Pathophysiology of Dyslipidemia and Its Management by PCSK9 Inhibitors: A Literature Review

Dyslipidemia is a disease of abnormal lipid levels in the blood that contributes to the atherosclerotic process. This atherogenic process leads to the formation of plaque and also leads to thromboembolic events and other vascular accidents. It is known that high-density lipoprotein cholesterol serves as a protective effect on the vessel wall and causes the reduction in the progression of atherosclerosis. And multiple interventions are directed in maintaining a higher level of the aforementioned lipoprotein cholesterol. While the low-density lipoprotein stays controversial but lowering its levels through various therapeutic agents is the main goal in the management of dyslipidemia. A newer group of drugs, proprotein convertase subtilisin/kexin type 9 inhibitors lowers the levels of low-density lipoprotein through modulating proprotein convertase subtilisin/kexin type 9 gene involved in cholesterol metabolism and affects the levels of the lipoproteins by controlling the receptors. The inhibitors of this gene decrease proprotein convertase subtilisin/kexin type 9-induced low-density lipoprotein receptor degradation in the lysosomes of hepatocytes increasing its recycling and expression on the cell surface, causing increased clearance of low-density lipoprotein from the circulation. These drugs Alirocumab, Evolocumab and along with other agents can be a novel approach in controlling dyslipidemic state. This review revisits the literature in understanding the pathophysiology of dyslipidemia along with its management by proprotein convertase subtilisin/kexin type 9 inhibitors, its mechanism of action, its pharmacokinetics, the results of the clinical trials and the limitations in its application.

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease of the walls of large and medium-sized arteries. It is characterized by destruction of the normal arterial skeleton, involving collagen, elastin and smooth muscles, using enzymes and substituting this with disorderly laid collagen and elastin, along with cholesterol and foam cells [1]. Although a lot of information regarding atherosclerosis has been gained in the last five decades, it remains as one of the leading causes of mortality in both genders and the leading reason for poor quality of life [2]. This atherogenic cholesterol accumulation that damages the vascular anatomy is related to abnormal levels of lipid, often referred as dyslipidemia. Out of other causes of atherosclerosis, dyslipidemia stands prominently as a major contributor in the accumulation of fat in the lumens, narrowing the diameter of the vessels, reducing the per unit time blood supply and a major source of thromboembolic events. Dyslipidemia alone is related to stroke, transient ischemic attacks, ischemic heart disease, pulmonary and systemic embolism. Modulating the triggering elements of dyslipidemia or controlling this disease process can help control many other conditions, serve in comorbid processes and can help in a better quality of life. In past few decades, with sedentary lifestyles, high caloric food intake and commercialized less organic food products, the tendency to develop dyslipidemia have increased many folds and the need for more effective regimens are needed. Statins have been used as a gold standard treatment for three decades now, although with knowing multiple aspects of the disease process, newer and more effectual treatment is needed. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a pro-
tease and is expressed predominantly in the liver that plays a vital role in cholesterol metabolism by regulating the levels of low-density lipoprotein (LDL) receptors. PCSK9-inhibitors inhibit PCSK9 induced low-density lipoprotein receptor degradation, increasing its recycling and expression on the cell surface, causing increased clearance of low-density lipoprotein from the circulation and can therefore prove to be the wonder drug in management of hypercholesterolemia and help prevent atherosclerosis and development of congenital heart defect (CHD).

**Atheroma Plaque Formation**

When high-density lipoprotein (HDL) is not able to take up excess cholesterol from tissue turnover or maybe from dietary cholesterol that is accumulated as lipid layer along the blood vessels and serves in the formation of an atheroma plaque. It will be the role of inflammatory cells to digest the cholesterol not picked up by HDL and when inflammatory cells, particularly the macrophages, take up cholesterol, they cannot metabolize and become foam cells and start releasing cytokines that cause an inflammatory environment of the vascular endothelium. When the intima gets inflamed, the repair would require fibrosis and collagen fibrous plaque would be formed on top of the necrotic core of lipids and cholesterol cleft – this fibrous plaque is called atheroma plaque [3]. Plaque formation is pathognomonic of atherosclerosis. Deposition of cholesterol in lesions in the arterial walls leads to the formation of plaques. This reduces the diameter of the arterial lumen thereby decreasing the volume of blood per time that can pass through [2].

