INTRODUCTION
Atherosclerosis is a condition affecting large and medium-sized arteries. It is characterized by the accumulation of various substances like lipids, inflammatory cells, smooth muscle cells and extra-cellular matrix in the sub endothelial space. It is the leading cause of death and disability in the developed world. CVD claims more lives in the US each year than the next five leading causes of death combined.

Blood pressure, plasma cholesterol and obesity are established cardiovascular risk factors, while plasma levels of interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), C-reactive protein (CRP), and leptin have also been implicated in atherogenesis. Although blood total cholesterol and low-density lipoprotein cholesterol (LDL-C) are the most frequent lipid components reported in clinical and epidemiological studies, little attention has been paid to levels of high-density lipoprotein cholesterol (HDL-C) and triglycerides. A recent systematic review showed epidemiological evidence of an inverse association between HDL-C levels and stroke risk. Hyperlipidemia and hyperglycemia trigger endothelial generation of reactive oxidant species, activating nuclear transcription factors (NFkB) which induce genes involved in atherosclerosis such as VCAM-1 and MCP-1.

Several pharmaceutical therapies have been developed traditionally to inhibit cholesterol synthesis. These agents, collectively referred to as statins, inhibit the enzyme 3-hydroxy3-methylglutaryl coenzyme A reductase to effectively reduce blood cholesterol levels and represent the standard of care for treatment of dyslipidemia. A new class of cholesterol-lowering therapeutics, called 2-azetidinones, decreases plasma cholesterol levels by blocking intestinal absorption of cholesterol. Co-administration of statins and ezetimibe produces dual-pathway inhibition resulting in an additive effect on plasma cholesterol reduction. This therapeutic strategy is particularly effective because the two drug classes decrease cholesterol by distinct mechanisms, inhibition of cholesterol synthesis (statins), and inhibition of cholesterol absorption (ezetimibe). Reduced plasma cholesterol in dogs was found due to either an additive or synergistic manner, depending on the statin employed.

The effectiveness of plasma lipid lowering in the clinic is well supported by a growing number of contributions, indicating the significant improvement in cardiovascular risk in primary and particularly in secondary prevention. While these studies have clearly indicated that the more potent agents for cholesterol reduction can provide a very effective help, other pathways of lipid metabolism have gained interest. But these agents give only symptomatic relief. So newer molecular target should be found by which we can treat the atherosclerosis in place of giving symptomatic relief by targeting lipid level as well as inflammation.

Up regulation of Sterol regulatory element binding protein (SREBP) that control lipid biosynthesis and uptake, Capsase-3 which is found in apoptosis and NF-kB that contribute in atherosclerosis associated inflammation is done by GSK-3. Hence GSK-3 inhibitors may be potential target for prevention of atherosclerosis. In this research we have tried to find out effectiveness of valproate, a potent GSK-3 inhibitor, in combination with ezetimibe. Combination of atorvastatin and ezetimibe was used as a standard treatment. Atorvastatin was selected because of its pleiotropic effect as a GSK-3 inhibitor.

Keywords: Atherosclerosis, Vascular injury, High fat fed.
**MATERIALS AND METHODS**

**ANIMALS**

Male Sprague Dawley rats of weighing 280-350 gm were procured from central animal facility of B. S. Patel Pharmacy College. The care and the use of these animals were in accordance with the guidelines of the CPCSEA. Experimental protocol was approved by Institutional Animal Ethic Committee (IAEC). The rats were divided into 6 groups.

**Surgical procedure for femoral artery injury**

The rats were anesthetized using ketamine (55 mg/kg, i.m) and diazepam (5 mg/kg, i.m) after shaving of lower ventral region 1.5 cm incision was made adjacent to femoral vessel. The femoral artery was separated carefully from the surrounding fascia and femoral vein and nerve using small curved micro forceps. When the femoral artery was isolated, it was clamped with the help of bull dog. The arteriotomy was performed in the isolated portion of the artery with the metal wire, whose surface was rough and the tip was made blunt and smooth. After making small hole in the wall of the artery metal wire was inserted into the lumen of the femoral artery at a fixed distance. And then it was rotated completely for 3 times and removed carefully. The metal wire was then withdrawn, and after allowing back bleeding through the arteriotomy site, the femoral artery was ligated proximal to the arteriotomy site using 4-0 silk sutures. Then the ligature was tied below the hole in the femoral artery. Thereafter slowly remove the bulldog clamps from the femoral artery. After visual inspection (to ensure adequate pulsation of the femoral artery), the surgical incision was closed, and the rats were allowed to recover from anesthesia. The incisions were closed with the help of the suturing needle. And antiseptic cream was applied on that day.

**ANTI-ATHEROSCLEROTIC ACTIVITY PROTOCOL**

**Glycogen Estimation from Liver**

From liver 60 percent potassium hydroxide at 100º C can extract glycogen, that is quantitatively precipitated from a 70 per cent of alcohol, and that the optimal condition for its conversion to glucose is in 2.2 per cent hydrochloric acid at 100º C. Following the conversion of glycogen to glucose, the Glucose can be estimated with the help of titration method. Then concentration of Glucose was converted into Glycogen percentage.

**Blood sampling and Serum separation:** Un-haemolysed sample of blood was collected from the clean tail tips in eppendorf tubes from the anaesthetized animals. The blood was allowed to clot at room temperature (37ºC) and centrifuged at 2500 rpm for 10 minutes at room temperature to separate the serum and subjected to biochemical estimation.

