Lipid lowering therapy with PCSK9 inhibitors

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2023-2024

Abstract

Lipids are common and ancient molecules. The lipid chemistry of the primary building blocks varies between the three domains of life on our planet. The atypical assembly, breakdown, or transit of their lipoprotein particles is frequently the cause of abnormal blood levels of triglycerides and cholesterol. The term "hypolipoproteinemia" refers to a low level of plasma lipoproteins, whereas "hypolipoproteinemia" refers to an elevated level. The risk of cardiovascular disease (CVD) can be accurately predicted by measuring the levels of plasma lipids. A proprotein convertase called PCSK9 aids in the liver's breakdown of LDL receptors. In certain cases, mutations in the PCSK9 gene result in familial hypercholesterolemia by decreasing the quantity of LDL receptors present on the hepatocyte surface. This decreases their ability to clear LDL cholesterol from plasma. In this short article, we reviewed the biochemistry of lipids and the new group of lipid lowering agent also known as PCSK9 inhibitors

Introduction

Lipids are ancient and widespread compounds found throughout various domains of life on Earth. They serve essential roles in biological systems, functioning as structural components of cell membranes, energy reservoirs, and signaling molecules. One of the primary distinctions in lipid chemistry among different organisms lies in the types of linkages and backbone structures present in their lipid molecules. For instance, while ester linkages are common in the lipids of bacteria, archaea, and eukaryotes, certain archaeal lipids feature ether linkages. Moreover, variations in the stereochemistry of glycerol, such as L-glycerol versus D-glycerol, can further differentiate lipids among organisms. Viruses, though not living entities themselves, often possess lipid envelopes derived from the host cell membrane during the viral assembly and budding process. These lipid envelopes are vital for viral infectivity, as they facilitate interactions with host cell membranes and promote fusion between viral and cellular membranes during entry (1).

Two primary biosynthetic routes are responsible for producing lipids. In the first pathway, a carbanion intermediate combines with acyl carrier protein intermediates derived from acetyl- and malonyl-CoA esters. This process leads to the formation of various lipids containing fatty acyl chains, including phospholipids, glycerolipids, and fatty acids. In plants, a similar mechanism is found in the polyketide biosynthesis pathway. In the second biosynthetic pathway, a carbocation intermediate reacts with branched-chain five-carbon pyrophosphate intermediates. This pathway results in the synthesis of specific lipid compounds. (2).

Lipids have evolved from these metabolic pathways to fulfill a diverse array of biological functions crucial for living organisms. While the exact count of lipid molecular species in nature remains undetermined, estimates suggest that their number could be in the thousands or even higher. This estimation considers various factors such as chirality, the positions of double bonds, the attachments of different head groups, as well as the incorporation of carbohydrates, amino acids, and other potential sources of chemical diversity (3).

The cell membrane is composed of lipids, which are integral to its structure and function. Typically, these lipids consist of a hydrophobic fatty acid tail, a phosphate group, and a glycerol backbone, with the glycerol portion being hydrophilic. As a result, phospholipids are considered amphipathic. Within the cell membrane, phospholipids are organized into a bilayer arrangement. This bilayer serves to protect the cell and acts as a barrier against specific chemicals. In this bilayer structure, the hydrophobic tails face inward, away from the surrounding aqueous environment, while the hydrophilic heads face outward, interacting with the surrounding water molecules. This arrangement allows the cell membrane to selectively control the passage of molecules into and out of the cell. For example, small nonpolar molecules and tiny polar molecules, such as oxygen and water, can easily diffuse across the lipid bilayer. However, larger or polar molecules, such as glucose, require assistance to traverse the lipid bilayer. Transport proteins embedded within the cell membrane facilitate the movement of these molecules across the membrane, allowing for selective transport based on the needs of the cell. Overall, the organization of phospholipids in the cell membrane plays a crucial role in regulating the passage of molecules and maintaining the integrity of the cell. (4).

