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Comparative Study of Ezetimibe and Atorvastatin Alone and in Combination on Lipid Profile in Rats

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ABSTRACT

Introduction: Coronary heart disease and hyperlipidemia is a global problem in today's world. A large number of people suffer from hypertension, atherosclerosis and all these has strong association with the hyperlipidemia. There are many drugs for the treatment of hyperlipidemia, but statins are most commonly used. But, with high dose of statins, the side effect is also there which restricts its use in high dose. Ezetimibe is a comparatively new drug for the treatment of hyperlipidemia having lesser adverse effect as compared to statin. This study has been planned to find out the comparative efficacy of Ezetimibe and Atorvastatin alone and in combination on the lipid profile in rats. **Methodology:** This study was conducted in SCB Medical College, Cuttack. 60 rats were fed on atherogenic diet. These were divided in six groups having ten rats in each group and followed for 12 weeks. Group I received only atherogenic diet. All other groups received drugs after four weeks. Group II received Ezetimibe 1mg/kg, Group III received Ezetimibe 2mg/kg, Group IV received Atorvastatin 4mg/kg, Group V received Atorvastatin 8mg/kg and Group VI received Atorvastatin 4mg/kg and Ezetimibe 1mg/kg. Blood lipid profile measured at zero week, four weeks and 12 weeks. **Results:** All the lipid profile parameters improved significantly with treatment groups as compared with control group. There was no significant difference in the level of different lipid parameters between Group V (Atorvastatin 8mg/kg) and Group VI (Atorvastatin 4mg/kg and Ezetimibe 1mg/kg). **Conclusion:** High dose of Atorvastatin is associated with more adverse effect. The efficacy of high dose of Atorvastatin is comparable to combination of low dose Atorvastatin with Ezetimibe. This combination has lesser side effects. So, this can be a good alternative.
Key words: Ezetimibe, Atorvastatin, Lipid profile.

1. INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the world (1) with the global burden of the disease continuing to increase. Approximately 7.25 million people die from CHD and 6.15 million from stroke each year (1). The Indians with coronary heart disease have a distinctive lipid profile, characterized by low level of High Density Lipoprotein-Cholesterol (HDL-C), hypertriglyceridemia and increased lipoprotein (a) (2). The management strategies for dyslipidemias, therefore, need to be addressed differently in the Indian population.

The United States National Cholesterol Education Program (NCEP) issued guidelines for the management of dyslipidemia in 1988, 1993 and 2001 (3). In the Atorvastatin Comparative Cholesterol Efficacy and Safety Study (ACCESS), only 43% of patients with CHD receiving the initial dose of Atorvastatin achieved ATP-II goals (4). At maximum titration (up to 80mg) 72% of the patients with CHD receiving Atorvastatin in ACCESS achieved the ATP-II goals, but Atorvastatin at maximum doses has been associated with increased incidence of elevated liver enzymes. Hepatotoxicity and myalgias have also been reported with high doses of statins (5). Ezetimibe is a novel cholesterol absorption inhibitor that prevents the absorption of dietary and biliary cholesterol without affecting the absorption of triglycerides or fat soluble vitamins. Ezetimibe inhibits cholesterol absorption in the intestine whereas statins inhibit cholesterol production primarily in the liver; so they have complementary effects. In pre-clinical models this new class of compound reduced diet induced hypercholesterolaemia in hamsters (6), monkeys (7), rats (8) and dogs (9). In combination with low doses of statins, Ezetimibe lowered Low Density Lipoprotein Cholesterol (LDL-C) as much as 60% in two weeks treatment in hypercholesterolemic patients(). The present study was undertaken to find out the efficacy of Ezetimibe and Atorvastatin alone and in combination on lipid profile in rats.

2. MATERIALS AND METHODS

Study area: The study was conducted in the animal house of Department of Pharmacology of SCB Medical College, Cuttack, India. Cuttack is a town in the state of Odisha in Eastern India.

Study design: It was a prospective interventional study.

