

Pharmacokinetics and pharmacodynamics of Atorvastatin alone and in combination with Lercanidipine in hyperlipidemic rats

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ABSTRACT

The present study was carried out to investigating the pharmacokinetics and pharmacodynamics of atorvastatin (AT) alone and in combination with lercanidipine (LER) in hyperlipidemic rats. The standard cholesterol diet was used to induce hyperlipidemia in Wister rats. The blood samples were collected (on 1st and 8th day) from AT alone and in combination with LER treated groups and were analyzed for various pharmacokinetic parameters and lipid profiles. Atorvastatin caused a marked reduction in the lipid profiles in hyperlipidemic rats. The combination of AT and LER in hyperlipidemic rats produced a significant change in lipid profiles (pharmacodynamics) and insignificant change in the pharmacokinetics of atorvastatin when compared with AT alone. The results of present study, suggests synergistic activity of lercanidipine with atorvastatin.

Key words: Atorvastatin, lercanidipine, hyperlipidemia and lipid lowering activity

INTRODUCTION

Atorvastatin (AT) is a synthetic lipid-lowering agent. It is a selective competitive inhibitor of HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, an important rate-limiting step in cholesterol biosynthesis (1). It is used in the treatment of hyperlipidemia or cardiovascular complications like coronary heart disease along with calcium channel blocker (CCB). Lercanidipine (LER) is a dihydropyridine calcium channel blocker (2), used for the treatment of mild to moderate hypertension. Hypertension and hyperlipidemia are two of the eight primary risk factors for coronary heart disease and their coexistence has been shown to be as high as 30% in many conditions (3). Due to the anti atherosclerotic properties of calcium channel blockers (4), atorvastatin along with lercanidipine was clinically used for treatment of atherosclerosis, causing a significant reduction in total cholesterol, low-density lipoprotein cholesterol, and plasma triglycerides in clinical studies (4). An elevation

of plasma lipids may be caused by a primary genetic defect or secondary to diet, drugs or diseases. Despite of differences in lipoprotein distribution and metabolism between humans and rats, hyperlipidemic rat models are extensively used in lipid research (5). The standard cholesterol diet has successfully been used to induce hyperlipidemia in rats in previous studies (6) and it was chosen as the hyperlipidemic model due to its convenience, reproducibility and availability. So far, the combination therapy of atorvastatin with lercanidipine in hyperlipidemic rats has not been reported. The present study investigated the effect of lercanidipine on pharmacokinetic and pharmacodynamics of atorvastatin in hyperlipidemic rats.

MATERIALS AND METHODS

Materials: Atorvastatin pure drug was a kind gift from Dr. Reddy's Pharmaceutical Ltd, Hyderabad. Lercanidipine pure drug was a kind gift from Nicholus Piramils India Ltd, Mumbai. HPLC grade acetonitrile, methanol and water were purchased from Qualigens Chemical Ltd. Ammonium Acetate (AR),

Cholesterol kit (Enzymatic Method), HDL-C kit were procured from Qualigens Diagnostics, Mumbai. Triglycerides kit was obtained from E-Merck Limited, Mumbai, India.

Experimental Animals

Wistar albino adult male rats weighing 200-220g were selected and housed in polypropylene cages in a room where the congenial temperature was $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. Before induction of hyperlipidemia, the weight of the individual animal and plasma cholesterol levels were estimated. The standard cholesterol diet along with butter (0.5 ml twice a day) was administered for 30 days to induce hyperlipidemia. At the end of the one month the blood was withdrawn from tail vein to analyze (7) for lipid profiles (TC, TG, LDL-C and HDL-C levels) to confirm the induction of hyperlipidemia.

Anti-hyperlipidemic studies

The hyperlipidemic rats were divided into four groups of six rats in each group.

Group I: (Non-HL) Control group of Non-Hyperlipidemic rats received a dose of 1.5% CMC.

Group II: (HL) Control group of Hyperlipidemic rats received a dose of 1.5% CMC.

