Study of Alteration in Serum Lipids by Enalapril and Ramipril in Albino Rabbits.

Shruti chandra*, Singh S.P2, Jain I.P2, Singh S2, Kushwaha V2.

1Department of pharmacology, Mahatma gandhi mission hospital and medical college, Aurangabad, India.
2Department of pharmacology, G S V M medical college, Kanpur, India.

* Corresponding author: Shruti Chandra.
E-mail id: dr.shruti1204@gmail.com

Abstract

Hypertension with dislipidemia is becoming a common morbidity, since ACE inhibitors are the first line of antihypertensive drugs so present study was undertaken with the aim to evaluate the possible effects of ACE inhibitor on lipid profile in albino rabbits. Rabbits were divided into 2 groups with 6 in each group. Rabbits of Group I were given Enalapril in dose of 0.50 mg/kg and of group II were given Ramipril in dose of 0.25 mg/kg for a period of 6 weeks. Lipid profile estimation (Serum Total cholesterol, serum HDL, serum LDL, serum Triglycerides and serum VLDL) was done at day 0, 7, 21 and 45 respectively. After analysis Rabbits of group-I (Enalapril) showed 7% decrease in serum cholesterol level at 45th day (P<.05). Serum HDL level increased by 10% and 20% at day 21 & 45 respectively (P<.05). Serum Triglyceride level increases by 8% at day 45 (P<.05). Serum LDL level decreases by 7.8% and 16% at day 21 and 45 respectively.(P<.05 ). There was no significant change in Serum VLDL level. Rabbits of group- II (Ramipril) showed increase in HDL level by 7 % & 12 % at day 21 and 45.(P<.05). Total cholesterol, triglycerides and VLDL levels were not significantly altered while serum LDL level decreases by 6.8 % at day 45. (P<.05). From our study it was concluded that enalapril had a favourable effect on serum lipid profile by decreasing total cholesterol, increasing serum HDL level. It may increase triglycerides, decrease LDL. Ramipril increase serum HDL and decreases LDL, there is no significant change in cholesterol, TG and VLDL levels.

KEY WORDS: ACE inhibitors, serum lipid profile, albino rabbits.

INTRODUCTION

Angiotensin II (AII) is an important regulator of cardiovascular function. The ability to reduce level of AII with orally effective ACE inhibitor represent an important advance in treatment of hypertension. The ACE inhibitors appear to confer a special advantage in the treatment of patient with diabetes, slowing the development and progression of diabetic
glomerulopathy. ACEn inhibitor decreases the production of AII, increases bradykinin level, and reduces sympathetic nervous system activity. A number of clinical trials have evaluated the possibility that ACE inhibitors may have particular advantages, beyond that of blood pressure control, in reducing cardiovascular and renal outcomes. They decrease proteinuria and retard the rate of progression of renal insufficiency in both diabetic and non-diabetic renal diseases. In most patients with hypertension and heart failure due to systolic and/or diastolic dysfunction, ACE inhibitor is recommended to improve survival. Experience from clinical trials suggest that drugs that target the renin-angiotensin system (RAS) may have metabolic advantage over drugs such as beta blockers and diuretics. Epidemiologic studies have established a strong correlation between elevated total cholesterol levels in serum and morbidity and mortality from myocardial infarction. Hyperlipidemia, in particular hypercholesterolemia, is regarded as an independent risk factor in the development of ischemic heart disease. One study has shown that fosinopril therapy for 6 months resulted in a reduction of micro albuminuria and an improvement in lipid profile and lipoprotein(a) [Lp(a)] levels in patients with type II diabetes. In the light of above facts present study had been undertaken with the aim to observe effects of certain ACE inhibitors on serum lipid profile in albino rabbits.

MATERIAL AND METHOD

Animals

Healthy albino rabbits of either sex 1.5-2.0 kg were used in the study. They were housed in iron cages & maintained under standard conditions (12 hours light & dark cycle, at room temperature 25 ± 3°C & 35—60% humidity). They were maintained on gram diet & water ad libitum. The care and use of these animals were in accordance with the guidelines of CPCSEA. Institutional Animal Ethics Committee approved the experimental protocols.

