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Comparing the effect of Monotherapies of Hyperlipidemia over Placebo Treatment

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Abstract:
This study was a placebo controlled, single centre prospective study to evaluate and compare the hypolipidemic activity of monotherapies of simvastatin (20 mg),ezetimibe(10 mg) and omega-3 fatty acids(4 g) over placebo treatment. Human dyslipidemic male subjects who had been selected based on the inclusionand exclusion criteria ,were divided into four groups of 21,20,22 and 20 subjects. The serum lipid profile level of these subjects were determined before and after 90 days treatments of placebo and above monotherapies. After 90 days of treatment, LDL cholesterol and total cholesterol levels were reduced significantly in simvastatin and ezetimibe groups while triglycerides level was significantly reduced and HDL cholesterol level increased in omega-3 fatty acids group compared to placebo group.

Keywords: simvastatin, ezetimibe, omega-3 fatty acids, hypolipidemic acids

Introduction

Atherosclerosis, [1]a disease state caused by abnormality of lipid metabolism. Dyslipidaemia, hypertension, insulin resistance, obesity, physical inactivity, cigarette smoking are the important risk factors for developing atherosclerosis. Hypercholesterolaemia is an important risk factor for coronary heart disease (CHD). The reduction of increased serum total cholesterol (TC), low-density lipoprotein cholesterol, triglycerides level and elevation of decreased HDL level reduces the risk of coronary artery disease.

According to NCEP ATP III Classification [2]of Total cholesterol (NCEP publication,2002), total cholesterol level less than 200mg/dl, LDL cholesterol level less than 100mg/dl, triglyceride level less than 150mg/dl and HDL level between 40-60 mg/dl are being considered as normal or optimal.

Serum cholesterol [3]is derived from biosynthesis (endogenous pathway) and intestinal uptake (exogenous pathway) of dietary and biliary cholesterol. Drug therapy with cholesterol-lowering medications, particularly 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), is effective in reducing the risk for cardiovascular disease and stroke in subjects. Statins,like simvastatin[4] which modulate only endogenous cholesterol, inhibit biosynthesis of cholesterol, deplete intracellular pools, and enhance removal of plasma LDL-C leading to significant reduction of serum LDL-C. However, not all subjects respond to statin treatment.

Ezetimibe[5] is the selective cholesterol absorption inhibitor which prevents the absorption of cholesterol by inhibiting the passage of dietary

and biliary cholesterol across the intestinal wall. Omega 3 fatty acids[6] obtained from fish liver oil are long chain highly polyunsaturated, principally eicosapentaenoate and docosahexaenoate reduces the triglycerides and LDL level by reducing the amount of cholesteryl esters in nascent VLDL and increases HDL level by the reducing the concentration of free fatty acids in plasma causing reduced net flux of cholesteryl esters from HDL to LDL and VLDL via reduced activity of the cholesteryl ester transfer protein. There is strong evidence that statin, ezetimibe or omega-3 fattyacid treatment decrease low density lipoprotein (LDL) cholesterol and triglycerides decreases mortality in patients with coronary heart disease (CHD)

Methodology

List of chemicals used and their sources:-

Materials used	Sources
1)simvastatin,ezetimibe tablets and omega-3 fatty acids capsules	Micro labs
2)Serum total cholesterol diagnostic kit	Span diagnostics Ltd
3)Serum triglycerides diagnostic kit	Span diagnostics Ltd
4) Serum HDL cholesterol diagnostic kit	Span diagnostics Ltd
5) ALT kit, AST kit.	Span diagnostics Ltd
6)CPK kit	Span diagnostics Ltd

Study design and data handling

A placebo controlled study in which subjects were randomized and received one of the following four treatments, daily for 90 days:

- Group I : Placebo
- Group II : Simvastatin 20 mg,
- Group III : Ezetimibe 10 mg,
- Group IV : Omega 3 fatty acids 4 g

The subjects were selected from the panel of subjects enrolled with the Centre of Clinical Research, seven days prior to the commencement of the study, subjects were screened based on the inclusion criteria of the study. On the basis of this preliminary screening, 96 subjects were selected based on the inclusion and exclusion criteria of the study. Selected subjects were tamilnadu (india) males,≥ 18 and ≤48 years, dyslipidemic ,with LDL - c levels between 129 and 200mg/dl,total cholesterol level(TC) between 200 and 280mg/dl ,triglycerides level between 150 and 350mg/dl and HDL level between 35 and 60mg/dl. .Exclusion criteria of the study were active liver disease, abnormal hematology, blood chemistry, urine analysis and liver transaminases, severe congestive cardiac failure, unstable angina, uncontrolled hypertension, uncontrolled endocrine or metabolic disease, impaired renal function No concomitant medication was allowed during the study phase. Subjects were also instructed to refrain from consuming alcohol, smoking or other stimulant drinks during this period.