The blood analysis carried out during routine cholesterol level assessment is known as the lipid profile. This lipid profile constitutes the following:

- **Low-density lipoprotein cholesterol (LDL-C)**
- **High-density lipoprotein cholesterol (HDL-C)**
- **Triglycerides (TGs) (blood fats)**

Customarily, during evaluation of LDL-C and triglycerides, values towards the lower end of the normal range are desired. This is due to the fact that these lipids are the main driving force behind the development and advancement of atherosclerosis. Conversely, a higher value of HDL-C, in most cases, is preferred as it offers a protective effect on the vessel walls and opposes the occurrence and progression of atherosclerosis [1, 4]. Studies carried on both men and women, suggests an inverse relationship between HDL-C and the risk of developing atherosclerosis, that is, a higher value of HDL-C means a lower risk for atherosclerosis while a lower value of HDL-C (1 milligram HDL-C per 1 deciliter of blood) indicates a greater risk for atherosclerosis [5]. Measurement of the cholesterol content of HDL-C was made possible due to advances in precipitation of apolipoprotein B (Apob) containing lipoproteins. These measurements were done in a large number of subjects and this enabled grand scale epidemiological studies to determine the relationship between HDL-C and coronary heart disease. The reports that initially compelled the strong inverse association between HDL-C and coronary heart disease lead to Framingham Heart Study [6]. Data interpreted from this study formed the basis for the widely endorsed concept of HDL as the good cholesterol. This led to a conclusion that HDL may possess properties that are protective against coronary heart disease, in turn leading to the conclusion that raising the level of HDL-C through medical intervention would lessen the risk of coronary heart disease (the HDL hypothesis).

**Controversy of HDL-C**

Even though HDL-C has been shown to possess qualities that are protective against atherosclerosis, the association between HDL-C and atherosclerosis has not been clearly defined. Cynicism regarding the HDL hypothesis has arisen due to certain unsuccessful clinical trials and human genetic studies [7]. Despite the fact that there are questions regarding the HDL hypothesis, the ability of HDL to predict cardiovascular events remains incontrovertible. Affirmation about HDL-C being a strong, consistent and independent predictor of cardiovascular incidences (myocardial infarction, ischemic stroke) has been displayed in multiple studies including various races and ethnicities all over the world [8,9]. Compelling information has also been gathered regarding the role of HDL-C in predicting the risk of cardiovascular events for patients with known history of cardiac diseases, for the purpose of secondary prevention. The predictive property of HDL-C for cardiovascular events in patients undergoing treatment was accurate in a few clinical trials while in some others it was inaccurate [10, 11]. Also, the randomized controlled trials and human genetic studies have provided much of the information that disputes the HDL hypothesis. Research about Mendelian disorders has contributed much to this matter. The three Mendelian disorders causing primary extreme low HDL-C include mutations in apoA1, ABCA1, and LCAT [12]. Although HDL-C levels are lower than the 5th percentile in all of these disorders, none of them are explicitly related to the development of premature coronary heart disease [12].

Furthermore, it has been proved that along with the part that LDL-C plays in raising the rate of cardiovascular events and, the supplementation of diet with statin therapy, exercise, and stoppage of smoking are extremely efficacious in lowering the incidence and prevalence of hyperlipidemia. Although there is a presence of dependable genetic, biological and epidemiological data as well as information from randomized trials, LDL-C remains a topic of controversy between national guidelines and clinical practice. Points of contention include measurement of LDL levels, benefits of population population-based screening, the net benefit to risk ratio for different drugs that decrease LDL, the usefulness of treatment targets and the safeness of vigorously lowering LDL [13].

Nonetheless, to this day, there have been various different drugs that are used for the treatment of dyslipidemia that acts by lowering LDL-C and triglycerides.

