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Research Article

EFFECT OF *EFAVIRENZ* AND *RITONAVIR* ON THE PHARMACOKINETICS OF *LOSARTAN* USING RAT MODEL

MADHUKAR. A, JAGADEESHWAR. K*, NAGULU. M, MOHD. TAJUDDIN BABA, V. CHENDRASHEKAR

Vaagdevi College of Pharmacy, Hanamkonda, Warangal, A.P, India Email: madhukar.mpharm@gmail.com, jagdishoncology@gmail.com

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ABSTRACT

Limited data are available about the effect of *Efavirenz* and *Ritonavir* (EFV and RTV) on *Losartan* pharmacokinetics. As patients may benefit by using these two agents in combination with *Losartan*, this study determined the extent and direction of this drug–drug interaction. A single dose of 10 mg/kg *Losartan* was treated with EFV, RTV (100 mg/kg, 10 mg/kg) daily. Pharmacokinetics profiles were determined on day 1 and 14 for *Losartan*. EFV significantly decreased *Losartan* area under the curve (AUC) by 18.8% on 1stday and 36% on 14thday (P<0.0001), (CI) 9.74% decreased on 1stday, 12.75% increased on 14thday. RTV significantly changed *losartan* area under the curve (AUC) by 1.53 decreased on 1stday and 7.73% increased on 14thday (P<0.001), (CI) 4.32% increased on 1stday, 9.74% decreased on 14thday, respectively. Hence, a single dose of *Efavirenz* with *Losartan* enhances the plasma drug concentration in a smaller amount. In repeated dose administration, *Efavirenz* decreases the bioavailability of *Losartan* there by decrease the antihypertensive activity. Where as *Ritonavir* with *Losartan* decreases the bioavailability of *Losartan* in smaller amounts on 1stday. In repeated dose administration *Ritonavir* increases the bioavailability of *Losartan* there by enhances the antihypertensive activity and these results were found to be statistically significant.

Keywords: Efavirenz, Ritonavir, Losartan, RP-HPLC, Pharmacokinetics.

INTRODUCTION

Free fatty acids (FFAs) are released in abundance form an expanded adipose tissue mass. In the liver, FFAs result in an increased production of glucose, triglycerides and secretion of very low density lipoproteins (VLDLs). Associated lipid/lipoprotein abnormalities include reductions in high density lipoprotein (HDL) cholesterol and an increased density of low density lipoproteins (LDLs). FFAs also reduce insulin sensitivity in muscle by inhibiting insulin mediated glucose uptake. Associated defects include a reduction in glucose partitioning to glycogen and increased lipid accumulation in triglyceride (TG). Increases in circulating glucose and to some extent FFA, increase pancreatic insulin secretion, resulting in hyperinsulinemia. Hyperinsulinemia may result in enhanced sodium reabsorption and increased sympathetic nervous system (SNS) activity and contribute to the hypertension[1].

Drugs that inhibit CYP450 enzymes, generally lead to decreased metabolism of other drugs metabolized by the same enzyme. The decreased metabolism can result in higher drug levels and increased potential for toxicity. When drugs that induce CYP450 enzymes are administered to a patient, the body responds by increasing the production of specific enzymes of the CYP450 system. The increased enzyme production can lead to increased metabolism and decreased concentrations of drugs metabolized via the same pathway. In general, the maximal effect of enzyme induction is apparent within 7 to 10 days[2].

Concomitant *Lopinavir/r* and phenytoin administration results in a two-way drug interaction. Phenytoin appears to increase *Lopinavir* clearance via induction of CYP3A4 and this is not off set by the presence of low dose *Ritonavir*. In addition, the lower *Ritonavir* concentrations may partially account for the decrease in *Lopinavir* exposure. Phenytoin clearance may be increased by *Lopinavir/r* via induction of CYP2C9[3]. *Lopinavir/r* probably decreases lamotrigine levels by induction of glucuronidation[4]. When *Paroxetine* combined with *Fosamprenavir/r*, *Fosamprenavir/r* significantly decrease the *Paroxetine* AUC by 55%, C_{max} by 51% and elimination half-life by 25%; the free fraction of *Paroxetine* increased by 30%[5]. The *Ketoconazole* AUC was increased 2.69-fold when given with *Fosamprenavir/r*[6]. Co-administration of *Lopinavir/r* with *Bupropin* resulted in significant decreases for both *Bupropion* C_{max} and AUC. Decreases were also observed for hydroxybupropion C_{max} by 31% and AUC 50%[7].