Group III: (AT) atorvastatin alone in Hyperlipidemic rats.

Group IV: (AT-LER) atorvastatin in combination with lercanidipine.

This study was carried out for 7 days and the oral dose of atorvastatin was 80 mg/kg and that of lercanidipine was 20 mg/kg once in a day. The protocol of the present study was approved by the institutional animal ethical committee, Vel's College of Pharmacy, Chennai. India.

Collection of Blood samples

On 1st and 8th day, blood samples of 0.5 mL were withdrawn at different time intervals through retro-orbital sinus into heparinized eppendorff tubes at 0.5, 1, 2, 4, 6, 8 and 24 hrs and equal amount of saline was administered to replace blood volume at every blood withdrawal time (8). Plasma was obtained by immediate centrifugation of blood samples using REMI ULTRA cooling centrifuge at 3000 rpm for 5 minutes at room temperature. All samples were stored at 4°C until analysis.

Method of analysis:

The plasma samples of atorvastatin (9-10) and lercanidipine (11) were estimated by High Pressure Liquid Chromatography (HPLC, Shimadzu LC-8A solvent delivery, SPD-10A VP UV-Visible spectrophotometer detector, Class CR-10 Data processor) methods available in the lit-

erature. To the rat plasma (0.5 ml) samples 2.5 ml of ice-cold absolute ethanol was added and centrifuged at 3000 rpm for 10 minutes. The precipitate was resuspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000-6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a stream of nitrogen at room temperature. Samples were reconstituted in 200 μl of mobile phase (50% acetonitrile: 0.1M ammonium acetate buffer, at pH 4). 20 μl of sample was injected for HPLC analysis. The peak area ratios obtained for different concentrations (0.05, 0.1, 0.5, 1, 5, 10, 20, 40 $\mu\text{g}/\text{ml}$) of the atorvastatin and lercanidipine were plotted against the concentration of two drugs. The slope of the plots determined by the method of least square regression analysis ($r^2=0.9999$) was used to calculate the atorvastatin and lercanidipine concentration in the unknown sample.

Biochemical analysis:

Plasma lipid levels include TC, TG and HDL-C were carried out using respective diagnostic commercial kits from Qualigens diagnostics, Mumbai, India and LDL-C in plasma was calculated as per Friedewald estimation(12-13), $\text{LDL-C} = \text{TC} - (\text{TG}/5 + \text{HDL}) - \text{C} = \text{mg}/\text{dl}$

Statistical Analysis:

The results were expressed as mean \pm SD. Statistical comparisons for the pharmacokinetic - pharmacodynamic study among, Non HL, HL, AT and AT-LER groups were carried out using one-way ANOVA followed by Bonferroni's test. Differences below $P < 0.05$ implied statistically significance (14).

RESULTS

Pharmacokinetic Analysis:

Pharmacokinetic parameters like area under the curve [AUC], elimination half life [$t_{1/2}$], volume of distribution [V/f], total clearance [CL/f], peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were calculated for each subject using a non compartmental pharmacokinetic model "WIN NONLIN". The plasma levels ($\mu\text{g}/\text{ml}$) of atorvastatin in AT and AT-LER groups at different time points, on day 1 and day 8 were shown in figure 1 and 2 and respective pharmacokinetic parameters were shown in table 1.

Pharmacodynamic Study:

The lipid profiles were estimated for all the groups on day 1 & day 8 at different time points. The average lipid profiles of atorvastatin alone and in combination with lercanidipine were shown in table 2 & 3. Standard cholesterol diet effectively induced hyperlipidemia by increasing the plasma TC,

Table 1: Pharmacokinetic parameters of plasma atorvastatin alone and combination with Lercanidipine on day 1 & 8 in hyperlipidemic rats

