by reverse phase HPLC- UV method. Our study results shows that single dose of studies of efavirenz with losartan increase the bioavailability of Losartan in smaller amounts. In multiple dose studies efavirenz decrease the bioavailability of Losartan there by decrease the antihypertensive effect. Whereas, ritonavir produces antagonist effect. Hence, we conclude that drug interaction takes place with antiretroviral drug Incombination with antihypertensive therapy. In future, our clinical research with HIV patients to confirm the possibility of drug-drug interactions.

Keywords: Antiretroviral drugs, Drug-Drug interaction, HIV Patients, Pharmacokinetic.

INTRODUCTION

Drug interactions have become an increasingly complex challenge for treating patients with HIV infection. Patients are often receiving therapy for co-morbid conditions and prophylaxis of opportunistic infections. Hence a combination of antiretroviral drugs with other diseases condition. The non-nucleoside reverse transcriptase inhibitor efavirenz [EFV]. Ritonavir is often used, in combination with antihypertensive therapy [Losartan]. Plasma concentrations of EFV

are known to show a high degree of inter-individual variability [1] . EFV and, to a certain extent, nevirapine, are metabolized by CYP2B6, [2,3] a cytochrome P450 isoenzyme characterized by wide inter-individual variability in hepatic expression and activity, which is in part due to extensive genetic polymorphism [4,5] . Whereas, losartan is longer acting active metabolite (E-3174) interfere with the binding of angiotensin-II to the angiotensin-II AT1-receptor by, themselves, binding reversibly to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin-II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systematic vascular resistance. The purpose of this research is to provide effect of efavirenz and ritonavir on the pharmacokinetics of losartan.

MATERIALS AND METHODS

Animals

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervision on experimentation on animals (CPCSEA) and were approved by the Institutional Animal Ethical Committee (IEAC), Vaagdevi College of Pharmacy, Warangal, Andhrapradesh, India. (1047/ac/07/CPCSEA, dated 24/04/2007) . Wister rats weighing between 180 to 200 gm of either sex were purchased from Mahaveera Enterprises [Hyderabad AP., India]. Animals had free access to standard pellet diet and water ad libitum.

Drugs

Losartan, Valsartan-Aurobindo, Hyderabad; Efavirenz, Ritonavir (Sun Pharmaceuticals ltd, Mumbai, India) were obtained as a gift samples. Acetonitrile and Methanol HPLC grade were purchased from Ranbaxy, Delhi. Water, Glaciel acetic acid (HPLC grade) were purchased from Qualigens Fine Chemicals, Mumbai, India.

HPLC description

A Cyberlab HPLC system used in the study consisted of a pump (Model LC-P100, Cyber lab corporation, USA) operating at 1ml/min, a syringe loading sample injector of 20µl capacity (Model 7725i) a C18 reverse phase column of 250 × 4.6 mm dimension and 4 µ particle size and a dual wavelength UV-Visible detector (Model LC-100).

Chromatographic conditions

The mobile phase consisted of 0.1% of glacial acetic acid in water and acetonitrile in the proportion of 50:50 v/v. The mobile phase was filtered through 0.22 µm membrane filter. The flow rate was 1ml/min and the effluent was monitored at 230nm.The total run time of the method was set at15 min.

Preparation of calibration curve of losartan

Preparation of stock solutions: A stock solution representing 100 µg/ml of losartan was prepared in water, and the solution was stored at -20˚C. The working standard solutions were prepared prior to use from stock solution by sequential dilution with water to yield final concentrations of 0.1, 0.5, 1, 5 and 10 µg/ml of losartan. The internal standard stock solution.

Extraction Procedure

A volume of 0.5ml blank plasma, 0.1ml of losartan concentrations of 100mg to 10 µg and 0.1ml of 25 µg of valsartan as an internal standard were added. Then the mixtures were gently vortex for 50sec, then add 0.5ml of acetonitrile. The mixture was gently shaken using cyclomixer for 1min and centrifuged for 6min at 13000 rpm. Then the supernatant was transferred into tube and they were evaporated to dryness. Add 0.1ml of mobile phase to reconstitute the drug and then 20 µl was injected into the HPLC.