**Protocol for Lipid Profile Study:**

Estimation of serum Total cholesterol, Triglyceride, and HDL-cholesterol were done by using Standard kit (Nicholas India Pvt. Ltd.) with semi-auto analyzer.
RESULTS:

A  Liver Glycogen content

B  Serum Total Cholesterol

C  Serum Triglyceride
Effect of Drug treatment on (A) liver glycogen content, (B) Total Cholesterol content, (C) Triglyceride content, (D) HDL content, (E) VLDL content (F) LDL content, (G) HDL LDL ratio and (H) Atherosclerotic Index in Normal Control, Sham Control with HFF, HFF and injury induced Disease Control, Disease Cont. - AT. (Atorvastatin), Disease Cont.-EZE. (Ezetimibe), Disease Cont.-VAL. (Valproate), Disease Cont.-EZE.- AT., Disease Cont-EZE.-VAL. treated rats. Values are expressed in Mean ± s.e.m. n=4-6. For one way ANOVA followed by Dunnett post test *p < 0.05, **p < 0.01, ***p < 0.001 Vs Disease Control Group.

**DISCUSSION**

The prime objective of this research project was to postulate the crucial molecular events linking hyperlipidemia and vascular dysfunction associated with atherosclerosis with main emphasis on Glycogen Synthase Kinase-3 (GSK-3). We have designed our research project to find out newer approach that can cure vascular dysfunction besides only treating elevated blood lipid level by targeting offending molecular target. This type of research is needed because no one perfect drug is available which fits in this role till now. According to World Health Organization in 1999, cardiovascular disease contributed to one third of all deaths, with 78% of those deaths occurring in low and middle income countries. This is the status of mortality due to atherosclerosis though we have potent drugs like statins, since more than four decades, which block cholesterol biosynthesis and lowering down blood lipid profile. There are other drugs as well but no one drug gives preventive therapy. We can cure vascular dysfunction by using combination of newly found drug along with conventional lipid lowering agent to mitigate the mortality issue related to the atherosclerosis. Since intima-media thickness (IMT) is a marker for early atherosclerosis
strongly associated with stroke \(^7\) and that can be measured by ultrasound analysis, it is possible to think for preventive drug therapy as well in advance in early stage of the atherosclerosis. By considering all these facts we have made attempt for both the preventive and curative therapy for newer drug and conventional drugs. We have design our research work to get the idea of effectiveness of combination therapy also.

Glycogen synthase is a rate-controlling step in insulin-stimulated muscle glycogen synthesis so glycogen measurement was performed to elucidate role of GSK-3 in hyperlipidemia state \(^8\). Decreased glycogen content in atherosclerotic rat liver indicates decreased glycogen synthase (GS) activity and increased activity of Glycogen Synthase Kinase as compare to normal animals. Treatment of atorvastatin (p < 0.01) and valproate (p < 0.01) in monotherapy and in combination with ezetimibe (p < 0.001) shows significant different from disease control animal both in curative and preventive therapy as compare to disease control (fig A). This shows effect of atorvastatin and valproate as a GSK-3 inhibitor and they may be newer drug for prevention of atherosclerosis.

Hypercholesterolemia is one of the most important risk factors for atherosclerosis and coronary heart disease (CHD). Plasma total cholesterol distributes among three major lipoprotein classes including low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL). Risk of CHD is positively correlated with LDL cholesterol (LDL-C), whereas it is inversely associated with cholesterol of HDL (HDL-C) \(^9\). From that LDL is the prime lipoprotein for consideration because it pays main role in atherosclerosis. Oxidized low-density lipoprotein (ox-LDL) causes endothelial dysfunction. Both simvastatin and lovastatin upregulated eNOS expression and completely prevented its downregulation by ox-LDL \(^10\). A decrease in the catabolism of VLDL triglycerides, chylomicron accumulation in the plasma due to impaired clearance could also be a contributing factor to the increased concentration of triglyceride. Lipolytic products of triglyceride-rich lipoproteins, such as free fatty acids (FFAs), have also been shown to increase the permeability of endothelial monolayers, enhance lipid accumulation in SMCs, promote endothelial production of inflammatory mediators (NF-κB, IL-8, ICAM-1), and alter the extracellular matrix synthesis in cultured SMCs. Studies with both endothelial cells \(^11\) and SMCs \(^12\) have demonstrated that increased fatty acids also increased oxidative stress and can increase NF-κB activation and the expression of NF-κB mediated genes including VCAM-1. Atherosclerotic control animals showed increase in serum triglyceride, total cholesterol, LDL and VLDL while decrease HDL level, compare to normal control rats, indicates abnormalities in lipid metabolism. Atorvastatin inhibits the enzyme 3-hydroxymethyl-glutaryl coenzyme A reductase to reduce blood cholesterol levels effectively with its pleotropic effect on oxidative stress and inflammation and represent the standard for treatment of dyslipidemia and inflammation associated vascular dysfunction. Ezetimibe, the first-in-class representative of the 2-azetidinones, blocks both dietary and biliary cholesterol absorption in the proximal jejunum in hamsters \(^7\). In our study Atorvastatin alone and in the combination with the Ezetimibe shows significant decrease in serum triglyceride (p < 0.001), total cholesterol (p < 0.001), LDL (p < 0.001), VLDL (p < 0.001) and atherosclerotic index VLDL (p < 0.001) while increase in HDL (p < 0.01) level and HDL LDL ratio both in curative and preventive therapy. Effect in preventive therapy is higher than curative therapy. While this type of effect for Ezetimibe we found in preventive therapy (p < 0.01). No significant hypolipidemic effect was found in the case of valproate treated animal (fig B-H). It is reasonably to conclude that, GSK-3 inhibitors has limited role as a hypolipidemic agent. So we have to develop newer analogue of statins that has higher GSK-3 inhibitory activity along with lipid lowering activity.

REFERENCES


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