**Novel Treatment of Dyslipidemia**

The safety and efficacy of statins have been proven in a large number of randomized clinical trials, therefore becom-
ing the drug of choice in treating atherogenic dyslipidemia. Nevertheless, even in the presence of optimum treatment with statins, there still remains a 60% to 80% risk of a cardiovascular event [14]. In recent times, there has been a development of a new class of lipid-lowering drugs with some of them being made ready for use in clinical practice. The proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor acts by increasing the production of low-density lipoprotein (LDL) receptors in hepatocytes. It achieves this by augmenting LDL receptor recycling [15]. The microsomal triglyceride transport protein (MTP) inhibitor and antisense oligonucleotide against apolipoprotein B (Apo-B) act by decreasing the Apo-B containing lipoprotein. This is accomplished by obstructing the hepatic very low density lipoprotein synthesis pathway [16,17]. The apolipoprotein A1 (ApoA1) mimetics act by mimicking the effect of apolipoprotein A1. Their effects are similar to the protective effect of HDL-C and can even overturn the progress of atherosclerosis [18]. However, some of the data gathered from clinical trials with Apo-A1 mimetics is contentious and further testing is required for their acceptability in humans [13]. Encouraging results were shown by the PCSK9 inhibitors in recent studies, including a critical decrease in LDL-C in familial hypercholesterolemia patients from the long term phase III trials.

History and Discovery of PCSK9
In the beginning, PCSK9 was classified as a new addition to the proprotein convertase family and was thought to be involved in some way in the differentiation of cortical neurons and in the process of hepatic regeneration [19]. The PCSK9 (proprotein convertase subtilisin/kexin type 9 [MIM 607786]) gene is responsible for encoding a cholesterol-regulated proprotein convertase. Selected missense mutations in PCSK9 were described in 2003, which gave rise to a new type of autosomal dominant hypercholesterolemia (MIM 603776) [20]. This revelation brought to light a hitherto unknown process that has a major impact on the circulating level of low-density lipoprotein cholesterol (LDL-C). The role of cholesterol in the regulation of PCSK9 was also noted [20].

What is PCSK9 gene? Function and Physiology
PCSK9 became the 9th substance to be classified as a part of the subtilisin family of kexin-like proconvertases [21]. The signal sequence (amino acids 1-30) in PCSK9, in a similar fashion to the other members of the group, precedes the prodomain (amino acid 31-152) and the catalytic domain [19]. PCSK9 is classified as a 72-kd kD protease and is expressed predominantly in the liver. Structural properties of PCSK9 include three recognizable domains, a catalytic domain, an N-terminal prodomain and a carboxyl-terminal domain whose function is as yet unknown. Similarities between the carboxyl-terminal domain and resistin, which is an inflammatory cytokine that is correlated with insulin resistance, were discovered on performing crystallographic studies [22]. PCSK9 plays a crucial part in cholesterol metabolism, which has led scientists in both academic and industrial circles to be highly optimistic regarding its role in the treatment of hypercholesterolemia. Its mechanism of action includes regulation of LDL receptor levels [23]. These receptors are plasma membrane glycoproteins that cause the removal of cholesterol-rich LDL particles from the plasma [24,25]. Reduction in the level of LDL receptors occurs due to gain-of-function mutations, leading to increased levels of LDL-C in plasma and increased risk of development of cardiovascular disease. Conversely, loss-of-function mutations cause an increase in the number of LDL receptors, therefore decreasing the level of LDL-C and providing a protective effect against coronary heart disease [26,27].

Role of PCSK9 in Controlling Cholesterol
The process by which LDL receptor levels are decreased by PCSK9 has perplexed scientists for a long time, but progress in understanding its mechanism of action has been made due to studies conducted in recent years. PCSK9 adheres to the LDL receptors which are present on the surface of cells. The elimination of LDL receptors involves internalization of this LDL receptor-PCSK9 complex. Within the cell, when this complex enters the endosome, the acidic environment causes an increase of about 150-fold in the affinity of PSCK9 for the LDL receptor [28,29]. The region which PCSK9 binds to on the LDL receptor is the epidermal growth factor (EGF)-like repeat A [28]. This region has been established to play a pivotal role in the recycling of the LDL receptor to the cell surface from the endosomes [30]. The binding site of PCSK9 on the LDL receptor is some distance away from its catalytic site, as disclosed by studies involving a crystal structure of PCSK9 bound to the EGF-like repeat A [31]. Consequently, this binding of PCSK9 to the LDL receptor leads to a shift in the location of the LDL receptors from the cell surface to lysosomes [32]. In this manner, PCSK9 seems to divert the route of internalized LDL receptors, leading them to the lysosomes for destruction instead of the usual pathway of recycling to the cell surface [28,29,32]. (Figure 1)

PCSK9 Inhibitors: A new Regulator of Cholesterol Trafficking
Praluent (Alirocumab) injection is a new kind of drug that was approved for use by the U.S. Food and Drug Administration on 24th July 2015. This drug became the first of its kind to be approved for the treatment of hyperlipidemia and belongs to a family of drugs known as proprotein convertase subtilisin/kexin type 9 inhibitors [33].

