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STUDY OF ALTERATION IN SERUM LIPIDS BY LISINOPRIL AND PERINDOPRIL IN ALBINO RABBITS

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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. The study was conducted in the Deptt. of Pharmacology & Therapeutics, GSVM Medical college, Kanpur. Rabbits were divided into 2 groups with 12 in each group. After 2 weeks of adaptation rabbits were given cholesterol rich diet for 8 weeks. After that animals of group I were continued with the same cholesterol enriched diet along with Lisinopril (0.25mg/kg/day PO) and group II were fed with cholesterol enriched diet along with Perindopril (0.20mg/kg/day PO) for 6 weeks more. Lipid profile estimation (Serum Total cholesterol, serum HDL, serum LDL, serum Triglycerides and serum VLDL) was done at day 0, 60 and day 102 respectively. Statistical analysis was carried out by using

paired 't' test. Serum total cholesterol, triglycerides, HDL, LDL and VLDL cholesterol, were increased significantly (P<0.05) in both the groups after 60 days of cholesterol feeding. Group I (Lisinopril) showed significant decrease in serum total cholesterol and LDL levels. HDL and Triglycerides levels increased significantly. The VLDL levels were also found to be increased but not found significant. Same results were obtained by Rabbits of group II (Perindopril). It was concluded that ACE inhibitor e.g Lisinopril, Perindopril had a favourable effect on serum lipid profile by decreasing total cholesterol, increasing serum HDL level and decreasing LDL levels by inhibiting ACE enzyme and various other mechanisms.

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

INTRODUCTION

Cardiovascular diseases, a major group of non-communicable diseases, have become a major public health problem in many developing countries.[1, 2] In today's world, most of the deaths are attributable to noncommunicable diseases & just over half of these are because of cardiovascular diseases.[3] According to World Health Report 2003, an estimated 16.7 million of total global deaths result from the various forms of cardiovascular diseases (CVDs). Out of these, 7.2 million are due to ischaemic (coronary) heart disease. That's why the WHO has drawn attention to the fact that coronary heart disease is our "***MODERN EPIDEMIC"***.[4] **Coronary heart disease** (CHD), also known as coronary artery disease (CAD), ischaemic heart disease and atherosclerotic heart disease, is the end result of the accumulation of atheromatous plaques within the walls of the arteries that supply the myocardium (the muscle of the heart) with oxygen and nutrients. The prospective community based 'FRAMINGHAM HEART STUDY' provides support for the concept that HYPERCHOLESTEROLEMIA, HYPERTENSION & other factors are correlated with the cardiovascular risk. Abnormalities in plasma lipoprotein and derangement in lipid metabolism rank among the most firmly established and best understood risk factor for atherosclerosis.[5] High plasma concentration of cholesterol, particular those of low-density lipoprotein (LDL), is one of the principal risk factor for atherosclerosis but decrease in HDL cholesterol or lower HDL: LDL ratio also causes the atherosclerosis and are associated with increased risk of CAD. Atherosclerosis is an inflammatory disease in which arterial wall thickens. The chronic inflammatory response in the walls of arteries is due to the accumulation of macrophages, white blood cells and promoted by low density lipoproteins(LDL), inadequate removal of fats and cholesterol from

the macrophages by functional high density lipoproteins (HDL).[6] Atherosclerosis may results into complications like stroke, transient cerebral ischemia, intermittent claudication.[7] Different cardiac consequences due to atherosclerosis of the coronary arteries are stable angina, unstable angina, myocardial infarction (MI), heart failure, arrhythmias and sudden death.[8] So the goals of treatment include relief of symptoms, inhibition or slowing of disease progression by treatment of lipid disorders and control of hypertension, prevention of future cardiac events such as myocardial infarction (MI), and improved survival.[9]

Angiotensin II (AII) is an important regulator of cardiovascular function. The ability to reduce level of AII with orally effective ACE inhibitor represents 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.[**10**]ACE 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 nondiabetic renal diseases. In most patients with hypertension and heart failure due to systolic and/or diastolic dysfunction, ACE inhibitor is recommended to improve survival.[**11]** 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.[12**]** 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. [13**]** One study has shown that fosinopril therapy for 6 months resulted in a reduction of microalbuminuria and an improvement in lipid profile and lipoprotein (a) [Lp (a)] levels in patients with type II diabetes.[**14]** 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. The drugs evaluated were Lisinopril and Perindopril.