Construction of calibration curve

The calibration curve was obtained by plotting peak area ratios of losartan to valsartan (y-axis) against losartan concentrations (x-axis). The slope of the plot determined by the method of least square regression analysis was used to calculate the losartan concentration in the unknown sample. A linear calibration curve in the range of 0.1 to 10 µg was established (r^2=0.998). It has been shown in Figure no 1.

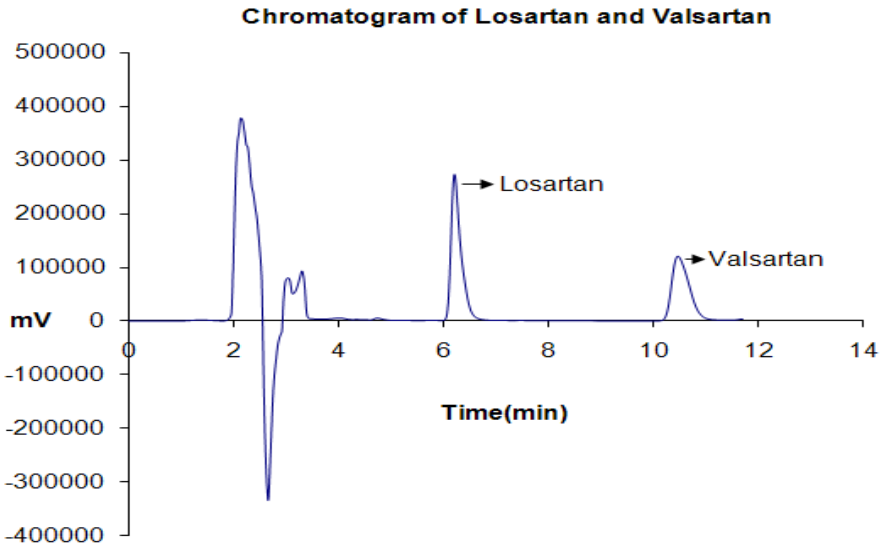

Fig 1: Chromatogram of Losartan and Valsartan

Pharmacokinetic studies in rats

Albino rats of either sex were randomly distributed into five groups of six animals in each group; they were housed in well ventilated aluminium cages and maintained on uniform diet and temperature with 12h light and dark cycle. Before the experiment all animals were fasted for 18hours and water ad libitum, water was withdrawn during experiment.

Group I (control) - 0,2ml of normal saline; p.o.
Group II- Administration of Losartan (10mg/kg;p.o)
Group III – Administration of Efavirenz (100mg/kg) for 13 days, 14th day administration of Efavirez (100mg/kg) followed by Losartan (10mg/kg) after 30minutes.
Group IV – Administration of Ritonavir [100mg/kg] orally followed by losartan (10mg/kg) after 30minutes, treated with Ritonavir (100mg/kg) for 13 days, 14th day administration of Ritonavir (100mg/kg) followed by losartan (10mg/kg) after 30 minutes.

Blood samples were withdrawn at 0, 1, 2, 4, 6, 8 hours time intervals from orbital sinuses using heparinized capillaries. Plasma was separated by centrifugation and stored in vials at −70°C until further estimated.

Analysis of Losartan: Losartan was estimated by reverse phase HPLC-UV method.

Statistical analysis:
Student post t-tests using Graph Pad Instant Software version and "RAMKIN and RAMLIN" software.

Treatment of bioavailability data:
The various pharmacokinetic parameters like elimination half life ($t_{1/2}$), overall elimination rate constant (Ke), area under concentration time curve (AUC), area under first movement curve (AUMC), apparent volume of distribution for fraction of dose absorbed (Vd/f) and systemic clearance for fraction of dose absorbed (Cls/f) for the drug under consideration were obtained in each subject from serum concentration verses time profile on an IBM compatible personal using RAMKIN and RAMLIN, a program developed based on the described in the following paragraphs.