Mechanism of Action
PCSK9 is classified as a serine protease and performs a critical part in the regulation of cholesterol metabolism in the liver. It achieves this by causing an increase in the destruction of LDL receptors. Surface expression of LDL receptors can be raised by inhibiting the process by using PCSK9 inhibitors which in turn decreases the degradation
Figure 1. Recycling of LDLR during the metabolism of LDL-C, in the absence (left) and in the presence (right) of PCSK9. Right side shows LDL-C particle attachment to the LDLR and subsequent endocytosis via clathrin-coated vesicle. LDL-C is then degraded in the endosome while LDLR is recycled back on the surface of hepatocyte. Left side shows binding of PCSK9 to the LDL-C-LDLR complex with subsequent internalization, however the presence of PCSK9 on LDLR directs this vesicle to the lysosomes where the LDLRs are degraded in an acidic environment. (LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9.)

Figure 2. Effect of PCSK9 inhibitors on LDL-C metabolism. Under physiologic conditions, LDLRs and PCSK9 are synthesized in hepatocytes and are secreted into the circulation. Here LDLRs binds LDL-C and is also attached by PCSK9. This complex is endocytosed and directed to lysosome by PCSK9 for destruction. Fewer LDLRs are recycled to the surface of hepatocytes to remove LDL-C. PCSK9 inhibitors, however, prevent this destruction of LDLRs in lysosome and more LDLRs are recycled back to remove LDL-C from the circulation. (LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9)
of LDL receptors (LDLRs). This leads to an increase in the recycling of LDLRs which subsequently causes a reduction in the levels of LDL-C (Figure 2). Various methods have been suggested for the inhibition of PCSK9 so far. Some of these include small interfering RNA, monoclonal antibodies, mimetic peptides and antisense oligonucleotides. Out of all of these, the most promising data on humans has indubitably been displayed by the fully humanized monoclonal antibody against PCSK9 [34].

Kinetics of PCSK9 Inhibitors

Alirocumab

Maximum serum concentration is achieved in 3-7 days and the serum concentration-time profiles for different injection sites, including the abdomen, upper arm and thigh are almost alike. On average, about 3-4 doses are required to achieve steady state concentration. When administered intravenously, the volume of distribution is 0.04 to 0.05 L/Kg. When given at a dose of 75mg to 150mg every fortnight, the median half-life (t1/2) was revealed to be between 17 to 20 days. Removal of Alirocumab occurs in two phases, based on its concentration in plasma. When the concentration in plasma is low, the main route by which Alirocumab is eliminated is by saturation of the targets (PCSK9) bound to the antibodies. On the other hand, when plasma concentration is high, the proteolytic pathways are the predominant mode of elimination [35].

Evolocumab

Evolocumab exhibits non-linear pharmacokinetic properties in absorption when the dosage is less than 140mg. Conversely, when the dosage is between 140mg to 420mg, it is noted that the pharmacokinetics become linear. Following administration of one dose, 3-4 days are required to achieve maximum concentration. Administration of a solitary dose of 420mg results in approximately 3.3 L ± 0.5 L of the volume of distribution. Dosing for nearly 12 weeks is needed to obtain a steady state in serum. Evolocumab has a half life (t1/2) somewhere between 11 days to 17 days. A Dosage dosage of 140mg once a fortnight and dosage of 420mg once monthly produced nearly identical results in maximal lowering LDL cholesterol levels and took about 14 days to take effect. Mild and moderate dysfunction of liver or kidneys does not produce any variation in the pharmacokinetics of Evolocumab according to data collected during clinical studies. On the other hand, no data is available regarding the effect on pharmacokinetics in individuals with severe hepatic and renal impairment [36].

Clinical Trials

In contrast to treatment with statins alone, the addition of monoclonal antibodies that inhibit PCSK9 along with statin therapy brings a 50%-60% further lowering of LDL-C, as shown by phase I, II, and III trials. Data collected from short-term trials show that PCSK9 inhibitors had a very low occurrence of harmful effects and were tolerated reasonably well. Data is being gathered by progressive phase III trials which will tell whether the drug is safe to use over a long period of time and its effectiveness in averting cardiovascular disease [37].