Subjects were stabilized as outpatients on an NCEP Step I diet, study treatments were administered orally with 200 ml of noncarbonated, room-temperature water, once daily in the morning for 90 days.

The protocol of the study was submitted to the Institutional Human Ethical Committee and the approval for conducting the same was obtained. Prior to the commencement of the study, each subject was provided with an information sheet giving details of the investigational drugs, procedure and potential risk involved and a written consent was obtained. They were instructed that they were free to withdraw their consent and to discontinue their participation in the study at any time without prejudice.

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All the subjects were made to assemble in the Centre of Clinical Research, the subjects were given code numbers and allocated to the treatment in accordance with the randomized code. Their pulse rates and blood pressures were recorded and disposable needles were used with strict aseptic precautions for blood collection. Blood samples (5 ml) were collected using disposable syringes in pre-heparinised centrifugal tubes at 0^{th} (before drug administration), 25^{th}, 50^{th} and 90^{th} days. The samples were centrifuged at 3500 rpm for 10 minutes to separate plasma. They were transferred into airtight containers and stored at -20^{0} C until starting of analysis.

The study was monitored by a physician and a clinical pharmacologist. The subjects were monitored for abnormal symptoms during the study period and for one week after the study period and if noticed, the details were entered in the case report sheets and tabulated at the end of the study.

Tolerability

Blood samples were collected on 0^{th} day (before dosing), 25^{th} day, 50^{th} day and 90^{th} day monitoring signs of muscle and liver injury. Vital signs (blood pressure, heart rate, and body mass index) were monitored during screening, on 0^{th} day (before dosing), 25^{th} day, 50^{th} day and 90^{th} day and at follow-up on day 15. Subjects were continually observed and questioned for possible adverse events.

Pharmacodynamics

Blood samples were collected for serum lipid profiles (LDL, TC, HDL, and TG) just before dosing on days on 0^{th} day (before dosing, baseline value), 25^{th} day, 50^{th} day and 90^{th} day. Lipid concentrations were determined by direct quantitative enzymatic colorimetric tests using validated commercial assay kits.

Cholesterol screening: Enzymatic methods used were the assays of choice for the measurement of cholesterol. They were easily adapted for use on auto analysers. Cholesterol reagents combined with the enzymes and other required components into a single photometric reagent. This reagent mixed with 10 microlitre aliquot of serum, incubated under controlled conditions for color development and absorbance measured in the visible portion of the spectrum generally at about 505 nm. The reagents typically use a bacterial cholesteryl ester hydrolase to cleave cholesteryl esters into cholesterol and fatty acid. The 3-OH group of cholesterol was then oxidised to a ketone and H_2O_2 in an oxygen requiring reaction catalysed by cholesterol oxidase. H_2O_2 with phenol and 4 amino antipyrine in a peroxidise catalysed reaction formed a colored dye.

Estimation of serum total cholesterol (Nicholas .V et al, 1956) Span diagnostic kit was used for the estimation of total cholesterol, which followed cholesterol oxidase / peroxidase method.

Triglycerides screening (Nicholas .V et al, 1956)[7] The first step was the lipase catalysed hydrolysis of triglycerides to glycerol and fatty acids. Glycerol was then phosphorylated in an ATP-requiring reaction catalysed by glycerokinase to glycerophosphate and adenosine di phosphate. Glycerophosphate was then oxidised to dihydroxyacetone and H_2O_2 in a glycerophosphate oxidase catalysed reaction. H_2O_2 was measured as shown above.

HDL screening: The concentration of HDL in plasma was assessed by determining the concentration of cholesterol associated with HDL. Polyanions like dextran sulphate when added to an aliquot of plasma react with positively charged groups on lipoproteins and formed a precipitate of the non-HDL lipoproteins within 10 minutes at

room temperature. This precipitate was removed by centrifugation and HDL cholesterol was measured enzymatically in the supernatant on an auto analyser.

Estimation of Serum High-Density Lipoprotein Cholesterol (HDL-C) ((Nicholas .V et al, 1956)

Span diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase method.

Estimation of Serum Low-Density Lipoprotein Cholesterol (LDL-C)

Using the data obtained including total cholesterol, HDL cholesterol and VLDL, the LDL cholesterol levels were calculated using the empirical equation of Friede Wald [8].