Overall elimination rate constant:
The overall elimination rate constant is the sum of individual rate constant associated with the loss of parent drug from the body. This is a quantitative index of the persistence of drug in the body and is calculated from the slope of the drug in biological fluid verses time, after subjecting it to liner regression analysis.

$$K = 0.693/t_{1/2}$$

Half life ([$t_{1/2}$):
Half life the drug is defined as the time required to reduce the concentration of drug in the body by 50%. It can be calculated from elimination rate constant, assuming the elimination to be a first order process.

$$t_{1/2}=0.693/Ke$$

Area under the curve (AUC) :
The area under the concentration time curve extended to infinite time represents bioavailability of the drugs. It is calculated by means of trapezoidal rule. It is under the zero moment curves.

$AUC_0^{\alpha} = AUC_0^{t} + C^*/K$
Where, C* is the concentration at last point t.

Area under first movement curve (AUMC):
This is again computed by means of trapezoidal rule and it is the area under the curve resulting upon plotting the product of concentration verses time.

Mean residence time (MRT):
Mean residence time represents the time for 63.2% of the administered dose to be eliminated. It is statistical movement along of half-life.

Where, AUMC is the area under first movement curve.

RESULTS

Drug interactions mostly occur when two or more drugs administered concomitantly, these interactions usually seen in clinical practice and the mechanisms of interactions are evaluated usually in animal models. We studied the influence of efavirenz and ritonavir on the pharmacokinetics of losartan rats.

Losartan is an angiotensin-I receptor blocker, Losartan and its longer acting active metabolite (E-3174) themselves binds to the receptors in vascular smooth muscle and in adrenal gland, causes markedly reduce the blood pressure.

Losartan is well absorbed and undergoes substantial first-pass metabolism; the systemic bioavailability of losartan is approximately 33%. About 14% of an orally-administered dose of losartan is converted to the active metabolite (E-3174). Metabolism occurs in the liver by Cytochrome P450 2C9 and 3A4.

From the concentration vs. time profile we calculate the pharmacokinetic parameters using Ramkin & Ramlin software. There is significant change in pharmacokinetic parameters on 14th day.

The mean (±SD) plasma concentration vs time profile of efavirenz and losartan following oral administration of multiple doses of Efavirenz (100mg) with Losartan (100mg) decreases the bioavailability of losartan and hence it has been shown in the Figure No:2 and 3.

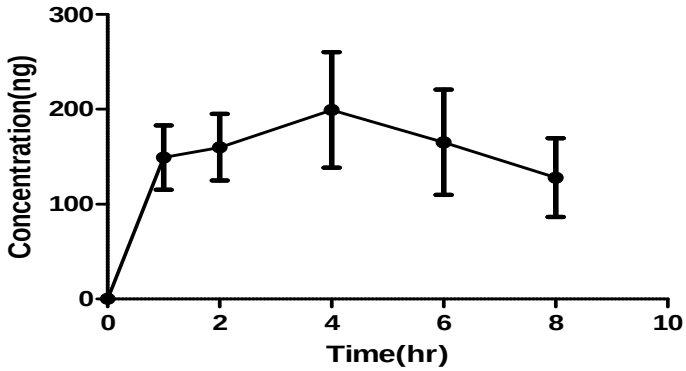

Fig 2: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Efavirenz by oral administration rats [1st day]

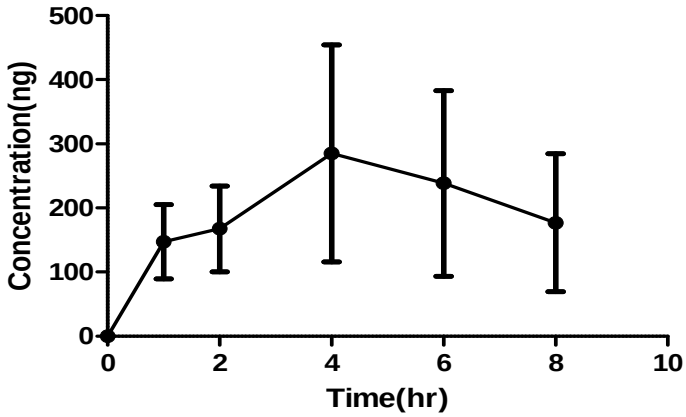

Fig 3: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Efavirenz by oral administration rats [14th day]

The derived pharmacokinetic parameters for efavirenz following administration of drug with losartan on 1st day and 14th day are represented in the table no:1