MATERIALS AND METHOD

Animals

The present study was conducted on normal adult healthy albino rabbits of either sex, weighing 1.5 - 2.0 kg. They were housed in iron cages & maintained under standard

conditions of 12 hours light & dark cycle, room temperature, 25 ± 3°C & 35-60% humidity. The animals were maintained on standard pellet diet & water ad libitum two weeks before start of experiment. The study was approved by Institutional Ethical Committee of G.S.V.M. Medical College, Kanpur.

Drugs

lisinopril 0.25mg/kg/day/PO and perindopril 0.20 mg/kg/day/PO

Dose calculations were done according to table from Paget & Barnes (1964).[15]

Experimental design

After 2-weeks period of adaptation, all the animals were divided into two groups, comprising of 12 rabbits in each and marked to permit individual identification. After that blood samples were taken from all the rabbits of both groups, for the estimation of basal serum lipid profile, which was considered as 'day 0' sample serving as a normal control for their respective groups. This was immediately followed by induction of experimental hypercholesterolemia in all the animals by administering the cholesterol enriched diet (standard diet containing 1% cholesterol and 10% ground nut oil) and water ad libitum for 8 weeks (60 days) and the 'day 60' sample (cholesterol fed control) was drawn again. After that animals of group I were continued with the same cholesterol enriched diet along with lisinopril and group II were fed with cholesterol enriched diet along with perindopril for 6 weeks more and at ' day 102' (14 weeks) (test), blood samples were drawn again to observe the effect of drugs on serum lipid profile.

Induction of hypercholesterolemia in rabbits

Rabbits were made hypercholesterolemic by feeding a high cholesterol fat diet. Deoxycholic acid was mixed thoroughly with powdered standard rabbit diet (100g/day/rabbit). Simultaneously 1% cholesterol was dissolved in 10% warmed ground nut oil and this oil solution was added slowly in to powdered mixture to obtain homogeneous soft cake. This cholesterol rich (HFD) preparation was molded in the shape of pellets of about 3g each and fed for 8 weeks by 100g/day/rabbit for the induction of hypercholesterolemia.[16]

Collection of blood samples

For estimation of serum lipid profile, blood samples were taken in the plain vials from the marginal vein of pinna of rabbits at 'day 0' (before induction of hypercholestrolemia), 'day 60' (before administration of drugs) and 'day 102'(after 6 weeks of drugs administration) by

using disposable syringes under aseptic conditions, after overnight fasting and then centrifuged at 3000 rpm for 10 minutes. The clear supernatant serum was taken for the estimation of serum lipid profile in each group. Estimations of serum triglyceride, total serum cholesterol & HDL cholesterol were done separately by using their respective reagent kits & by their respective enzymatic methods with the help of UV spectrophotometer while the estimation of serum LDL & VLDL cholesterol were done by the methods noted against them.

Estimation of serum lipid profile

Total Serum Cholesterol & HDL Cholesterol & Serum Triglyceride Estimation

Estimation of total serum cholesterol level was done by using Cholesterol oxidase-phenol-aminophenazone (CHOD-PAP) method & HDL cholesterol by Polyethylene glycol Cholesterol oxidase-phenol-aminophenazone (PEG-CHOD-PAP) method by using a span diagnostic reagent kit (code no. LG 052) and serum triglyceride level was estimated by using glycerol phosphate oxidase-phenol-aminophenazone (GPO-PAP end point assay) method, by using span diagnostic reagent kit (code no. LG 062). Serum LDL Cholesterol was calculated on the basis of Friedwald's equation.

Serum LDL cholesterol (mg/dl) = Total cholesterol - (HDL+Triglyceride/5)

Serum VLDL Cholesterol

VLDL(mg/dl) = Total cholesterol - (HDL + LDL)

Statistical analysis

Data of changes in serum lipid profile were expressed as the mean ± SE (standard error). The values of day '0' were compared statistically with 'day 60' and value of 'day 60' were compared with 'day 102' in both the groups using paired-t test and value of $P<0.05$ was considered to be statistically significant.