Wax represents another category of lipids. These are esters composed of a fatty acid and a long-chain alcohol. Waxes serve protective functions, particularly

evident in plants where leaves are often coated with wax to prevent water loss and protect against environmental stresses. In humans, cerumen, commonly known as earwax, plays a similar protective role by shielding the skin of the ear canal. Steroids comprise another class of lipids characterized by their structure of four fused rings. Among them, cholesterol stands out as a significant type. Produced primarily in the liver, cholesterol serves as a precursor to various steroid hormones, including cortisol, estrogen, and testosterone. Furthermore, cholesterol is present in cell membranes, where it intercalates within the lipid bilayer, influencing membrane fluidity and integrity. (5).

Biochemistry

When lipids are transported in the plasma, the contrast between their affinity for fats and aversion to water becomes apparent. Triglycerides and cholesterol, being nonpolar lipid molecules, require assistance to move through the polar environment of the plasma. Lipoprotein particles serve as the key facilitators for the hydrophobic transport of lipids in water. The structure of lipoproteins comprises triglycerides, cholesterol, phospholipids, and apolipoproteins. Apolipoproteins primarily function as carrier proteins, aiding in the transport of lipids, but they also play roles in facilitating the exchange of lipid components among lipoproteins and act as cofactors for enzymes involved in lipid metabolism. Several types of lipoproteins are involved in the transport of lipids, each serving distinct roles at different stages of lipid transport. These include chylomicrons, low-density lipoproteins (LDL), high-density lipoproteins (HDL), intermediate-density lipoproteins (IDL), and very-low-density lipoproteins (VLDL). Each lipoprotein type is specialized for specific functions in the lipid transport process. (6).

Chylomicrons are large, triglyceride-rich particles that are synthesized in the endoplasmic reticulum of enterocytes in the small intestine. They play a crucial role

in transporting dietary cholesterol and triglycerides from the intestines to the liver and other peripheral tissues throughout the body. The assembly process of chylomicrons relies on an apolipoprotein known as Apo B-48. This protein is essential for the formation and secretion of chylomicrons from enterocytes into the lymphatic system and subsequently into the bloodstream. The presence of Apo B-48 is necessary for the efficient absorption of dietary fats and fat-soluble vitamins, as it facilitates the packaging of triglycerides and cholesterol into chylomicron particles. These chylomicrons then serve as vehicles for the transport of lipids from the intestine to various tissues where they are utilized for energy or storage purposes. (7).

Triglyceride-rich lipoproteins, known as VLDLs (very-low-density lipoproteins), are primarily synthesized in the liver. The synthesis of VLDLs heavily relies on a protein called Apo B-100. These VLDL particles transport triglycerides synthesized in the liver to various tissues throughout the body, including muscle and adipose tissue. When triglycerides are extracted from VLDL particles by muscle and adipose tissue, they form intermediate-density lipoprotein (IDL) particles, which are rich in cholesterol. IDL particles can further combine with VLDL particles to generate LDL (low-density lipoprotein) particles, which are particularly high in cholesterol. LDL particles are often referred to as "bad cholesterol" because they contribute to the buildup of cholesterol in the arteries, increasing the risk of cardiovascular diseases. Apo B-100 plays a crucial role in the metabolism of LDL particles by serving as a ligand for the LDL receptor-mediated absorption of LDL particles by the liver and other organs. In contrast, high-density lipoprotein (HDL) particles are rich in phospholipids and cholesterol. HDL particles help transfer cholesterol from peripheral tissues back to the liver, where it can be eliminated from the body. HDL cholesterol is often referred to as "good cholesterol" due to its role in

cholesterol clearance and its association with reduced risk of cardiovascular diseases. (8).