Study technique: 60 albino rats weighing between 150—200 grams were selected for this study. The rats were kept in the departmental animal house and they were acclimatized to the environment of animal house before beginning the experiment. During the study period, all the rats received hypercholesterolemic diet and water ad libitum. Then they were divided into six groups of ten rats each in separate cages. Blood samples were collected from the lateral or dorsal veins of the tails of the rats with the help of scalp vein for the estimation of serum lipid profile at the start of the experiment, after four weeks and after 12 weeks. Group I served as control group which did not receive any drug (only hypercholesterolemic diet). Drugs were given to other groups starting at four weeks of the experiment and continued till the end of the experiment (up to 12 weeks). The six groups were as follows: a) Group I: Only atherogenic diet; b) Group II: Atherogeic diet + Ezetimibe 1mg/kg body weight; c) Group III: Atherogeic diet + Ezetimibe 2mg/kg body weight; d) Group IV: Atherogeic diet + Atorvastatin 4mg/kg body weight; e) Group V: Atherogeic diet + Atorvastatin 8mg/kg body weight; f) Group VI: Atherogeic diet + Ezetimibe 1mg/kg body weight+ Atorvastatin 4mg/kg body weight. Serum lipid profile was estimated using autoanalyzer.

The Institutional Animal Ethics Committee permission was taken before performing the experiment.

Statistical analysis: After data collection it was entered in Microsoft Excel sheet and validated. Then the generated clean data sheet was copied into SPSS (version 16.0). Analysis was done in SPSS (version 16.0). Paired and unpaired t test were used for assessing the statistical significance.

3. RESULTS

For the experiment for analyzing the lipid lowering effect of Ezetimibe and Atorvastatin in different doses and the combination of both drugs, total 60 rats were used. The comparative effects between different groups were analyzed using one way analysis of variance (ANOVA) test. The levels of different blood lipid parameters in 12 weeks were compared. From the Table 1 it is clear that the level of blood total cholesterol was found to be significantly less in all treated groups as compared to the control group (Group I, receiving atherogenic diet, not receiving any drug) and the p value was found to be highly significant (<0.001). The difference total cholesterol level between the groups receiving Ezetimibe 1 and 2mg/kg doses were not significant (p=0.056). But the total cholesterol level was found to be significantly lower in Group IV (Atorvastatin 4mg/kg), Group V (Atorvastatin 8mg/kg) and Group VI (combination of Atorvastatin 4mg/kg and Ezetimibe 1mg/kg). There was no significant difference between the level of total cholesterol in Group III (Ezetimibe 2mg/kg) and Group IV (Atorvastatin 4mg/kg). But the total cholesterol level was significantly less in Group V (Atorvastatin 8mg/kg) and Group VI (Atortastatin 4mg/kg and Ezetimibe 1mg/kg) as compared to Group III (Ezetimibe 2mg/kg). The total cholesterol level was found to be significantly less in Group V and Group VI as compared to Group IV (p<0.001). There was no significant difference between the level of total cholesterol between Group V (atorvastatin 8mg/kg) and Group VI (Atorvastatin 4mg/kg and Ezetimibe 1mg/kg). Table 2 shows that the level of low density lipoprotein (LDL) cholesterol at 12 weeks was found to be significantly less in all treatment groups as compared to control

	Sum of square	F value	p value
Between groups	23087.262	765.931	<0.001
Within group	316.499		

Groups	Mean interval	p value	95% confidence interval	
			Lower	Upper
Group I versus Group II	42.6	<0.001	39.547	45.653
Group I versus Group III	45.61	<0.001	42.557	48.663
Group I versus Group IV	47.35	<0.001	44.297	50.403
Group I versus Group V	54.41	<0.001	51.357	57.463
Group I versus Group VI	54.4	<0.001	51.347	57.453
Group II versus Group III	3.01	0.056	-0.043	6.063
Group II versus Group IV	4.75	<0.001	1.697	7.803
Group II versus Group V	11.81	<0.001	8.757	14.863
Group II versus Group VI	11.8	<0.001	8.747	14.853
Group III versus Group IV	1.74	0.595	-1.313	4.793
Group III versus Group V	8.8	<0.001	5.747	11.853
Group III versus Group VI	8.79	<0.001	5.737	11.843
Group IV versus Group V	7.06	<0.001	4.007	10.113
Group IV versus Group VI	7.05	<0.001	3.997	10.103
Group V versus Group VI	0.01	1	-3.043	3.063

Table 1. ANOVA showing comparison of total cholesterol value at 12 weeks between different treatment groups

	Sum of square	F value	p value
Between groups	16644.489	405.073	<0.001
Within group	431.446		