Observations & results

TABLE:-I Effect of Lisinopril on serum lipids (Group I)

TREATMENT	TOTAL CHOLESTEROL (Tc)	TRIGLYCERIDE (TG)	HDLc	LDLc	VLDLc
Normal control (day '0')	91±2.01	86.33±1.45	28.41±2.29	45.31±2.29	17.26±0.29
Cholesterol fed control (day '80')	202.08±2.86*	128.25±2.59*	40.58±1.54*	135.85±3.23*	25.65±0.51*
Cholesterol+ lisinopril (day 102)	191.66±3.01#	132.66±3.0#	48.5±0.83#	116.63±3.14#	26.53±0.60

Tc = Total cholesterol, HDLc = HDL - cholesterol, LDLc = LDLcholesterol, VLDLc = VLDLcholesterol, Values are mean ± SEM

Levels of significance *P < 0.05 when compared with normal control rabbits, #P<0.05 when compared with cholesterol fed control rabbits

TABLE II :- Effect of Perindopril on serum lipids (Group II)

Treatment	TOTAL CHOLESTEROL (Tc)	TRIGLYCERIDE (TG)	HDLc	LDLc	VLDLc
Normal control (day '0')	87.25±1.36	83±1.72	28.83±1.29	41.81±1.30	16.6±0.35
Cholesterol fed control (day '80')	188.67±2.10*	122.25±1.82*	39.58±1.18*	124.63±2.59*	24.45±0.36*
Cholesterol+ perindopril (day 102)	183.33±1.93#	125.08±1.79#	44.17±1.22#	114.15±2.25#	25.01±0.36

Tc = Total cholesterol, HDLc = HDL - cholesterol, LDLc = LDLcholesterol, VLDLc = VLDLcholesterol, Values are mean ± SEM.

Levels of significance *P < 0.05 when compared with normal control rabbits, #P<0.05 when compared with cholesterol fed control rabbits.

These observations indicated Serum total cholesterol, triglycerides, HDL, LDL and VLDL cholesterol, were increased significantly (P<0.05) in both the groups after 60 days of cholesterol feeding. Group I showed significant decrease in serum total cholesterol from 202 mg% to 191 mg% and LDL levels from 135 mg% to 116 mg%. HDL levels increased from 40 mg% to 48.5 mg%. Triglyceride levels increased to 132.6 mg% from 128 mg%. The increase was significant. The VLDL levels were also found to be increased but not found significant. Rabbits of group II showed significant decrease in total cholesterol level and serum LDL level. Total cholesterol became 183 mg% from 188 mg%. Serum LDL levels decreased to 114 mg% from 124 mg%. Triglyceride level increased from 122 mg% to 125 mg% and HDL levels were found to be increased from 39.5 mg% to 44 mg%. Both results were significant. Serum VLDL levels increased slightly.

DISCUSSION

We observed that after administration of Lisinopril Rabbits of group I showed significant decrease in serum total cholesterol and LDL levels. There was significant increase in HDL

and triglyceride levels. The VLDL levels were also found to be increased but not found significant. Similar results were obtained by Anichkov A D et al in a prospective randomized blinded study. HDL cholesterol tended to increase and fasting glucose tended to decrease in the lisinopril group.[17] Another longitudinal study showed that maximum lisinopril doses significantly reduced proteinuria, serum total cholesterol, LDL and triglycerides without substantially affecting serum HDL and renal hemodynamics.[18] Fogari R et al observed that lisinopril increased HDL-C and decreased TC and LDL-C. The study concluded favourable effect of lisinopril on serum lipid profile.[19]

Williams LL et al observed contrary results as compared to our results. HDL cholesterol was depressed in those taking hydralazine alone and in combination with lisinopril in patients with essential hypertension.[20] Rabbits of group II showed similar results as group I. Bezrodna L et al compared the effects of perindopril on lipids profile. Perindopril reduced plasma level of triglycerides and increased HDL-C as observed by Bezrodna L et al.[21] Contrary to above results we found that perindopril increased serum triglyceride levels but it showed similar effect on HDL levels by increasing it. Bak J F, Gerdes L U, Sorensen N S et al observed that total cholesterol, triglycerides and HDL levels were not altered by perindopril but we found that perindopril decreased total cholesterol and LDL levels while HDL and triglyceride levels were increased.[22]

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.[23] NA promote hydrolytic release of fatty acid and glycerol from triglycerides in adipose tissue by activating hormone sensitive lipase.[24] 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.[25] This increase in glucose production promote insulin release from beta cells of pancreas. Insulin in turn promotes lipogenesis.[26] Thus blockage of conversion of AI to AII may result in reduced lipid synthesis. Drugs used in our study i.e. Lisinopril and Perindopril 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.[27] In our study, we found profound reduction in total cholesterol levels by the drugs. 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.[28] 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.[29] 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).[30] 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.[31] We observed that Lisinopril and Perindopril increased serum triglyceride levels.

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.

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Conflicts of Interest

The authors have no conflict of interests related to this work.

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