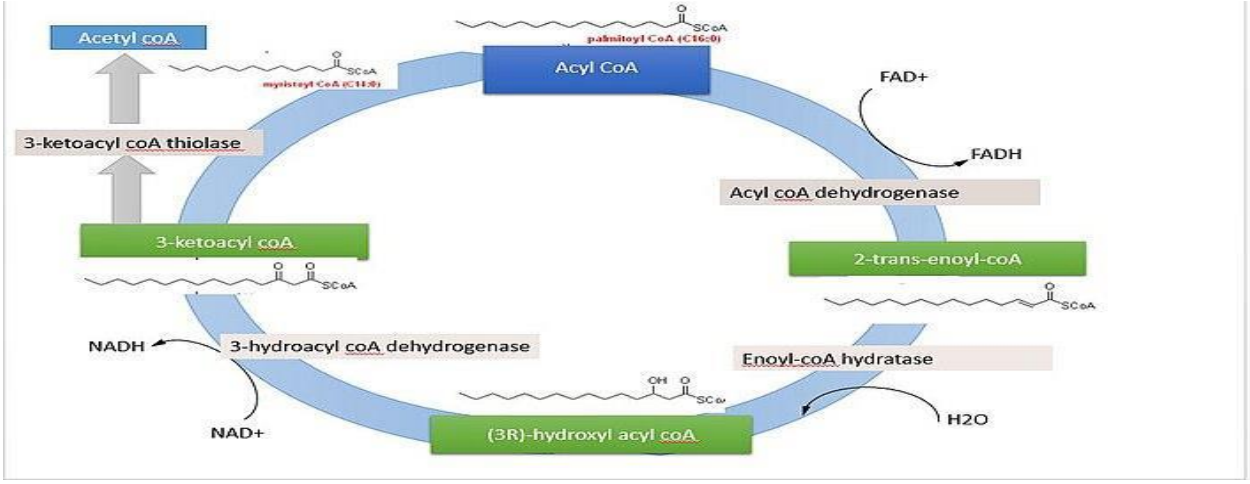


Figure 1. metabolism of lipids

To sum up, there are two ways that plasma lipids are transported. One is an external route that facilitates the movement of cholesterol and triglycerides from the small intestine through food. Triglycerides are broken down in the small intestine by bile acids including cholic acid and enzymes. First, the duodenum releases the hormone cholecystokinin (CCK) in response to the early digestion products like free fatty acids. In addition to inducing the pancreas to release pancreatic digesting enzymes into the gut, CCK activity also promotes the gallbladder's emptying, which results in the release of bile into the small intestine. Bile acids have a detergent effect that helps emulsify fats so that the increased surface area makes it simpler for water-soluble digestive enzymes to hydrolyze them. With the aid of mixed micelles produced throughout the process, one significant enzyme, pancreatic lipase, breaks down triglycerides to form free fatty acids and monoacylglycerol, which are absorbed by the intestinal mucosal cells. (9).

Fatty acids with 12 or fewer carbons are absorbed into the bloodstream directly from the gut mucosal villi and utilized for energy in the liver. Longer-chain

fatty acids are re-esterified in the intestinal mucosa to form triglycerides, which are then transported via chylomicrons to tissues for energy production or storage. Chylomicrons interact with lipoprotein lipase, releasing free fatty acids for cellular use. Remnants of triglyceride breakdown are taken up by the liver, where cholesterol is released for various metabolic processes. (10).

The alternative route involves the endogenous system, which is how triglycerides and cholesterol leave the liver and other non-intestinal tissues and enter the bloodstream. Triglycerides are made by the liver from free fatty acids and carbs. The VLDL core then releases these triglycerides into plasma. In tissue capillaries, the VLDL particles engage with lipoprotein lipase, resulting in the breakdown of the triglyceride core and the release of free fatty acids. A portion of the leftover particles bind to the hepatic cells after being extracted from the plasma. The remaining leftover particles, on the other hand, change into LDL particles, which subsequently supply cholesterol to tissues like the kidneys, skeletal muscle, adrenal glands, gonads, and lymphocytes that contain LDL receptors.Fat can also be broken down for energy as needed, in addition to the previously listed uses. For the release of fatty acids, adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL) are activated by either the release of glucosegon (when fasting) or adrenaline (during exercise) (11).