Groups	Mean interval	p value	95% confidence interval	
			Lower	Upper
Group I versus Group II	36.56	<0.001	32.996	40.124
Group I versus Group III	38.52	<0.001	34.956	42.084
Group I versus Group IV	39.73	<0.001	36.166	43.294
Group I versus Group V	46.42	<0.001	42.856	49.984
Group I versus Group VI	46.0	<0.001	42.436	49.564
Group II versus Group III	1.96	0.635	-1.604	5.524
Group II versus Group IV	3.17	0.113	-0.394	6.734
Group II versus Group V	9.86	<0.001	6.296	13.424
Group II versus Group VI	9.44	<0.001	5.876	13.004
Group III versus Group IV	1.21	0.944	-2.354	4.774
Group III versus Group V	7.9	<0.001	4.336	11.464
Group III versus Group VI	7.48	<0.001	3.916	11.044
Group IV versus Group V	6.69	<0.001	3.126	10.254
Group IV versus Group VI	6.27	<0.001	2.706	9.834
Group V versus Group VI	0.42	1	3.144	3.984

Table 2. ANOVA showing comparison of LDL cholesterol value at 12 weeks between different treatment groups

	Sum of square	F value	p value	
Between groups	49244.972	1867.0	<0.001	
Within group	276.901			
Groups	Mean interval	p value	95% confidence interval	
			Lower	Upper
Group I versus Group II	10.21	<0.001	7.355	13.065
Group I versus Group III	12.13	<0.001	9.275	14.985
Group I versus Group IV	60.19	<0.001	57.335	63.045
Group I versus Group V	61.21	<0.001	58.355	64.065
Group I versus Group VI	60.69	<0.001	57.835	63.545
Group II versus Group III	1.92	0.396	-0.935	4.775
Group II versus Group IV	49.98	<0.001	47.125	52.835
Group II versus Group V	51.0	<0.001	48.145	53.855
Group II versus Group VI	50.48	<0.001	47.625	53.335
Group III versus IV	48.06	<0.001	45.205	50.315
Group III versus Group V	49.08	<0.001	46.225	51.935
Group III versus Group VI	48.56	<0.001	45.705	51.415
Group IV versus Group V	1.02	0.929	-1.835	3.875
Group IV versus Group VI	0.5	0.998	-2.355	3.355
Group V versus Group VI	0.52	0.998	-2.335	3.375

Table 3. ANOVA showing comparison of triglyceride value at 12 weeks between different treatment groups

	Sum of square	F	p value	
Between groups	118.850	20.325	<0.001	
Within group	61.399			
Groups	Mean interval	p value	95% confidence interval	
			Lower	Upper
Group I versus Group II	-3.56	<0.001	-4.905	-2.215
Group I versus Group III	-3.85	<0.001	-5.195	-2.505
Group I versus Group IV	-4.05	<0.001	-5.395	-2.705
Group I versus Group V	-3.6	<0.001	-4.945	-2.255
Group I versus Group VI	-2.99	<0.001	-4.335	-1.645
Group II versus Group III	-0.29	0.994	-1.635	1.055
Group II versus Group IV	-0.49	0.923	-1.835	0.855
Group II versus Group V	-0.04	1	-1.385	1.305
Group II versus Group VI	0.57	0.853	-0.775	1.915
Group III versus IV	-0.2	0.999	-1.545	1.145
Group III versus Group V	0.25	0.998	-1.095	1.595
Group III versus Group VI	0.86	0.457	-0.485	2.205
Group IV versus Group V	0.45	0.948	-0.895	1.795
Group IV versus Group VI	1.06	0.215	-0.285	2.405
Group V versus Group VI	-0.61	0.809	-1.955	0.735

Table 4. Unpaired t test showing comparison of HDL cholesterol value at 12 weeks between different treatment groups

group (Group I). LDL cholesterol level was not significantly different between Group II and III and Group II and IV. But the LDL level was significantly less in Group V and Group VI as compared to Group II. There was no significant difference between LDL level of Group III and Group IV; but its level was significantly less in Group V and VI as compared to Group III. LDL cholesterol level was significantly less at 12 weeks in Group