Calculation:

Serum LD Lcholesterol = Total cholesterol – HD Lcholesterol - Triglyceride/5

Estimation of Serum Glutamate Pyruvate transaminase (ALT)

-(Reitman S,et al 1957)[9]

The normal range of values for ALT (SGPT) is about 5 to 40 units per liter of serum. It is found to be distributed mainly in the liver and to a lesser extend in the kidney and muscles. ALT level elevated in liver damage and myocardial infarction.

Serum glutamate pyruvate transaminase, SGPT also called as Alanine transaminase ALT was determined by using Reitman and Franker method.

Alanine amino transferase in serum catalyses,

a –keto glutarate +L-alanine ALT $\longrightarrow$ L-glutamate +pyruvate

Pyruvate with 2, 4 DNPH resulted in brownish red colour complex in an alkaline medium. The colour intensity was directly proportional to the SGPT concentration in the serum and was measured photometrically at 505nm under alkaline condition.

Estimation of Glutamate Oxaloacetate Transaminase (SGOT)

The normal range of values for AST (SGOT) is about 5 to 45 units per liter of serum. AST level elevated in myocardial infarction,muscular dystrophy,and liver necrosis .

Serum oxaloacetate transaminase, SGOT also called as aspartate amino transaminase. AST was determined by using Reitman and Frankel method-(Reitman S,et al,1957)[9]

Aspartate amino transferase in serum catalyses,

a–keto glutarate +aspartate AST $\longrightarrow$ glutamate +oxaloacetate

Oxaloacetate, with DNPH resulted in brown colour which was measured under alkaline condition .

Assay & Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

ESTIMATION OF CREATINE KINASE: (Marco Machado et al, 2009)[10]

Creatine kinase (CK) is a key metabolic enzyme. Two different sub-units of CK occur, M and B. The CK holoenzyme exists as MM and BB homodimers and an MB heterodimer. High expression levels of the MB isoform in heart explain its use as biomarker for heart disease. Likewise, CK-MM can be used as a specific biomarker for skeletal muscle injury.

Principle of the test: Coupled enzymatic method (at 37^0 C) was used to measure the CK activity (Oliver-Rosalki method). NADH absorbance measured on a spectrophotometer at 340 nm. .

Creatine phosphate + ADP $\xrightarrow{ck}$ ATP+creatine

ATP+ glucose $\xrightarrow{HK}$ glucose-6 phosphate + ADP

NAD^+ + glucose-6 phosphate $\xrightarrow{G\text{-}6 - PDH}$ gluconate – phosphate + NADH

CK catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate,to

form ATP and creatine.The auxillary enzyme hexokinase(HK) catalyzes the phosphorylation of glucose by the ATP formed ,to produce ADP and glucose-6-phosphate.The glucose-6-phosphate is oxidized to 6-phosphogluconate with the concomitant production of NADH.The rate of NADH formation ,measured at 340nm, is directly proportional to serum CK activity.

Reference range normal: Males-upto 160 U/L.

Females- upto 130 U/L.

Statistical analysis

Mean, standard deviation or standard error, coefficient of variation, ANOVA and dunnets t test were used for evaluating changes in the lipid parameters LDL-C, TC, HDL-C, and in each group after 25th, 50th and 90th days compared with base line values.

Results and Discussion

Among the 155 subjects screened, 96 subjects were selected based on study inclusion and exclusion criteria. During an initial 15 day washout phase of the study, the participants received no anti-hyperlipidaemic medication and were stabilized on the NCEP Step I diet. After washout phase, subjects were randomized to one of the four treatment groups.

The treatment regimen consisted of distribution of subjects, demographics and baseline characteristics were comparable among the four treatment groups. Study treatments were administered orally with 200 ml of noncarbonated water, once-daily dosing in the morning for 90 consecutive days.

In my present study 83 subjects successfully completed the study. 13 subjects discontinued during treatment. The adverse event was minimal and there were no clinically significant changes or trends in vital signs, clinical laboratory tests (particularly in the enzymes assessing muscle and liver injury) with any of the treatments indicating that the drugs administered were well tolerated.

The primary goals present study is to confirm the safety and tolerability of the selected drugs in the mono therapies provides consistent and predictable reductions in LDL-C levels.