Clinical importance of lipids

The atypical assembly, breakdown, or transit of their lipoprotein particles is frequently the cause of abnormal blood levels of triglycerides and cholesterol. The term "hypolipoproteinemia" refers to a low level of plasma lipoproteins, whereas "hypolipoproteinemia" refers to an elevated level. The risk of cardiovascular disease (CVD) can be accurately predicted by measuring the levels of plasma lipids. As an illustration, hyperlipoproteinemia is linked to an increased risk of ischemic vascular

disease, atherosclerotic cardiovascular disease, and the formation of fatty deposits under the skin called xanthomas and xanthelasmas. An elevated risk of coronary heart disease and elevated plasma triglycerides are associated with elevated plasma concentrations of LDL and total cholesterol. A higher incidence of atherosclerotic heart disease is correlated with elevated levels of VLDL. Nonetheless, because high levels of HDL cholesterol can prevent the body from accumulating too much cholesterol, they may offer protection against atherosclerotic heart disease (12).

Medication treatments exist that aid in lowering plasma levels of lipids. Nonetheless, controlling dietary intake and lifestyle modifications is crucial, either prior to or in addition to initiating medication. A lower-calorie diet, exercise, and, if a smoker, giving up may be some of these adjustments. Statins, fibrates, bile acid sequestrants, omega-3 fatty acids, cholesterol-absorption inhibitors, and PCSK 9 inhibitors are common treatment alternatives (13).

Statins are the option that is most frequently administered among these. Through competitive inhibition of HMG-CoA reductase, the rate-limiting enzyme for cholesterol formation, they can reduce the manufacturing of cholesterol, mainly in the liver. Statins help absorb and break down low-density lipoproteins (LDL). They have decreased the death rates of coronary patients and advanced both primary and secondary prevention of coronary heart disease (14).

Recently, a new groups of drugs were introduced with a very promising future and decreasing both the levels of plasma lipids and it outrageous morbidity and they are known as **proprotein convertase subtilisin/kexin type 9** inhibitors (PCSK9 inhibitors) which they are the principle objective in our short review.

Background

The identification of certain families displaying the clinical phenotype of autosomal dominant hypercholesterolemia (ADH), also known as familial hypercholesterolemia (FH), played a crucial role in uncovering the genetic basis of PCSK9 inhibition. ADH/FH is characterized by elevated levels of plasma LDL cholesterol (LDL-C), premature development of atherosclerosis, and related complications. Significantly, individuals with this condition did not exhibit mutations in genes encoding the LDL receptor or apolipoprotein B (APOB), which are traditionally associated with familial hypercholesterolemia. Instead, researchers observed a distinct genetic locus on chromosome 1 that appeared to be associated with the disorder. Ultimately, this research led to the discovery of mutations in the PCSK9 gene as a cause of autosomal dominant hypercholesterolemia. PCSK9 encodes a protein involved in the regulation of LDL receptor levels on the surface of cells. Mutations in PCSK9 can result in impaired LDL receptor function, leading to elevated LDL-C levels and increased risk of cardiovascular disease. Inhibiting PCSK9 activity has since emerged as a promising therapeutic approach for lowering LDL-C levels and reducing cardiovascular risk in individuals with FH and other forms of hypercholesterolemia. (15).

Abifadel and associates subsequently demonstrated that autosomal dominant hypercholesterolemia was caused by mutations in the PCSK9 gene (16). Research eventually revealed that PCSK9 is essential to the LDL receptor 3's life cycle. Hepatocyte surface clathrin-coated pits are home to a concentration of LDL receptors. An endocytic vesicle is formed when the pit pinches off from the cell surface after attaching to LDL. The LDL receptor can change conformation in the endosome, releasing the LDL particle. The receptor is then recycled back to the hepatocyte surface, where it can continue to pull LDL from the bloodstream.

Notably, LDL receptors have a 100-or so recirculation capacity. The LDL particle is transported to the lysosome for degradation in the interim. Hepatocytes release PCSK9, which attaches itself to the LDL receptor's epidermal growth factor-like repeat A (EGF-A) domain. Following internalization of the PCSK9–LDL receptor complex, PCSK9 blocks the conformational shift in the LDL receptor. The LDL receptor then proceeds to the lysosome with the LDL, where it is subsequently eliminated (17).