Table 1: Percentage change of each pharmacokinetic parameter in rats

	AUC	**Ke**	**$t_{1/2}$**	**Cl**	**Vd**
1st day	1.8%↑se	12%↓se	6.2%↑se	26%↓se	16.5%↓se
14th day	24%↓se	36%↑se	9%↓se	15%↑se	6.0%↓se

Concurrent administration of losartan with or without efavirenz as shown in the table no 5. It was proved that significant decrease in AUC, t ½ and Vd on the day 14th .This shows that the value of 1.8,6.2,16.5 and 24,9,6.

Pharmacokinetics parameters of ritonavir following administration of losartan with or without ritonavir as also shown in the table no.3.

Table 3: Comparison of Pharmacokinetic parameters of Losartan following pretreatment with Ritonavir by oral administration rats [n=6]

Parameter	**Losar**	**Losar + Rito [1st day]**	**Losar + Rito [14th day]**
AUC[ng/ml/h]	3000±1320	2954±1396	3232±1417
$t_{1/2}$[hr]	5.72±0.53	5.461±0.40	5.3996±0.80
Cl[ml/h]	3.911±1.64	4.08±1.87	3.53±1.39
Ke[hr^{-1}]	0.1219±0.01	0.127±0.009	0.1163±0.015*
Vd[ml]	33.11±16.71	32.60±16.53	31.49±15.57

**- Significant at P<0.05*

There was a significant decrease in AUC, on the first day and on 14th day increase in the presence of ritonavir (P<0.5) on concurrent ritonavir administration were also resulted in marked

reduction of clearance and constant Ke. To evaluate the metabolites kinetics of efavirenz and ritonavir, the apparent half life of the metabolite was with or without losartan 6.3,5.9 and 5.4.5.7 on the 1st day and 14th day was found to be 5.93,5.98 and 5.39,5.72 respectively.

Table 2: Comparison of Pharmacokinetic parameters of Losartan following pretreatment with Efavirenz by oral administration rats [n=6]

Parameter	Losar	Losar + Efa [1st day]	Losar + Efa [14th day]
AUC[ng/ml/h]	2392±703.8	2436±755.7	1918.819±1865*
$t_{1/2}$[hr]	5.988±1.348	6.39±0.63	5.93±1.00
Cl[ml/h]	4.48±1.25	3.53±1.81	4.41±2.64
Ke[hr^{-1}]	0.1228±0.038	0.1094±0.0116	0.194±0.0187
Vd[ml]	38.95±14.57	33.42±17.64	36.76±20.30

**- Significant at P<0.05*

This shows that on multiple doses of efavirenz decrease the bioavailability of losartan. Whereas, multiple dose of ritonavir increases the bioavailability losartan. This indicates that synergetic effect of ritonavir and antagonist effect of efavirenz in a combination therapy. It has been shown in Figure 4 and 5.

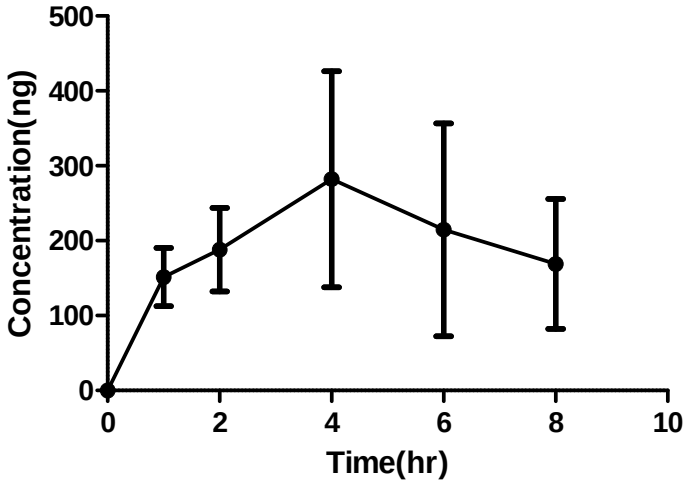

Fig 4: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Ritonavir by oral administration rats [1st day].