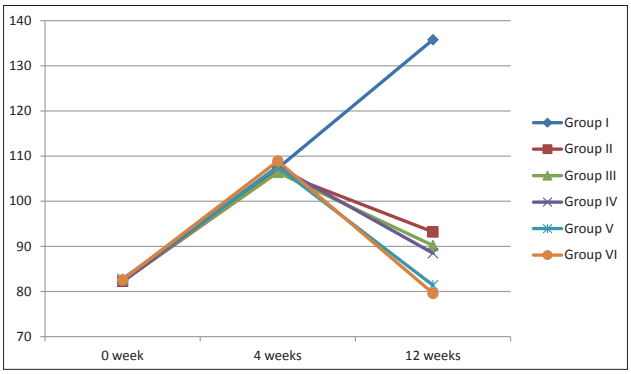

Figure 1. Change of mean total cholesterol value in different groups

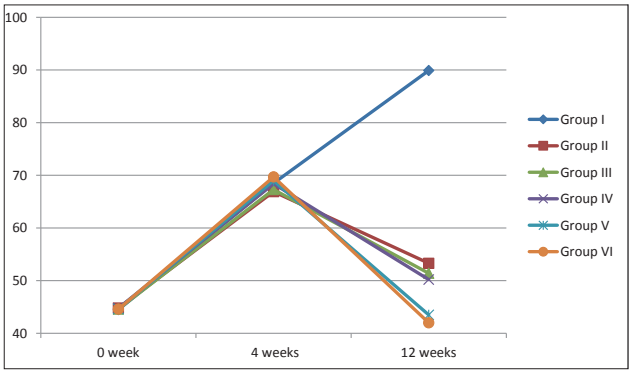

Figure 2. Change of mean LDL cholesterol value in different groups

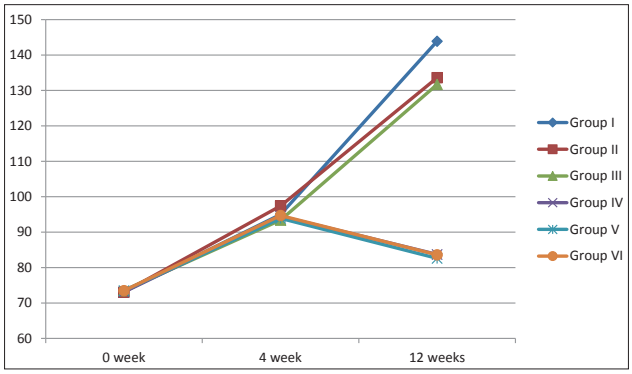

Figure 3. Change of mean Triglyceride in different groups

V and VI as compared to Group IV. There was no significant difference between LDL cholesterol level at 12 weeks between Group V (Atorvastatin 8mg/kg) and Group VI (Atorvastatin 4 mg/kg and Ezetimibe 1 mg/kg). According to Table 3, the level of triglyceride at 12 weeks was found to be significantly less in all treatment groups as compared to control group (Group I). The difference between triglyceride level of Group II and Group III was not significant at 12 weeks; but the triglyceride level was found to be significantly less in Group IV, V and VI as compared to Group II. The triglyceride level was significantly less in Group IV, V and VI as compared to Group III also. There was no significant difference between triglyceride level in Group IV with Group V and VI as well as between Group V and Group VI. High density lipoprotein (HDL) cholesterol is considered to be good cholesterol for health. Its high level is good for health. Table 4 shows that its level was found to be significantly higher at 12 weeks in all treatment groups (Group II to Group VI) as compared to control group. But among the different treatment

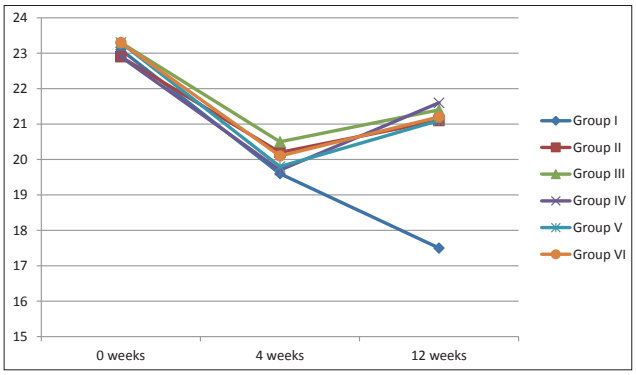

Figure 4. Change of mean HDL cholesterol in different groups

groups its level was not significantly different; i.e. in all cases p value is more than 0.05. Figure 1 to 4 indicates the mean level of different lipid parameters in different treatment groups. It is clear that in control group (fed on atherogenic diet without receiving any drug) the total cholesterol, LDL cholesterol and triglyceride continued to be increased and the HDL cholesterol continued to be decreased throughout the 12 weeks time period. But in all other groups treatment was started at 4 weeks. So, up to 4 weeks the trend of change of different lipid parameters were like control group. But in these groups it is seen that after 4 weeks the blood level of total cholesterol, LDL cholesterol and triglyceride decreased and HDL cholesterol increased. It is also observed from the figures that the difference of level of different lipid parameters at 12 weeks between Group V (Atorvastatin 8mg/kg) and Group VI (Atorvastatin 4mg/kg) is very less.