Parameters	AT alone (Day 1)	AT+LER ^a (Day 1)	AT alone (Day 8)	AT+LER ^b (Day 8)
C _{max} (µg/ml)	23.44 ±0.53	23.98±0.33	29.81±1.03	30.59±0.3
T _{max} (h)	2±0	2±0	2±0	2±0
AUC _{0-t} (µg/ml/h)	235.05±7.33	243.36±3.49	325.88±3.67	329.92±0.77
AUC _{0-a} (µg/ml/h)	286.63±6.75	292.51±5.47	379.67±3.63	386.09±4.99
T _{1/2} (h)	9.65±.12	9.19±0.47	8.58±0.06	8.81±0.35
CL/f (ml/h/kg)	1414.8±15.82	1386.35±25.30	1035.95±36.27	1028.54±0.33
V/f (ml/kg)	20257.51±677.50	19548.81±122.95	12137.01±5250.33	14094.28±95.69

^a P>0.05 Vs AT alone on day 1 ^b P>0.05 Vs AT alone on day 8

TG and LDL-C levels and decreasing the plasma HDL-C levels. In groups of III, IV, the lipid profiles were significantly changed than group II. Atorvastatin alone and in combination with lercanidipine statistically reduced the hyperlipidemia.

Atorvastatin alone and in combination with lercanidipine, significantly reduced the plasma cholesterol, triglycerides, low density lipoprotein-cholesterol levels in standard diet induced hyperlipidemic rats (P< 0.001), but the change in the lipid levels was more in AT-LER treated group. There was a significant difference in change of lipid profiles in AT and AT-LER treated groups (P< 0.005) (table 2 & 3).

Table 2: Pharmacodynamic parameters of atorvastatin alone and in combination with lercanidipine on day 1 (n = 6)

Parameters	TC	TG	LDL	HDL
I:Normal rats	83.16 ± 2.71	123.5 ± 2.16	21.5 ± 1.76	32.16 ± 3.06
II: HL Control	179.16 ± 3.97 ^b	264.5 ± 9.48 ^b	264.5 ± 9.48 ^b	56.5 ± 1.64 ^b
III:AT alone	163.16 ± 8.18 ^a	251.5 ± 10.09 ^a	61.33 ± 4.88 ^a	61.5 ± 2.42 ^a
IV: AT+ LER	153.83 ± 2.40 ^a	236.66± 10.76 ^a	51.83 ± 3.06 ^a	64.66 ± 1.75 ^a

Table 3: Pharmacodynamic parameters of atorvastatin alone and in combination with lercanidipine on day 8 (n = 6)

Parameters	TC	TG	LDL	HDL
I:Normal rats	83.66 ± 2.91	125.8 ± 2.56	25.5 ± 1.96	33.18 ± 3.25
II: HL Control	175.16 ± 3.77 ^b	260.5 ± 9.35 ^b	72.16 ± 5.15 ^b	56.5 ± 1.64 ^b
III:AT alone	108.5 ± 1.37 ^a	134.5 ± 1.04 ^a	30.66 ± 0.51 ^a	61.5 ± 0.83 ^a
IV: AT+ LER	106.83 ± 1.32 ^a	119.83 ± 0.72 ^a	26.66 ± 2.42 ^a	64.66 ± 1.03 ^a

Values are in mean ± SD (µg/ml);(n =6); ^a = p < 0.05 Vs Group II, ^b = p < 0.05 Vs Group I

(TC=total cholesterol, TG=triglycerides, LDL =low density lipoprotein cholesterol and HDL= high density lipoprotein cholesterol)

DISCUSSION

Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-COA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic low density lipoprotein (LDL) receptors on the cell surface for enhanced uptake and catabolism of low density lipoprotein (LDL) and also decreases triglycerides (TG) lev-

els but, increases high density lipoprotein - cholesterol (15). It was reported that the atorvastatin therapy produced a statistically significant changes in total cholesterol, LDL-C, TG, HDL-C within 24 hours (13).reported that the atorvastatin therapy produced a statistically significant changes in total cholesterol, LDL-C, TG, HDL-C within 24 hours (13).