According to sequencing research, some families exhibiting the symptoms of autosomal dominant hypercholesterolemia had mutations in PCSK9 that caused a gain-of-function, which increased the degradation of LDL receptors and decreased the amount of LDL-C that the liver removed from the bloodstream. On the other hand, researchers hypothesized that people with loss-of-function variations in PCSK9 should have increased LDL receptor recirculation back to the hepatocyte surface and increased elimination of LDL-C from the bloodstream, protecting them against atherosclerosis (18).

Cohen and associates found that those with nonsense mutations in PCSK9 had circulating LDL-C levels that were about 40% lower than those of people without the mutations (19).

PCSK9 Mechanism of action

Familial hypercholesterolemia stems from mutations in the PCSK9 gene, situated on chromosome 1. Numerous studies have confirmed this, underscoring the urgency to develop methods for PCSK9 inhibition. The liver serves as the primary site of expression for the enzyme encoded by the PCSK9 gene. (20).

The proprotein convertase PCSK9 plays a critical role in the liver's breakdown of LDL receptors. In some patients, mutations in the PCSK9 gene lead to familial

hypercholesterolemia, reducing the number of LDL receptors on hepatocyte surfaces. Consequently, their ability to clear LDL cholesterol from the plasma diminishes. Conversely, certain PCSK9 mutations result in unusually low plasma LDL-cholesterol levels and reduced risk of atherosclerosis. Monoclonal antibodies designed to inhibit PCSK9 activity can prevent LDL receptor degradation and improve LDL-cholesterol clearance. Administering a specific PCSK9 antibody via injection can lower LDL cholesterol levels for several weeks. (21)

