

1. INTRODUCTION

1.1. Atherosclerosis

Atherosclerosis is a disease of medium and large arteries characterized by deposition of lipid-rich plaques onto the inner walls of arteries. This progressive process silently and slowly blocks arteries, setting the blood flow at risk. Atherosclerosis remains asymptomatic for long time period because the arteries broaden at all of the plaque locations, thus causing no consequence on blood flow. Therefore symptoms do not appear until enough narrowing of an artery occurs, due to clots. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. Over a time period, plaque becomes harder and narrows the arteries. Signs and symptoms are observed only after severe contraction of arteries causing lowered blood flow to different organs. This limits the flow of oxygen-rich blood to various body parts and shows symptoms in the form of cardiovascular diseases like heart attack, stroke and peripheral vascular diseases.¹⁴¹

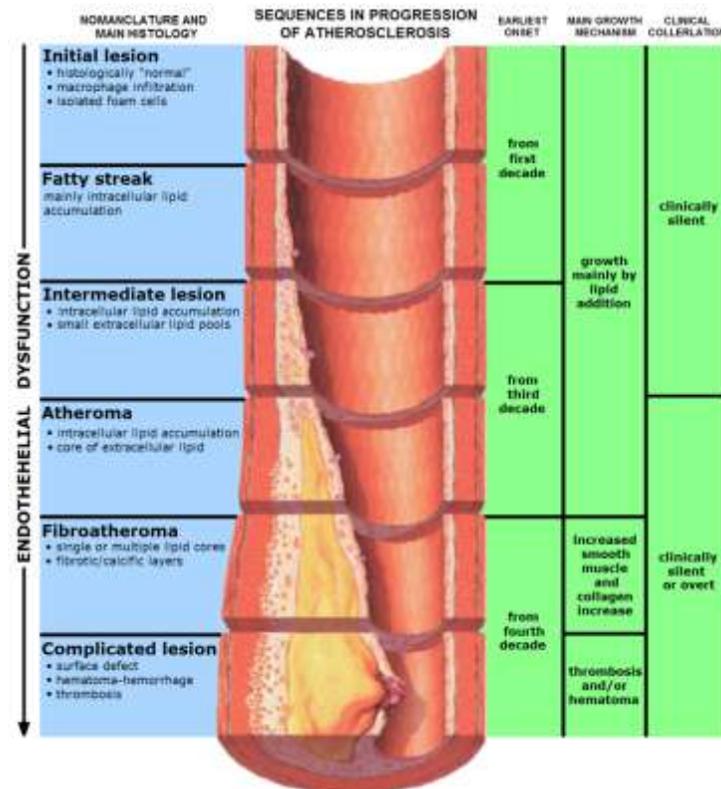


Figure 1: The progression of atherosclerosis.

Atherosclerosis is caused in the endothelial cells by inflammation of the vessel walls in reaction to the retained low-density lipoprotein (LDL) particles. Lipoproteins in the blood differ in size and literature shows that small dense LDL (sdLDL) particles are more eligible to pass through the endothelial cells, and able to go behind the monolayer of endothelium. These LDL particles are more vulnerable to oxidation by free radicals, and this risk is higher when the particles are present in the wall than when they are in the bloodstream. The LDL has a half life of only two to three days, and its content includes 3,000 to 6,000 fat molecules, including: cholesterol, phospholipids, cholesteryl esters, triglycerides etc which change with time.

As LDL particles are more prone to oxidation inside the vessel wall, the endothelial cells respond to it by attracting monocyte white blood cells, which on entering into the arterial walls get transformed into macrophages. The intake of oxidized LDL particles by macrophages activates a sequence of immune responses which produce an atheroma over a period of time. The macrophages and T-lymphocytes take up the oxidized LDL, and form specialized foam cells. If these foam cells fail to deal with the oxidized LDL and lack the recruitment HDL particles to remove the fats, the LDL grows and finally ruptures, leaving behind cellular membrane remnants, oxidized materials, and fats including cholesterol in the artery wall. This further attracts more white blood cells, and thus the cycle continues causing inflammation to the artery. Initially due to the plaque, the muscle cells of the blood vessel get stretched to compensate the additional volume by increasing the separation between the plaque and the lumen. This makes the wall stiffer and less acquiescent to stretching with each heart beat.

Cholesterol levels are affected by different factors such as rate of endogenous cholesterol synthesis, biliary cholesterol excretion and dietary cholesterol absorption.³ To treat this condition, usually the non-pharmaceutical measures such as stopping smoking and practicing regular exercise are obtained primarily. If these methods do not work, medicines are generally the next step.

Different lipid lowering strategies, particularly HMG-CoA reductase inhibitors (statins) or cholesterol synthesis inhibitors have been reported¹⁶ and are presently in therapeutic use. The patients who receive a statin monotherapy, do not achieve the decisive treatment goals.¹⁶ Moreover, augmenting the dose of statins may also cause adverse effects such as augmented concentrations of enzymes in liver, muscle problems and most importantly an increased risk of

diabetes. The other lipid lowering agents include fibrates and bile acid sequestrants. The fibrates belong to a class of amphipathic carboxylic acids that are mainly used in hypercholesterolemia. The major adverse effect of fibrates is increased risk of gallstones as they increase the cholesterol content of bile. However, the bile acid sequestrants may cause problems in the gastrointestinal tract such as constipation, diarrhea, bloating and flatulence.¹⁴²

Towards the development of better therapeutic agents to treat this condition, the research to discover novel lipid lowering agents is continuing. The novel approaches are presently targeting the process of inhibition of absorption of intestinal cholesterol. The plant sterols and stanols,¹⁴³ ACAT inhibitors,¹⁴⁴ microsomal triglyceride transfer protein (MTP) inhibitors,¹⁴⁵ and Niemann-pick C1-L1 ligand inhibitors (NPC1-L1)¹⁴⁶ are compounds that decrease intestinal cholesterol absorption.

The esterification of cholesterol is observed to be a rate limiting step in the intestine for the absorption of cholesterol. Decisive work from different researchers¹⁴⁷⁻¹⁴⁹ eventually led to the conclusion that Acyl CoA: cholesterol O-acyltransferase (ACAT; EC 2.3.1.26)¹⁵⁰ is one of the primary enzymes responsible for the cholesterol esterification in intestinal mucosal cells and it plays very important role in absorption of cholesterol from the intestine.

1.2. Acyl Coenzyme A: Cholesterol O-Acyl Transferase (ACAT)

The acyl-coenzyme A: cholesterol O-acyltransferases (ACAT), also known as sterol O-acyltransferase (SOAT), is a small family of enzymes comprising three homologous members, namely acyl-coenzyme A: cholesterol O-acyltransferase 1 and 2 (ACAT-1 and ACAT-2), and acylcoenzyme A: diacylglycerol acyltransferase 1 (DGAT-1). It is an intracellular protein located in the endoplasmic reticulum that converts cholesterol into cholesteryl esters.



ACAT has generated much interest as a potential means of exploring the atherosclerotic disease process by both lipid and non-lipid mechanisms. The tissue distribution of ACAT-1 and ACAT-2 is quite different. ACAT-1 is found in macrophages, adrenal glands, hepatocytes, enterocytes, renal tubular cells and neurons, whereas ACAT-2 has been observed in the apical region of the intestinal villi and in the hepatocytes. ACAT-1 can accumulate in macrophages and

biological activity data obtained from this laboratory, it was aimed to develop a predictive pharmacophore model along with a 3D-QSAR model for ACAT inhibitors and attempt to identify the enzyme structure to identify the possible active sites in the enzyme for better designing of drugs.