Drugs

Drugs used in present study included Enalapril (0.50 mg/kg) and Ramipril (0.25 mg/kg)

Groups

Rabbits were divided into 2 groups with 6 rabbits in each. Each of 6 rabbits of Group 1 received Enalapril (0.5 mg/kg/day/PO) by feeding cannula for 6 weeks. Rabbits of group 2 were given Ramipril (0.25 mg/kg/day/PO) for 6 weeks.

Sampling

Blood samples were taken from the marginal vein of pinna of rabbits after overnight fasting. Blood samples were collected at day 0 (before the drugs administration). Since day 1 drug to be studied was given for 6 weeks. At day 7, at day 21 and at day 45 (after 7, 21 and 45 days of drugs administration), blood samples were collected for estimation of serum lipid profile.

Lipid profile estimation

Estimation of total serum cholesterol, Triglyceride & HDL cholesterol were done separately by using their respective reagent kits. Estimation of total serum cholesterol level was done by using Cholesterol oxidase-phenol-amino phenazone (CHOD-PAP) method & HDL cholesterol by Polyethylene glycol Cholesterol oxidase-phenol-amino phenazone (PEG-CHOD-PAP) method by using a span diagnostic reagent kit (code no. LG 052). Estimation of serum triglyceride level was done by using glycerol phosphate oxidase-phenol-amino phenazone (GPO-PAP end point assay) method, by using span diagnostic reagent kit (code no. LG 062). The equipment used was U V Spectrophotometer. Estimation of serum LDL & VLDL cholesterol were done by using calculation method.

The value of serum LDL cholesterol (mg/dl) was calculated on the basis of Friedwald’s equation-

Serum LDL cholesterol(mg/dl) = Total cholesterol - (HDL+Triglyceride/5)
Serum VLDL cholesterol was calculated by the following equation-
VLDL Cholesterol(mg/dl) = Total cholesterol - (HDL + LDL)
**Statistical analysis**
The statistical analysis was carried out by using paired t test. The values obtained at day 7, day 21 and day 45 (after drug administration) were compared with the day 0 (before drug administration) value in both the groups. All values were expressed as the mean ± SEM. Statistical significance was set at P<0.05.

**RESULTS**

**Rabbits of group-I [TABLE-I] [GRAPH-I]**
Mean total serum cholesterol at day ‘0’ was 220 mg% which showed 7% decrease in serum cholesterol level at 45th day i.e. 205.6 when compared with the level on day 0, found to be statistically significant (P<0.05). Mean serum HDL level at Day ‘0’ was 41.8 mg% which increased by 10% and 20% at day 21 & 45 respectively (P<0.05). Mean serum Triglyceride level at day ‘0’ was 117.1mg% which increased by 8% at day 45 (P<0.05). Mean serum LDL level at day ‘0’ was 155.4 mg%, found to be decreased by 7.8% and 16% at day 21 and 45 respectively(P<0.05). There was no significant change in Serum VLDL level.

**Rabbits of group- II [TABLE-II] [GRAPH-II]**
There was significant increase in mean serum HDL level by 7 % & 12 % at day 21 and 45 (P<0.05 ). Mean value was 42.5 mg%. Mean Total cholesterol level at day ‘0’ was 213.1 mg%, mean serum triglyceride at day’0’ was 119.8 mg% and mean serum VLDL level at day’0’ was 23.9 mg%. These values were not significantly altered while serum LDL level decreased significantly by 6.8 % at day 45. (P<0.01).