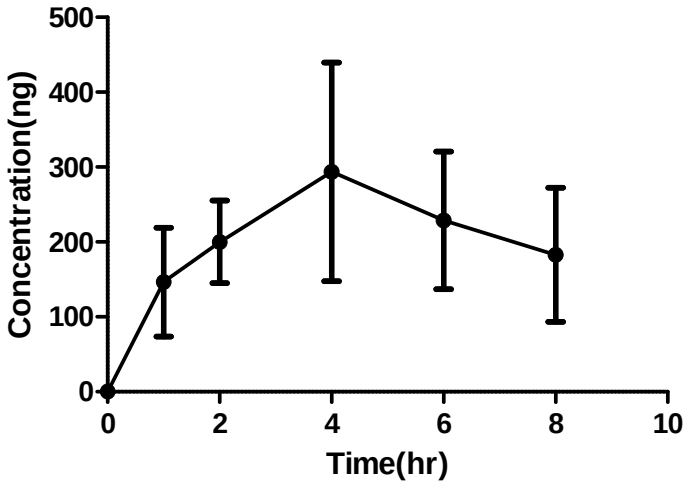

Fig 5: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Ritonavir by oral administration rats [14th day]

DISCUSSION

Evidence is emerging that the incidence of drug interaction evens increases over the last few years in HIV infected adults. Elinore et al [6] reported that increased sedation with atazanavir/ritonavir. Atazanavir or atazanavir/ritonavir may increase Buprenorphine and Buprenorphine metabolite concentrations and might require a decreased Buprenorphine dose. Stormer et al 2002; Chandler et al 2003; was reported that with concurrent efavirenz administration, the observed marked increase in the t_{max} of proguanil which is indicative of a slower rate or prolongation of absorption of the antimalarial may be attributable to the modulation of intestinal p-glycoprotein by efavirenz. It has been demonstrated that efavirenz is not a p-glycoprotein substrate but can slightly induce p-glycoprotein functionality and expression probably through induced cell stress [7,8]..

Whereas, bruce and Alice 2006 reported that patients developed somnolence and impaired cognition that required a decrease in Buprenorphine dose within 1-2 days of beginning a HAART regimen that included ATV/r [300/100mg daily the dose tested in this study as well [9,10]. In the past study wattanagoon et al 1987 Heisby et al 1900, reported the presence of efavirenz was associated with an increase, though not significantly ($P>0.05$) of the cycloguanil apparent terminal half –life. This trend is in line with the fact that, elimination of cycloguanil being formation rate-limited is determined by elimination of the parent compound. Also cycloguanil is a terminal metabolite and hence cannot undergo metabolic drug-drug interactions [11,12].

In current study single dose of efavirenz increased the AUC of losartan by 1-8%, Elimination rate constant decreased by 12%, Elimination half life increased [6,2%], Clearance decreased 26% and volume of distribution decreased 16.5%.

In single dose studies, efavirenz increased the AUC of losartan by 1.8%, elimination rate constant decreased 12%, elimination half life increased 6.2%, clearance decreased 26% and volume of distribution decreased 16.5%.

In multiple dose studies, efavirenz decreased the AUC of losartan by 24%, elimination rate constant increased 36%, elimination half life decreased 9%, clearance increased 15% and volume of distribution decreased 6%, the values are significant at $p<0.05$.
In single dose studies, ritonavir decreased the AUC of losartan by 1.5%, elimination rate constant increased 4.2%, elimination half-life decreased 4.5%, clearance decreased 4.4% and volume of distribution increased 1.5%.

In multiple dose studies, ritonavir increased the AUC of losartan by 7.1%, elimination rate constant decreased 4.6%, elimination half-life increased 5.6%, clearance decreased 10.2% and volume of distribution increased 4.9%, the values are significant at $p<0.05$. This indicates that synergetic effect of ritonavir and antagonist effect of efavirenz in a combination therapy.

CONCLUSION

The interaction described in this report was the drug - drug interaction between efavirenz with losartan verses ritonavir with losartan medications that has been conventional prescribed in HIV patients. In future, concominant treatment with this medication warrants clinically monitoring and possibly to do well designed clinical studies in humans need to confirm their interaction.

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