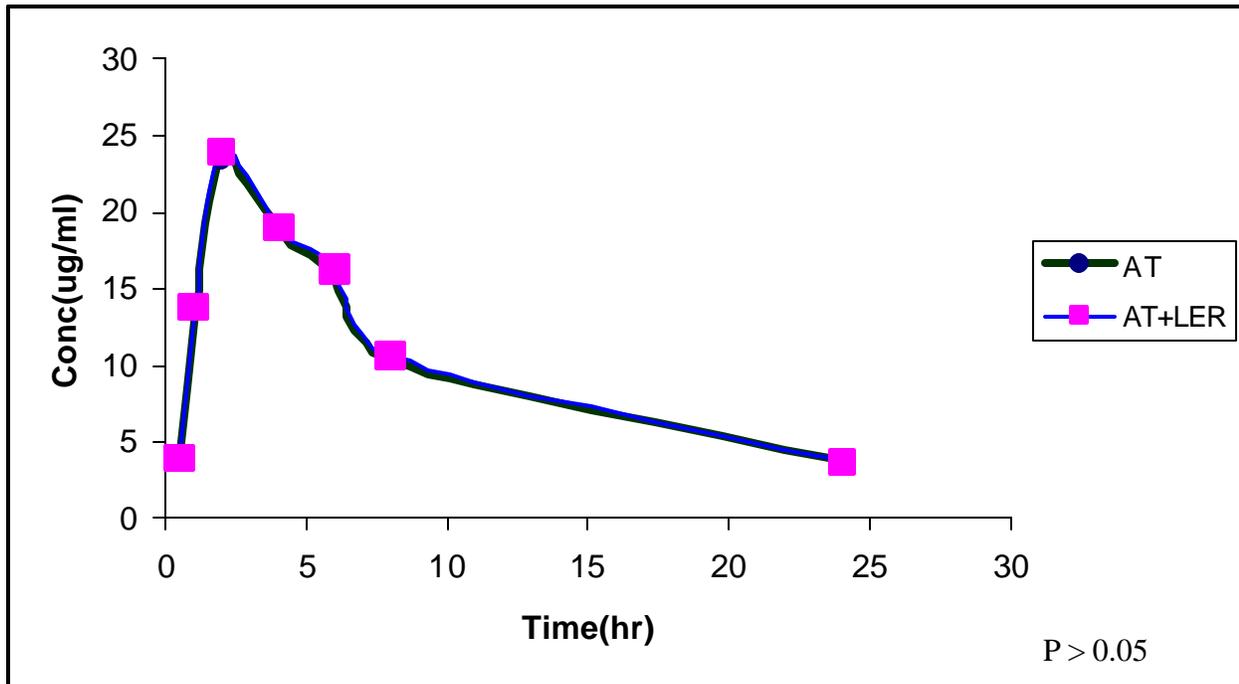


Fig.No.1 Mean Plasma Concentrations ($\mu\text{g/ml}$) of atorvastatin alone and combination with lercanidipine on day 1 in hyperlipidemic rats (n = 6)