**TABLE I (GROUP I)**
Lipid profile at day 0 (before) , day 7, 21 &45 (after) receiving enalapril (0.50 mg/kg/day/po)

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>DAY 0</th>
<th>DAY 7</th>
<th>DAY 21</th>
<th>DAY45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum total cholesterol level</td>
<td>220.6±3.3</td>
<td>217.5±2.0</td>
<td>213.3±1.6*</td>
<td>205.6±1.6***</td>
</tr>
<tr>
<td>% DECREASE</td>
<td>1.4</td>
<td>3.3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Serum HDL level</td>
<td>41.8±2.8</td>
<td>43.3±3.0</td>
<td>46±2.6**</td>
<td>50±2.2**</td>
</tr>
<tr>
<td>% INCREASE</td>
<td>3.5</td>
<td>10</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Serum triglycerides level</td>
<td>117.1±4.3</td>
<td>118.1±4.0</td>
<td>120.8±3.3</td>
<td>126.0±2.9*</td>
</tr>
<tr>
<td>% INCREASE</td>
<td>0.8</td>
<td>3.1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Serum LDL level</td>
<td>155.4±4.6</td>
<td>150.5±3.0*</td>
<td>143.1±3.4**</td>
<td>130.4±2.7***</td>
</tr>
<tr>
<td>% DECREASE</td>
<td>3.1</td>
<td>7.8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Serum VLDL level</td>
<td>23.4±0.8</td>
<td>23.6±0.8</td>
<td>24.1±0.6</td>
<td>25.2±0.5*</td>
</tr>
<tr>
<td>% INCREASE</td>
<td>0.8</td>
<td>3.1</td>
<td>7.6</td>
<td></td>
</tr>
</tbody>
</table>

* Significant (P<0.05) ** Highly Significant (P<0.01) *** Very Highly Significant (P<0.001)
TABLE II (GROUP II)
Lipid profile at day 0 (before), day 7, 21 & 45 (after) receiving ramipril (0.25 mg/kg /day/po)

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>DAY 0</th>
<th>DAY 7</th>
<th>DAY 21</th>
<th>DAY 45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum total cholesterol level</td>
<td>213.1±3.3</td>
<td>214.0±2.6</td>
<td>212.0±2.3</td>
</tr>
<tr>
<td></td>
<td>% DECREASE</td>
<td>0.4</td>
<td>0.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Serum HDL level</td>
<td>42.5±3.3</td>
<td>44±2.9</td>
<td>45.1±3.0*</td>
</tr>
<tr>
<td></td>
<td>% INCREASE</td>
<td>3.5</td>
<td>6.1</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>Serum triglycerides level</td>
<td>119.8±4.7</td>
<td>120.8±4.7</td>
<td>120.5±4.4</td>
</tr>
<tr>
<td></td>
<td>% INCREASE</td>
<td>0.8</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Serum LDL level</td>
<td>146.7±4.2</td>
<td>145.8±3.3</td>
<td>142.7±2.8</td>
</tr>
<tr>
<td></td>
<td>% DECREASE</td>
<td>0.6</td>
<td>2.7</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Serum VLDL level</td>
<td>23.9±0.9</td>
<td>24.1±0.9</td>
<td>24.1±0.8</td>
</tr>
<tr>
<td></td>
<td>% INCREASE</td>
<td>0.8</td>
<td>0.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Significant (P<0.05) ** Highly Significant (P<0.01) *** Very Highly Significant (P<0.001)

GRAPH I
Lipid Profile At Day 0 (Before), Day 7, 21 & 45 (After) Receiving Enalapril (0.50 Mg/Kg /Day/Po)
**DISCUSSION**