4. DISCUSSION

Ezetimibe is a member of new class of lipid altering agents that inhibits the absorption of dietary and biliary cholesterol without affecting the absorption of triglycerides or fat soluble vitamins (7). No clinically relevant drug-drug interactions have been associated with Ezetimibe administration, including statins. Combining Ezetimibe with a statin offers the theoretical advantage of incremental lipid lowering resulting from complementary mechanism of action (10). Ezetimibe is orally active and potent with a unique mechanism of action that differs from other classes of cholesterol reducing compounds. It localizes at the brush border of small intestine and inhibits the absorption of cholesterol, leading to a decrease in the intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol store and increase in clearance of cholesterol from the blood. Ezetimibe has no clinically meaningful effect on the plasma concentrations of the fat soluble vitamins and did not impair adrenocortical steroid hormone production. Statins inhibit 3-Hydroxy 3-Methyl Glutaryl Co Enzyme-A enzyme (HMG CoA) and thereby inhibit the synthesis of cholesterol. Combining this treatment in all cases resulted in complementary and additive improvements in LDL-C, HDL-C and Triglyceride. The mean serum total cholesterol in rats at the start of the experiment (zero week) varies between 81.15 to 82.2mg/dl which is almost same as the value (82.73mg/dl) reported by Sankhala et al 1992 (11). The concentration of mean serum triglyceride, HDL-cholesterol, LDL-cholesterol in rats at the start of the experiment were between 72.3 to 73.69 mg/dl, 22.58 to 22.93mg/dl, 43.82 to 45.21mg/dl respectively. These findings were almost same as reported by Sankhala et al 1992 (11). There was an increase in mean total cholesterol, mean triglyceride, mean LDL-cholesterol and a decrease in mean HDL-cholesterol in rats fed on atherogenic diet at 4 and 12 weeks. There was a significant less value in total cholesterol and LDL-cholesterol in rats receiving Ezetimibe at doses 1mg/kg and 2mg/kg body weight (as compared to control). This is comparable with the findings of Von Heek et al 2003 (8). In rats receiving Atorvastatin 4mg/kg and 8mg/kg body weight, it was observed that there was a significant less value in total cholesterol, triglyceride and LDL-cholesterol compared with the control group. Conde K et al (1996) observed similar findings in hypercholesterolemic guinea pigs receiving different doses of Atorvastatin (12). High dose of Atorvastatin produced significant lowering of LDL cholesterol and total cholesterol without significantly affecting triglyceride and HDL-cholesterol; but high dose of Atorvastatin is associated with more chances of adverse effects.

Rats treated with Ezetimibe at dose of 1mg/kg body weight in combination with constant dose of Atorvastatin (4mg/kg body weight) showed a very significant less level of total cholesterol, LDL-cholesterol and triglyceride. Harry R et al (2001) observed similar findings in hypercholesterolemic dogs treated with different doses of Ezetimibe in combination with Atorvastatin (13). These findings suggest that co-administration of Ezetimibe and Atorvastatin offers a highly efficacious new treatment option for patients with hypercholesterolemia. Ezetimibe inhibits cholesterol absorption in the intestine. This inhibition is associated with compensatory increase in cholesterol synthesis. The observed increase in hepatic cholesterol synthesis might explain the favourable effect of co-administering Ezetimibe and statins according to a study by Davies HR Jr et al (2001) (14). Thus, because statins can reduce the compensatory increase in hepatic cholesterol synthesis, induced by Ezetimibe, the combinations of Ezetimibe and statins results in an incremental lowering of LDL cholesterol concentration (Davies HR Jr et al (2001) (14). High dose of Atorvastatin is associated with more adverse effect. This study highlighted the fact that the efficacy of high dose of Atorvastatin is comparable to combination of low dose Atorvastatin with Ezetimibe. So, this combination is a good alternative as compared to high dose statin as this combination has lesser side effects. Further larger study on human beings is needed.

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