Table 1: A Baseline characteristics of subjects

Parameter (n=number of subjects)	Placebo (n=21)	Simvastatin (S) (n=20)	Ezetimibe (E) (n=22)	Omega-3 fattyacids (o) (n=20)
Age(years), mean	29.57	31.30	28.86	29.40
Body mass index, kg(m^2), mean	27.05	28.45	26.95	26.80
AST(U/litre)	26.19	26.20	24.86	25.20
ALT(U/litre)	26.86	29.20	27.95	26.85
CPK(IU/litre)	75.05	110.8	99.45	98.20
Heart Rate	71.05	71.45	70.64	72.55
SBP(mm Hg)	121.9	123.6	122.3	122.3
DBP (mm Hg)	84.38	82.65	83.91	83.15

Base-line characteristics: The mean age of the subjects,mean body mass index,mean AST ,mean ALT,mean CPK,mean,mean heart rate,mean systolic and diastolic pressures were given in table 1-A for all the four groups.

Table 1: B. Changes in clinical characteristics after 90 days

Parameter	Placebo (n=21)	Simvastatin (S) (n=20)	Ezetimibe (E) (n=22)	Omega-3 fattyacids (o) (n=20)
Body mass index, kg(m^2), mean	27.10	27.50	26.18	25.90
AST	26.24	26.80	25.64	26.15
ALT	27.19	32.95	30.55	29.70
CPK	74.95	111.6	101.2	101
Heart Rate	71.10	70.40	70.05	71.50
SBP (mm Hg)	121.2	121.8	120.6	121.1
DBP (mm Hg)	84.05	81.70	83.55	82.30

Changes in clinical characteristics after 90 days

There were no significant changes seen in mean body mass index, heart rate ,AST,ALT and CPK levels of groups after 90 days compare to base(table 1-B) All the groups showed slightly reduced systolic blood pressure except placebo group.The diastolic blood pressure reduced slightly only in simvastatin,omega-3 fatty acids monotherapy groups.

TABLE 2: Summary of safety data

Adverse effect	Placebo group	Simvastatin (S)	Ezetimibe (E)	Omega-3 fatty acids (O)
Drug related	N=0	N=2	N=0	N=0
Serious adverse effect	0	0	0	0
death	0	0	0	0
Discontinued due to adverse effect	0	0	0	0
Allergic rash	0	1	0	1
Gastro intestinal related	0	3	1	0
Hepatitis related	0	0	0	0

N=number of subjects

Safety report: No serious adverse event seen in any group of subjects.Each one subject from simvastatin and omega-3 fatty acids group showed allergic rash and three subjects from simvastatin group and one subject from ezetimibe group were seen with gastrointestinal disturbances.(ref;Tab-2)

TABLE No 3: Effect of placebo on lipid profiles

Lipoproteins mg/dl	Placebo control , n=21			
	base	25days	50days	90days
LDL	133.0 ± 0.2009	132.7 ± 0.1737	132.5 ± 0.1313	132.8 ± 0.2059
TC	232.4 ± 0.1107	232.9 ± 0.3079	232.8 ± 0.2573	232.4 ± 0.2634
TG	287.0 ± 0.1756	287.0 ± 0.0690	286.7 ± 0.2218	287.4 ± 0.1887
HDL	43.19 ±0.1636	42.76 ± 0.2172	42.62 ± 0.1086	43.00 ± 0.1952

Effect of placebo on lipid profiles: (Tab. 3)

In placebo treated group,there was not much changes in lipid profiles levels.(reductions of 0.2%,0.37%,0.15% of LDL,0.21%,0.17%,0% of TC,0%,0.13%,0.13% of TG and 0.9%,1.3% and 0.43% variation of HDL in 25th,50th and 90th days.).

TABLE 4: EFFECT OF 20 mg OF SIMVASTATIN ON LIPID PROFILES

Lipoprotein mg/dl	Simvastatin 20 mg n=20			
	Base	25 days	50 days	90 days
LDL	134.4 ± 0.7687	131± 0.8223	125 ± 0.7539	94.25 ± 0.9144
TC	235 ± 0.7236	223.3 ± 1.057	210.3 ± 1.024	186.1± 1.192
TG	285.9 ± 1.349	280.9 ± 1.082	271.8 ± 1.339	233.3 ± 2.027
HDL	38.85 ± 0.658	40.85 ± 0.4057	41.85 ± 0.3015	41.85 ± 0.4935

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TABLE 5: EFFECT OF 10mg OF EZETIMIBE ON LIPID PROFILES

Lipoprotein mg/dl	Ezetimibe 10 mg n=22			
	Base	25 days	50 days	90 days
LDL	144.1 ± 0.7838	139.3 ± 0.7740	132.7 ± 0.7068	110.3 ± 1.905
TC	236.6 ± 1.211	233.6 ± 1.154	222.5 ± 1.886	186.2 ± 2.104
TG	257.7 ± 1.485	249.3 ± 1.784	237.7 ± 1.620	211.5 ± 2.071
HDL	41.36 ± 0.6630	41.36 ± 0.6014	41.09 ± 0.6410	41.59 ± 0.6601