Co-administration of *Lopinavir/r* with *Irinotecan, Lopinavir/r* reduces the clearance of irinotecan by 47%[8]. High incidence of adverse events when a higher than standard dose of the *Lopinavir/r* tablets either 600/150 or 800/200 mg twice daily was combined with *Rifampicin* 600 mg once daily[9]. When given without *Ritonavir, Ketoconazole* increased *Darunavir* AUC by 155%, C_{max} by 78% and C_{min} by 179%, compared with *Darunavir* alone. In the presence of *Ritonavir, Ketoconazole* increased *Darunavir* AUC by 42%, C_{max} by 21% and C_{min} by 73%, relative to *Darunavir/r* treatment[10].

Co-administration of *Tiprinavir/r* with *Rosuvastatin, Tiprinavir/r* increased *Rosuvastatin* AUC by 37% and increases C_{max} by 2.23-fold. *Atorvastatin* AUC was increased by 9.36-fold and C_{max} increased by 8.61-fold[11]. *Ritonavir*-containing regimen administrated with a triamcinolone injection for osteoarthritis. There was profound and persistent hyperglycaemia and hypothalamic-pituitary adrenal suppression almost certainly due to inhibition of triamcinolone metabolism by *Ritonavir*[12].

MATERIALS AND METHOD

Drugs and chemicals

Losartan – Aurobindo Pharma, Hyderabad, India. *Ritonavir* (Sun Pharmaceuticals Ltd, Mumbai, India) were obtained as a gift samples. Acetonitrile (HPLC grade) - Ranbaxy Fine Chemicals Ltd, S.A.S Nagar. Glacial acetic acid (HPLC grade) - S.D. Fine Chem Ltd, Mumbai, India. Methanol (HPLC grade) - Ranbaxy, Delhi, India. Water (HPLC grade) - Qualigens Fine Chemicals, Mumbai, India.

Equipments

HPLC (contain C_{18} column 250 × 4.6 mm, packed with 5 µm), Cyber labs. Micropipettes (Tarsons), Sonicator (Hwashin Technology, Korea), Biofuge (Hearus instrument- Germany), Microcentrifuge tubes (Tarsons), and Heparinised capillaries.

Experimental animals

Experiments were performed with albino rats procured from Mahaveera Enterprises (Hyderabad, A.P., India), weighing between 180 to 210gms. The animals were housed in colony cages (four per cage) under conditions of standard lighting, temperature (22±1^{o}c) and humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. During this period, we provided food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee (IEAC), Vaagdevi College of Pharmacy, Warangal, and A.P., India. (1047/ac/07/CPCSEA, dated 24/04/2007)

HPLC method description

A Cyber lab 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 C_{18} reverse phase column of 250 X 4.6mm dimension and 4µ 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 0.22µm membrane filter. The flow rate was 1 ml/min and the effluent was monitored at 230nm. The total run time of the method was set at 15 min.

Preparation of calibration curve of losartan

Preparation of stock solution*s*: A stock solution representing 100µg/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 10µg/ml of losartan. The internal standard stock solution was prepared by dissolving 1mg of valsartan in 100ml water and this solution was stored at -20°C.

Extraction procedure

A volume of 0.5ml blank plasma, 0.1ml of Losartan concentrations of 100ng to 10µg and 0.1ml of 25µg 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 13000rpm. 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 20µl 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 10µg was established (r^2 =0.998).

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 - 0.2 ml of Normal Saline; p.o.

Group II - Losartan (10mg/kg; p.o.).

Group III - Administration of Efavirenz (100mg/kg) orally followed by Losartan (10mg/kg) after 30 minutes, treated with Efavirenz (100mg/kg) for 13 days, 14th day administration of Efavirenz (100mg/kg) followed by Losartan (10mg/kg) after 30 minutes.