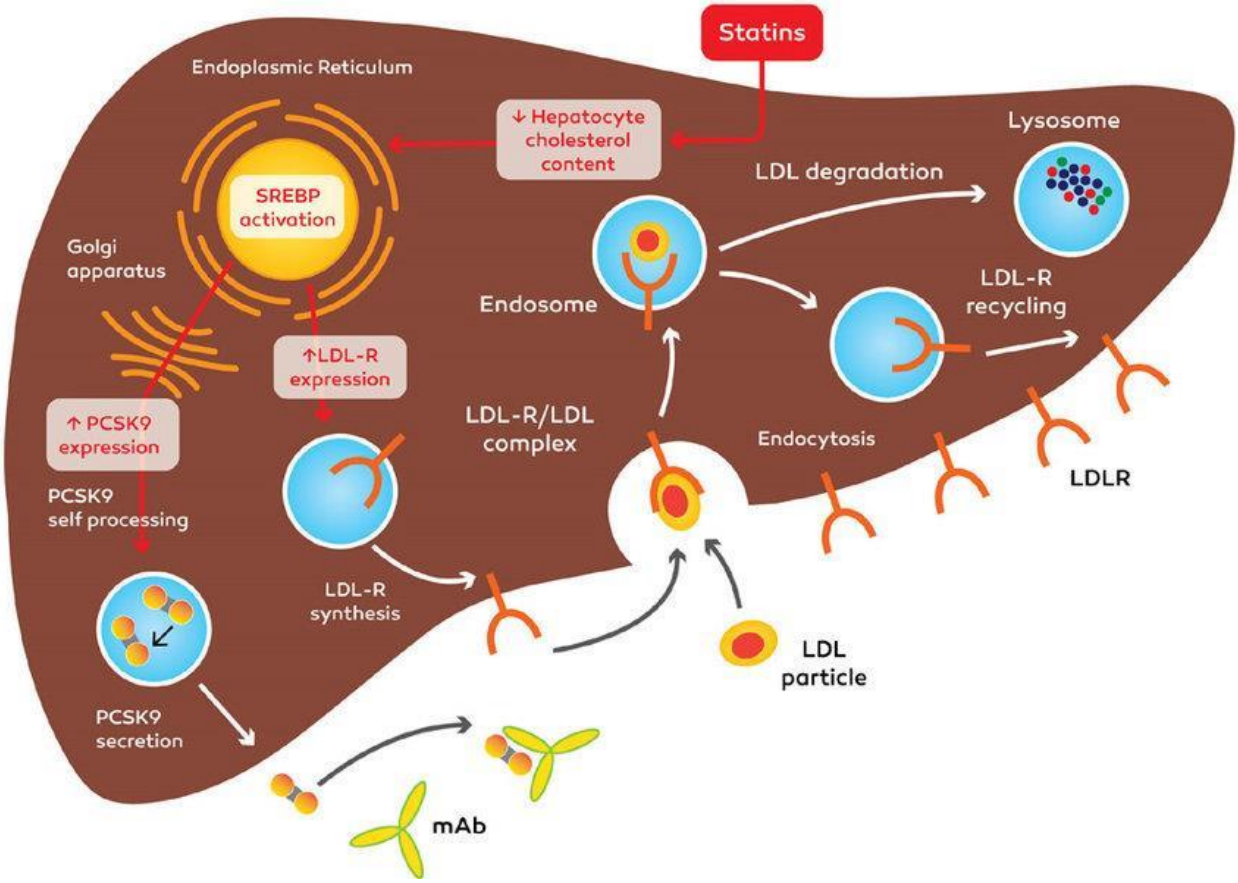


Figure 2. PCSK9 Mechanism of action

Lipid lowering therapy with PCSK9 inhibitors

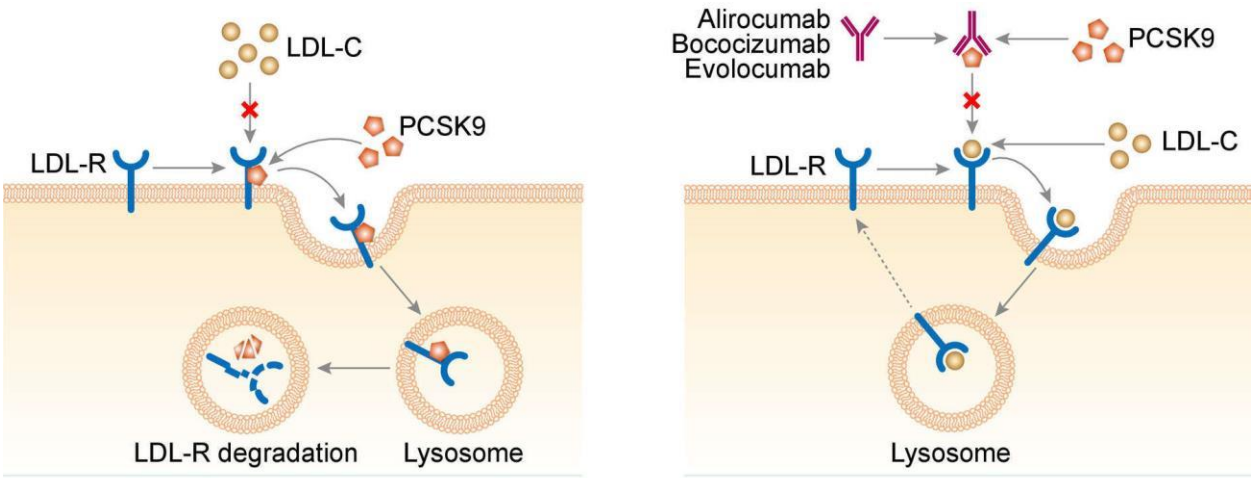
A new class of drugs known as PCSK9 inhibitors effectively lower low-density lipoprotein (LDL) cholesterol, often referred to as "bad" cholesterol. Their

introduction has the potential to revolutionize the management of atherosclerotic risk. Elocumab (Repatha) and Alirocumab (Praluent) are two FDA-approved PCSK9 inhibitors. Studies have shown that PCSK9 inhibitors can have a profound impact and, in certain cases, may even prevent heart attacks and strokes. These drugs can be used alone or in combination with statins, another class of cholesterol-lowering medications. However, it's important to note that PCSK9 inhibitors come at a higher cost compared to other cholesterol medications. Current guidelines and consensus statements recommend PCSK9 inhibitors as suitable second- or third-line treatments, or as an alternative therapy for patients with established atherosclerotic cardiovascular disease (CVD) or familial hypercholesterolemia who have persistent high cholesterol levels or are intolerant to statins. (22).