TABLE 6: EFFECT OF 4G OMEGA-3 FATTY ACIDS ON LIPID PROFILES

Lipoprotein mg/dl	Omega-3-fatty acids(4g) group n=20			
	Base	25 days	50 days	90 days
LDL	138.5 ± 1.123	133.1±1.015	125.8 ± 1.160	117.1±1.274
TC	233.7 ± 1.170	227 ± 1.316	217.1 ± 1.808	193.8 ± 1.329
TG	302.9 ± 1.421	275.4 ± 1.437	253.4 ± 1.457	202.1± 5.275
HDL	41.20 ± 0.6553	42.50 ± 0.5596	44.40 ± 0.5047	47.60 ± 0.5252

TABLE 7: Comparison of effect of treatments on lipid profiles at the end of study (percentage of change in lipid level at the end of study compare to base values)

Sl. No	Treatment	LDL ↓	TC ↓	TG ↓	HDL ↑
1	P	0.15	0	0.13	0.43
2	S	29.87	20.80	18.39	7.72
3	E	23.45	21.30	17.92	0.55
4	O	15.45	17.07	33.27	15.53

P - placebo, S - simvastatin, E – ezetimibe, O – omega - 3 fatty acids
↓ ---decrease, ↑ ---increase

Mono therapy with ezetimibe, Simvastatin and Omega 3 fatty acids

Ezetimibe has 18.5% of LDL, 12.2 % of TC and 14.9 % of TG reductions and also no effect on HDL observed in 25th day of initiating mono therapy. On day 50, ezetimibe alone reduced 23.4 % of LDL, 13.6 % of TC and 14.5 % of TG reduction and also 7.2 % HDL was increased. On day 90, ezetimibe reduced 22.6 % of LDL, 17.3 % of TC and 18.2 % of TG and also 9.2 % HDL was increased (Ref:Tab 5 and 7).

Simvastatin has a 7.36 % of LDL, 10.2-% of TC and 7.2 % of TG reductions and also no effect on HDL observed in 25th day of initiating mono therapy. On day 50, Simvastatin alone reduced 17.1 % of LDL, 14.3 % of TC and 9.2 % of TG reduction and also 8.0 % HDL was increased. On day 90, Simvastatin reduced 20.8 % of LDL, 15.5 % of TC and 12.7 % of TG and also 10.3 % HDL was increased (Ref; Tab 4 and 7).

On day 25, omega-3 fatty acids alone reduced 3.89% of LDL. On day 25, omega-3 fatty acids alone reduced 2.86% of TC. On day 25, omega-3 fatty acids alone reduced 9.07% of TG. On day 25, omega-3 fatty acids alone increased 3.15% of HDL .On day 50, Omega 3 fatty acids alone reduced 1.1 % of LDL, 7.0 % of TC and 22.9 % of TG and also 32.5 % HDL was increased. On day 90, Omega 3 fatty acids reduced 3.2 % of LDL, 11.2 % of TC and 28.8 % of TG and also 53.2 % HDL was increased(Ref: Tab 6 and 7).

Conclusion

The reduction of elevated serum total cholesterol and low-density lipoprotein cholesterol (LDL) reduces the risk of coronary artery disease, resulting in a decrease in cardiovascular mortality.

Among the monotherapies of simvastatin, ezetimibe and omega-3 fatty acids, simvastatin exhibited best LDL cholesterol reduction at the end of the study. Simvastatin, ezetimibe and omega-3 fattyacids provided similar reduction of total cholesterol (TC) .

omega - 3 fatty acids exhibited best triglycerides(TG) reduction and HDL cholesterol elevation at the end of the study among the above three monotherapies.

Compared to placebo treatment all the three monotherapies exibit good efficiency of lipid reduction in mixed hyperlipidemia with different mechanisms of action.

Combinaion therapy of simvastatin and ezetimibe will be an very good alternative for LDL reduction for subjects who need better LDL reduction than with simvastatin or ezetimibe alone.

Combination therapy of simvastatin or ezetimibe with omega-3 fatty acids would produce a greater reduction in triglycerides levels than mono therapies.

No groups in this study showed elevation of AST or ALT ≥ 3 x ULN nor CK ≥ 5 x ULN. This showed no incidence of myopathy or rhabdomyolysis.

Goals of future studies are to establish the efficacy and tolerability of drug therapies with large populations with primary hypercholesterolaemia.

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