Group IV - Administration of Ritonavir (10mg/kg) orally followed by Losartan (10mg/kg) after 30 minutes, treated with Ritonavir (10mg/kg) for 13 days, 14th day administration of Ritonavir (10mg/kg) followed by Losartan (10mg/kg) after 30 minutes.

Blood samples were withdrawn on first day and 14th day at 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.

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), apparent volume of distribution for fraction of dose absorbed (Vd) and systemic clearance for fraction of dose absorbed (Cl) for the drug under consideration were obtained in each subject from plasma concentration verses time profile and statically work done by Student t-test, followed one-way ANOVA.

RESULTS AND DISCUSSION

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 CYP450 2C9 and 3A4.

The working standard solutions were prepared from stock solution of losartan concentrations as 0.1, 0.5, 1, 5 and 10µg/ml and the retention time was found in rang of 6.2-6.4min. The internal standard 25µg/ml of valsartan was prepared from stock solution and the retention time was found in rang of 9.4 - 10.4 min.

The linearity of the detector response was found to be linear from 0.1 to 10 µg /ml of concentrations for losartan standard with a correlation coefficient value is (r^2) = 0.998, which shows that the method is capable of producing good response in UV-detector.

DETERMINATION OF PLASMA DRUG CONCENTRATIONS

Table 1: Linearity of Losartan in rat plasma

Conc. (µg/ml)	I.S Conc. (µg/ml)	Drug Area	I.S Area	PAR
0.1	25	4836.3	191585.2	0.02524
0.5	25	39870.3	220329.9	0.18095
1	25	62168.6	195770.0	0.31756
5	25	357150.7	274484.8	1.30116
10	25	676720.0	252619.5	2.67881

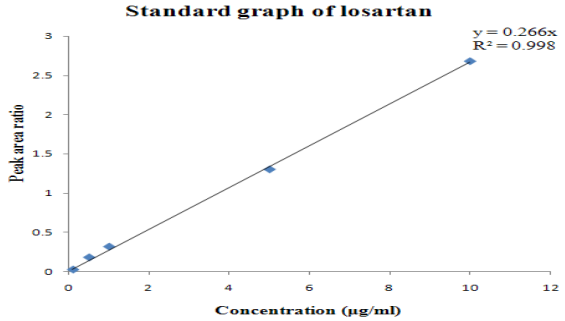

Fig. 1: Correlation Coefficient (r^2) of *Losartan* rat plasma

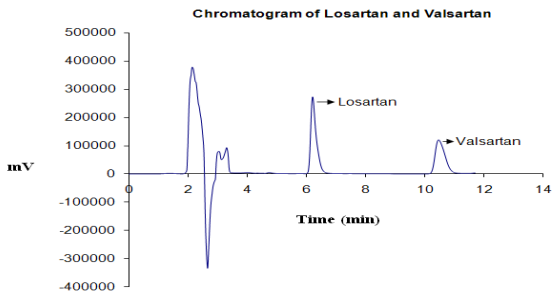

Fig. 2: Chromatogram of Losartan and Valsartan

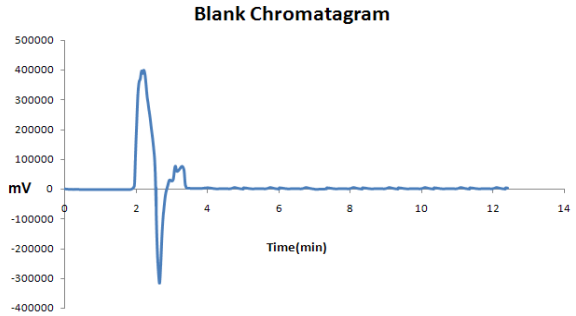

Fig. 3: Blank chromatogram

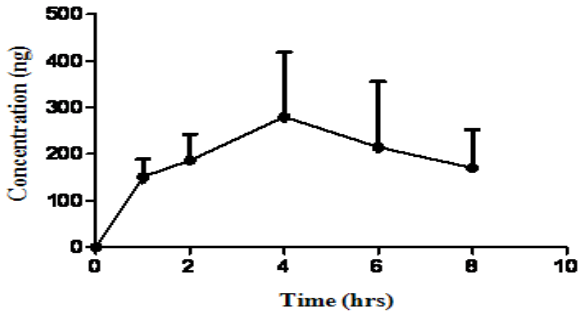

Fig. 4: Mean ± SD plasma concentration-time profile of Losartan in rats following oral administration