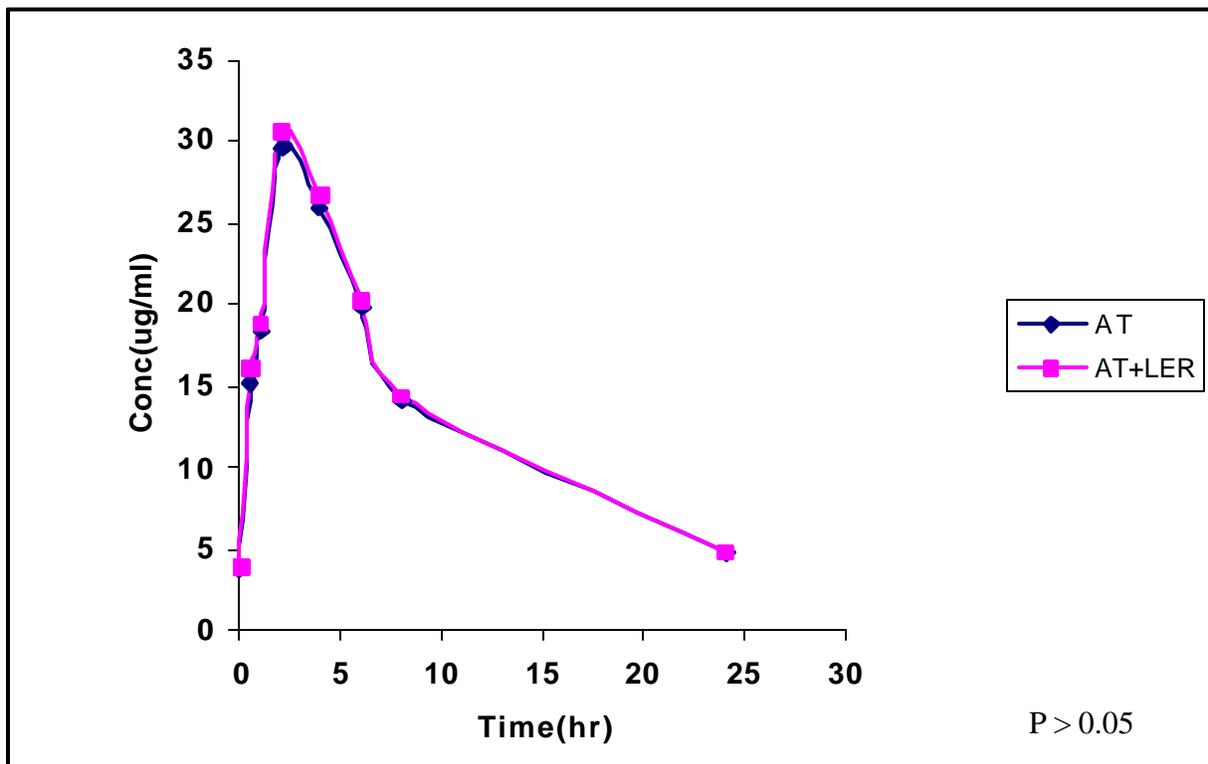


Fig.No.2 Mean Plasma Concentrations ($\mu\text{g/ml}$) of atorvastatin alone and combination with lercanidipine on day 8 in hyperlipidemic rats (n = 6)

The present study investigated the pharmacokinetic interaction of atorvastatin with lercanidipine in hyperlipidemic rats. The observed peak plasma concentrations (C_{max}) of atorvastatin was $29.81 \pm 1.03 \mu\text{g/ml}$ at 2 hr after oral administration on day 8, but in combination with lercanidipine it was insignificantly changed to $30.5 \pm 0.30 \mu\text{g/ml}$ ($P > 0.05$). There was no interaction in pharmacokinetic parameters of atorvastatin, in combination with lercanidipine on day 1 & 8 (Table 1). The AUC_{0-24} of atorvastatin was insignificantly increased to 1.21% in when it combined with lercanidipine on day 8. There was a slight decrease (0.67%) in the clearance (CL/f) rate of atorvastatin in combination with lercanidipine.

As there was no significant pharmacokinetic interaction between atorvastatin and lercanidipine, the significant difference in lipid levels of AT, AT+LER groups suggests the synergistic lipid lowering activity of lercanidipine which may be suitable for the patients of hypertension associated with hyperlipidemia. In support of our investigations the lipid lowering activity of calcium channel blockers was also reported (16-17). It can be suggested that the combination of these two drugs have potential benefits in safety, efficacy and tolerability than individual drugs.

CONCLUSION

It was concluded that therapy of atorvastatin with lercanidipine does not alter the pharmacokinetics of atorvastatin, but significant change in lipid profile was observed in hyperlipidemic rats. The current study demonstrated this combination therapy produces marked reduction in total lipid profile levels which were compared to atorvastatin alone. The characteristic changes in pharmacodynamic profiles may be advantageous for the management of atherosclerosis.

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