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

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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 1st day and 36% on 14th day (P<0.0001). (CI) 9.74% decreased on 1st day, 12.75% increased on 14th day. RTV significantly changed Losartan area under the curve (AUC) by 1.53 decreased on 1st day and 7.73% increased on 14th day (P<0.001), (CI) 4.32% increased on 1st day, 9.74% decreased on 14th day, 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 further by decrease the antihypertensive activity. Where as Ritonavir with Losartan decreases the bioavailability of Losartan in smaller amounts on 1st day. 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 from 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 FFAs, 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.

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.

Concomitant Lopinavir/r and phenytoin administration results in a two-way drug interaction. Pheynitoin 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 Losartan exposure. Phenytoin clearance may be increased by Lopinavir/r via induction of CYP2C9. Lopinavir/r probably decreases lamotrigine levels by induction of glucuronidation. When Paroxetine combined with Fosamprenavir/r, Fosamprenavir/r significantly decrease the Paroxetine AUC by 55%, Cmax by 51% and elimination half-life by 25%; the free fraction of Paroxetine increased by 30%; The Ketoconazole AUC was increased 2.6-fold when given with Fosamprenavir/r. Co-administration of Lopinavir/r with Bupropion resulted in significant decreases for both Bupropion Cmax and AUC. Decreases were also observed for hydroxybupropion Cmax by 31% and AUC 50%. Co-administration of Lopinavir/r with Irinotecan, Lopinavir/r reduces the clearance of irinotecan by 47%. 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. When given without Ritonavir, Ketoconazole increased Darunavir AUC by 155%, Cmax by 70% and Cmin by 179%, compared with Darunavir alone. In the presence of Ritonavir, Ketoconazole increased Darunavir AUC by 42%, Cmax by 21% and Cmin by 73%, relative to Darunavir/r treatment.

Co-administration of Tipranavir/r with Rosuvastatin, Tipranavir/r increased Rosuvastatin AUC by 37% and increases Cmax by 2.23-fold. Atorvastatin AUC was increased by 9.36-fold and Cmax increased by 8.61-fold. Ritonavir-containing regimen administrated with a triamcinolone injection for osteoarthitis. There was profound and persistent hyperglycaemia and hypophagic-pituitary adrenal suppression almost certainly due to inhibition of triamcinolone metabolism by Ritonavir.

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 C18 column 250 × 4.6 mm, packed with 5 µm), Cyber labs. Micropippettes (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 Mahavera 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°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 (IAEC), 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 C18 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 solutions: 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 ofEfavirenz (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 and14th 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 (t1/2), 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 (CI) for the drug under consideration were obtained in each subject from plasma concentration verses time profile and statically 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. 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.
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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Losar</th>
<th>Losar + Efa (1st day)</th>
<th>Losar + Efa (14th day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/ml/h)</td>
<td>3000±132.0</td>
<td>2436±755.7</td>
<td>1918±19×10^6.5***</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>5.93±1.00</td>
<td>6.39±0.63</td>
<td>3.57±0.53</td>
</tr>
<tr>
<td>Cl (ml/h)</td>
<td>3.91±1.64</td>
<td>3.53±1.81</td>
<td>4.41±2.64</td>
</tr>
<tr>
<td>Ke (hr⁻¹)</td>
<td>0.1198±0.01</td>
<td>0.1094±0.0116</td>
<td>0.194±0.0187***</td>
</tr>
<tr>
<td>Vd (ml)</td>
<td>36.76±20.30</td>
<td>33.42±17.64</td>
<td>33.11±16.71</td>
</tr>
</tbody>
</table>

*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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st day</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/ml/h)</td>
<td>3000±132.0</td>
<td>2954±139.6</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>5.93±1.00</td>
<td>5.46±0.40</td>
</tr>
<tr>
<td>CL (ml/h)</td>
<td>3.91±1.64</td>
<td>4.08±1.87</td>
</tr>
<tr>
<td>Ke (hr⁻¹)</td>
<td>0.1198±0.01</td>
<td>0.127±0.009</td>
</tr>
<tr>
<td>Vd (ml)</td>
<td>36.76±20.30</td>
<td>32.60±16.53</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Losar</th>
<th>Losar + Rito (1st day)</th>
<th>Losar + Rito (14th day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/ml/h)</td>
<td>3000±132.0</td>
<td>2954±139.6</td>
<td>3232±141.7**</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>5.93±1.00</td>
<td>5.46±0.40</td>
<td>5.99±0.80</td>
</tr>
<tr>
<td>CL (ml/h)</td>
<td>3.91±1.64</td>
<td>4.08±1.87</td>
<td>3.53±1.39</td>
</tr>
<tr>
<td>Ke (hr⁻¹)</td>
<td>0.1198±0.01</td>
<td>0.127±0.009</td>
<td>0.107±0.015</td>
</tr>
<tr>
<td>Vd (ml)</td>
<td>36.76±20.30</td>
<td>32.60±16.53</td>
<td>38.49±15.57</td>
</tr>
</tbody>
</table>

*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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st day</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/ml/h)</td>
<td>3000±132.0</td>
<td>2954±139.6</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>5.93±1.00</td>
<td>5.46±0.40</td>
</tr>
<tr>
<td>CL (ml/h)</td>
<td>3.91±1.64</td>
<td>4.08±1.87</td>
</tr>
<tr>
<td>Ke (hr⁻¹)</td>
<td>0.1198±0.01</td>
<td>0.127±0.009</td>
</tr>
<tr>
<td>Vd (ml)</td>
<td>36.76±20.30</td>
<td>32.60±16.53</td>
</tr>
</tbody>
</table>

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.

Whereas efavirenz decreases the bioavailability of losartan thereby 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 then 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.

REFERENCES