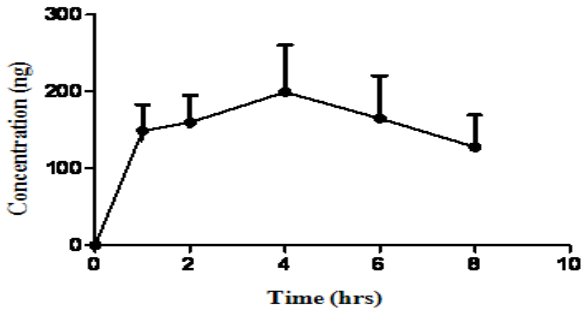

Fig. 5: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Efavirenz by oral administration rats (1st day)

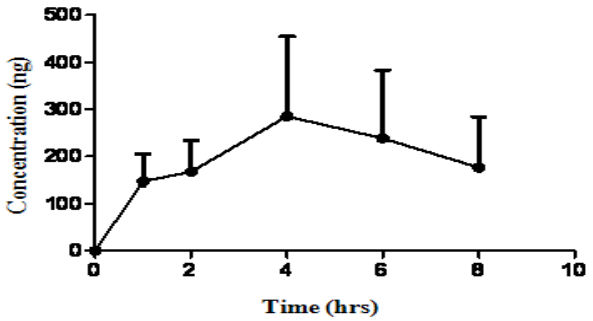

Fig. 6: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Efavirenz by oral administration rats (14th day)

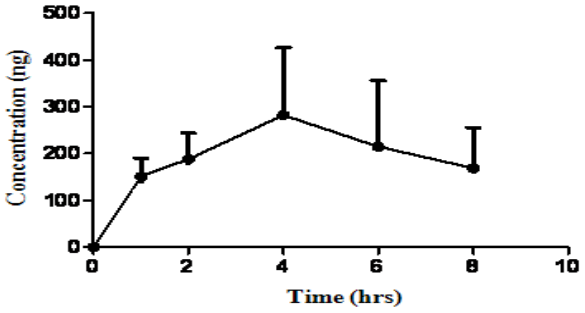

Fig. 7: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Ritonavir by oral administration rats (1st day)

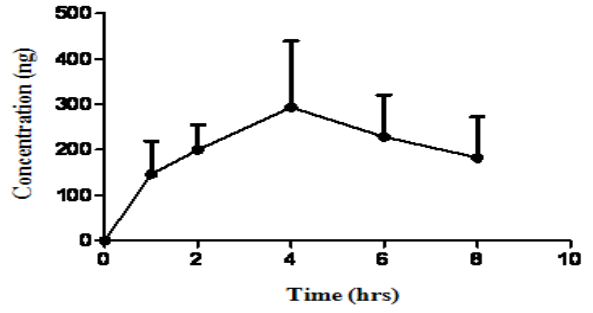

Fig. 8: Mean ± SD plasma concentration-time profile of Losartan following pretreatment with Ritonavir by oral administration rats (14th day)

From the concentration *Vs* time profile we calculate the pharmacokinetic parameters; there is significant change in pharmacokinetic parameters on 1st and 14th day.

In our single dose studies, efavirenz decreased the AUC of losartan by 18.8%, elimination rate constant decreased by 8.6%, elimination half-life increased 7.75%, clearance rate decreased by 9.74% and volume of distribution decline 9.92%. In our repeated dose studies, efavirenz decreased the AUC of losartan by 36%, elimination rate constant increased by 61%, elimination half-life decreased by 39%, clearance rate increased 12.75% and volume of distribution decline 9.9% these values are significant.