Of the several monoclonal antibodies developed to target PCSK9, two—evolocumab and alirocumab—have received approval for clinical use. Both are fully human monoclonal antibodies administered subcutaneously. Evolocumab can be administered at a dose of 140 mg every two weeks or 420 mg once monthly. Clinical studies have shown that both dosing regimens produce similar reductions in plasma LDL-C levels. In phase III clinical trials, evolocumab demonstrated a remarkable reduction in plasma LDL-C levels of over 60%. Importantly, the decline in plasma LDL-C values was consistent across all clinical subgroups studied. Additionally, evolocumab exhibited consistent efficacy in reducing LDL-C levels regardless of the baseline plasma PCSK9 levels. This finding indicates that the effectiveness of evolocumab is not influenced by the initial levels of PCSK9 in the bloodstream.. (23).

Phase III clinical trials have employed a range of alirocumab dosage regimens. One regimen involved titrating the dosage of alirocumab based on the decreases in plasma LDL-C levels that were attained. Patients would begin on 75 mg of the drug

every two weeks, and if the LDL-C level was ≥70 mg/dl, the dose would be increased to 150 mg every two weeks. Regardless of whether this treatment was administered as monotherapy, in conjunction with statin therapy, or to patients who were intolerant of statins, it generally produced 45–50% reductions in LDL-C levels. In these trials, the highest dose of alirocumab (150 mg every two weeks) decreased plasma LDL-C levels by almost 60% (24).



Physiology of PCSK9

Mechanism of action of anti-PCSK9 mAb

Abbreviations
PCSK9: proprotein convertase subtilisin kexin type 9
LDL-C: low-density lipoprotein cholesterol
LDL-R: low-density lipoprotein receptor
mAb: monoclonal antibody

Figure 3. pharmacokinetics of Evolocumab and Alirocumab

Adverse effects of PCSK9 inhibitors

There were no discernible variations in the incidence of side events between the treatment and placebo groups in the phase II clinical studies including evolocumab or alirocumab. There were no serious or potentially fatal side effects from the active ingredient in any of the patients who received these medications. Injection-site reactions, characterized by pain or localized rash, were the most frequent adverse events reported in the active-treatment groups (2–9%); upper

respiratory tract infections, nasopharyngitis (4–15%), and moderate gastrointestinal problems, including diarrhea (4%) and nausea (4-6%), were also common. In all trials, there were no antibodies against evolocumab found at the conclusion of treatment. Anti-histamines were able to treat an allergic reaction that was documented in one instance involving alirocumab (25).

Systemic effects

Concerns have been raised regarding potential adverse effects on LDL metabolism and PCSK9 due to the presence of PCSK9 in organs beyond the liver, including the neurological system, pancreas, and intestines. Studies conducted on Pcsk9 knockout (Pcsk9–/–) mice have suggested that PCSK9 inhibition could lead to several negative outcomes. One concern is the possibility of increased visceral obesity, as well as impaired glucose tolerance. Additionally, there are concerns about increased susceptibility to hepatic viruses. While these findings from animal studies are noteworthy, it's essential to interpret them cautiously and recognize that findings in mice may not directly translate to humans. Further research, particularly clinical studies in humans, is needed to fully understand the potential risks associated with PCSK9 inhibition and its effects on various organ systems beyond the liver. (26).

Conclusions

PCSK9 inhibitors are now proven to be valid additions to the clinicians' armamentarium for the treatment of dyslipidaemia. These medications have no side effects other than injection-site responses, dramatically lower the risk of severe vascular events, and lower plasma LDL-C levels by about 60%. Individuals with a high burden of ASCVD benefit more from PCSK9 inhibition in terms of relative and/or absolute risk reductions, as they are more likely to experience significant vascular events. Lowering plasma LDL-C to levels of about 20 mg/dl was the

therapeutic benefit observed in PCSK9 inhibition trials, indicating that more aggressive LDL-C targets ought to be used. In the event that PCSK9 siRNA inhibitors' safety and clinical efficacy are confirmed, these treatments may provide a means of treating dyslipidemia more quickly and effectively, as well as a chance to virtually eradicate cardiac disease.