We had observed that administration of Enalapril (Group-I)‘decreased’ the total serum cholesterol, the decrease was more after long term use of drug i.e. after 21 days (3.3%) and 45 days (7%). HDL levels increased which were significant on long term use i.e 10% after 21 days and 20% after 45 days. Increase in triglyceride levels were significant after 45 days (8%). Decrease in serum LDL levels were profound after 21 (7.8%) and 45 days (16%). Increase in serum VLDL levels were not much after short term use although it were found to be significant after 45 days of drug administration. Similar results were obtained by Nandeesha H, Pavithran P et al on serum lipid in newly diagnosed essential hypertensive men by antihypertensive therapy. In the enalapril group, they found a significant reduction in Total Cholesterol, TG, VLDL-C, non HDL-C, and TG to HDL-C ratio after treatment. A randomized double blind clinical trial done by Grimm H R, Flack M J, Grandits A G et al showed that decreases in triglycerides and increases in HDL cholesterol by enalapril. Significant decrease was found in the levels of very low-density lipoprotein (VLDL) fraction at 8 and 12 weeks by enalapril as demonstrated by Sasaki J, Arakawa K in patients with mild essential hypertension. There are some evidences that enalapril does not alter serum lipid profile as observed by Santos L E, Souza P K et al. They observed effect of enalapril on body weight and composition in young rats. The results showed that enalapril treatment is able to reduce body fat on both diets, without alteration in serum lipid profile. Administration of Ramipril (Group-II) led to ‘decrease’ in total cholesterol levels and increase in serum HDL levels after 21(6.1%) and 45(12.5%) days of drug administration significantly. Serum triglycerides and serum VLDL levels were almost unaffected. After long term administration i.e. 45 days serum LDL levels decreased significantly (6.9%). This decrease was not much after short term use of drug. Ramipril caused significant reduction of C-reactive protein and oxidized low-density lipoprotein cholesterol serum levels (p<0.001) in patients with Type 2 Diabetes Mellitus as observed by Koulouris S, Symeonides P, Triantafyllou K et al. Similar results regarding serum HDL levels i.e. HDL levels were increased significantly was demonstrated by Finta M K, Fischer J M, Lee L et al rabbits. However they found that total cholesterol levels were elevated in the rabbits fed the atherogenic diet containing ramipril. Significantly increased levels of triglyceride as observed in our study may be due to reduced production of angiotensin II (AII) which in turn decrease release of noradrenaline (NA) from the sympathetic nerve terminals. NA promote
hydrolytic release of fatty acid and glycerol from triglycerides in adipose tissue by activating hormone sensitive lipase\(^{13}\) thus decrease in NA release by ACE inhibitors may result in increased triglyceride deposition. NA also causes increase in glycogen breakdown resulting in increase in hepatic glucose level.\(^{14}\) This increase in glucose production promote insulin release from beta cells of pancreas. Insulin in turn promotes lipogenesis.\(^{15}\) Thus blockage of conversion of AI to AII may result in reduced lipid synthesis. Drugs used in our study i.e. enalapril and ramipril reduced levels of total cholesterol and serum LDL levels. Insulin by favouring upregulation of the expression of HMG CoA reductase gene may result in increase in cholesterol synthesis.\(^{16}\) In our study, we found profound reduction in total cholesterol levels by enalapril while ramipril also decreased cholesterol levels but to a lesser extent. This may be due to blockade of NA release resulting in decrease in insulin levels which in turn may result in decreased cholesterol synthesis. There are evidences that adipose tissue expresses components of the renin-angiotensin system and expresses AII receptors.\(^{17}\) Furthermore, adipose tissue is an important physiological target of AII. AII has been shown to modulate adipocyte lipid metabolism, increase inflammatory gene expression and decrease adiponectin expression.\(^{18}\) In addition, adipose tissue has been shown to secrete a number of factors that can directly impact the vessel wall and vessel wall cells. One of the factors highly expressed in the adipose organ is apolipoprotein E (apoE).\(^{19}\) ApoE has important effects on lipoprotein composition and systemic lipoprotein metabolism. one study done by Rao P, Huang Z H, and Mazzone T et al had shown that AII treatment of adipocytes stimulate triglyceride synthesis and suppress hydrolysis. Therefore, the suppression of apoE expression by AII could also be a homeostatic response to limit further triglyceride accumulation in adipocytes.\(^{20}\) in our study enalapril and ramipril increased serum triglyceride levels significantly after 45 days of drug administration.

**CONCLUSION**

Thus ACE inhibitors by blocking production of angiotensin II and its various effects, significantly altered serum lipid profile. Reduction of total cholesterol level, LDL, VLDL and increase in level of HDL is beneficial for individuals having cardiovascular risk.

**REFERENCES**