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)	3000±132.0	2436±755.7	1918.819±186.5***
$T_{1/2}$ (hr)	5.93±1.00	6.39±0.63	3.57±0.53
Cl (ml/h)	3.911±1.64	3.53±1.81	4.41±2.64
Ke (hr^{-1})	0.1198±0.01	0.1094±0.0116	0.194±0.0187***
Vd (ml)	36.76±20.30	33.42±17.64	33.11±16.71

*significant p<0.01, **very significant p<0.001, ***extremely significant p<0.0001

Table 3: Percentage change of each pharmacokinetic parameter in rats (from table-2)

	AUC	Ke	$t_{1/2}$	Cl	Vd
1st day	18.8% ±se	8.6% ±se	7.75% ±se	9.74% ±se	9.92% ±se
14th day	36% ±se	61% ±se	39% ±se	12.75% ±se	9.9% ±se

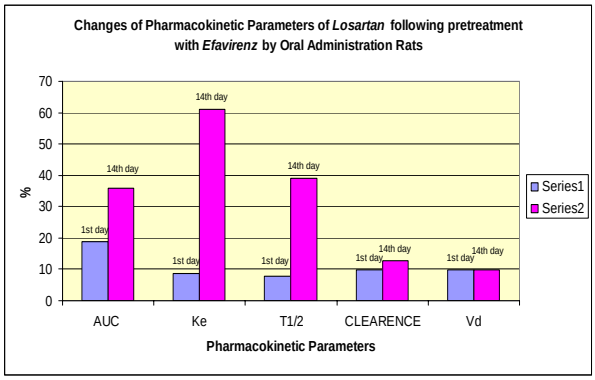

Fig. 9: Percentage change of each Pharmacokinetic parameters of Losartan following pretreatment with Efavirenz by oral administration rats

In single dose studies, ritonavir decreased the AUC of losartan by 1.53%, elimination rate constant raised by 6%, elimination half-life lowered by 7.9%, clearance rate decreased by 4.32% and volume of distribution decreased by 11.3%. In repeated dose studies, ritonavir increased the AUC of losartan by 7.73%, elimination rate constant decreased by 10.18%, elimination half-life increased by 1.11%, clearance rate decreased 9.74% and volume of distribution increased 4.7% these values are very significant.

Table 4: 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±132.0	2954±139.6	3232±141.7**
$T_{1/2}$ (hr)	5.93±1.00	5.461±0.40	5.996±0.80
Cl (ml/h)	3.911±1.64	4.08±1.87	3.53±1.39
Ke (hr^{-1})	0.1198±0.01	0.127±0.009	0.1076±0.015
Vd (ml)	36.76±20.30	32.60±16.53	38.49±15.57

*significant p<0.01, **very significant p<0.001, ***extremely significant p<0.0001

Table 5: Percentage change of each pharmacokinetic parameter in rats (from table-4)

Day	AUC	Ke	$t_{1/2}$	Cl	Vd
1st day	1.53 ±se	6% ±se	7.9% ±se	4.32% ±se	11.3% ±se
14th day	7.73% ±se	10.18% ±se	1.11% ±se	9.74% ±se	4.7% ±se

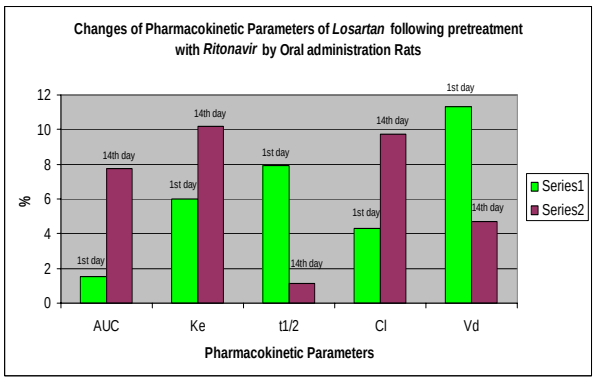

Fig. 10: Percentage change of each Pharmacokinetic parameters of *Losartan* following pretreatment with *Ritonavir* by oral administration rats

CONCLUSION

Hence we conclude that a single dose of efavirenz with losartan enhance the plasma drug concentration in a smaller amounts.

In repeated dose administration reveals efavirenz decreases the bioavailability of losartan there by decrease the antihypertensive activity and these results were found to be statistically significant. Whereas ritonavir with losartan decreases the bioavailability of losartan in smaller amounts. In repeated dose administration ritonavir increases the bioavailability of losartan there by enhances the antihypertensive activity and these results were found to be statistically significant.

In future, our studies needs well designed controlled clinical research in HIV patients to confirm the possibility of drug-drug interactions.

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