In comparison to a placebo, Alirocumab, and Evolocumab, which are both administered by injection, cause a massive decrease in the level of LDL-C (39 to 62% decrease for Alirocumab and 47 to 56% for Evolocumab). Level of LDL-C decreased to less than 25mg per deciliter on two successive readings in about 37% of subjects being treated with Evolocumab and about 24% of subjects being treated with Alirocumab during programs for developing the drugs [38]. During studies conducted on animals, it was found that inhibition of PCSK9 in zebrafish embryos leads to disorganized neuronal growth and can be fatal [39]. However, lack of PCSK9 in mice does not cause any neurological problems and their growth occurs normally [40]. In humans, individuals who are heterozygous for loss-of-function mutations in PCSK9 appear to have no health issues and have similar life spans as individuals with no mutations [26,41]. A study was conducted on PCSK9 involving 18 subjects, 10 females and 8 males, and was divided into two time periods along with being placebo-controlled. The subjects were given two doses of placebo at a gap of two weeks after which they were administered 5 doses of 150mg of Alirocumab at a gap of two weeks each. Effect of Alirocumab was to decrease LDL-C, separated by ultracentrifugation, by 55.1%. LDL-ApoB was decreased by 56.3% and reduction in plasma Lp lipoprotein (a) was 18.7%. An 80.4% rise in LDL-ApoB FCR (Fractional catabolic rate) along with a 23.9% decrease in LDL-ApoB PR (Production rate) appears to be the main cause of the reduction of LDL-ApoB. The LDL-ApoB is related to a 46.1% rise in LDL-ApoB FCR linked with a 27.2% reduction in the formation of LDL from IDL. There was no variation in apo(a) PR ApoA1 PR while the FCR of apo(a) ApoA1 was prone to increase. No effect of Alirocumab was observed on FCRs or PRs of VLDL-ApoB and VLDL-TG (Triglyceride) as well as on plasma TG after meals or ApoB48 levels [42].

A trial conducted to investigate the effectiveness of PCSK9 inhibitors, known as the Therapy for Easing Lipid Levels (MENDEL) trial, included 406 subjects with hypercholesterolemia and intolerance to statin therapy. The subjects were randomly divided into groups and each group was allocated a treatment regimen. These included a fortnightly dose of 70mg, 105mg and 140 mg of Evolocumab; a monthly dose of 280mg, 350mg, and 420mg of Evolocumab; fortnightly or monthly dose of placebo or a single daily dose of Ezetimibe. LDL-C levels were decreased in all groups with Evolocumab and the maximum effect was shown by the dosage of 140mg every fortnight (~51 %) without any harmful side effects being observed [43]. Massive improvement in LDL-C level by Alirocumab and Evolocumab were reported in one review. The results were more powerful for Alirocumab, as compared to Evolocumab, in subjects at high risk of a cardiovascular event who had poor LDL-C levels. Conversely, in subjects with heterogeneous familial hypercholesterolemia and subjects with varied risk of a cardiovascular event with deranged LDL-C values, Evolocum-
Clinical uses of PCSK9 Inhibitors

One of the chief advantages of PCSK9 inhibitors is its usage in therapy for familial hypercholesterolemia in people overly sensitive to statins and also in people whose low-density lipoprotein-cholesterol LDL-C levels remain high even after they have been put on the maximum dose of statins that can be safely prescribed. Interestingly, combination therapy of low dose statins with PCSK9 inhibitors is more efficacious for decreasing LDL-C levels and preventing adverse effects of statins. This is due to the fact that combination of PCSK9 inhibitors with both low dose and high dose statins have shown effects nearly identical to each other [46].

Potential Barriers

Large-scale use of PCSK9 inhibitors may be prevented by the presence of various probable hurdles. First of all, the efficacy of statins in treating hypercholesterolemia has been confirmed by the results of several long-term studies, which makes statins a better option over the PCSK9 inhibitors, specifically being conducted to gather data that can be used to illuminate the clinical advantages of these drugs as well as potential adverse effects. The possible advantages from the remarkable decrease in LDL-C levels achieved by these medications should be cautiously evaluated in contrast to their consumption. The most frequently reported side effects include allergic reactions and elevated liver enzymes [46].

CONCLUSION

An essential aspect of assessing pharmaceutical drugs belonging to a new class is to demonstrate proof of their intended effect on the subject, which in this case is a decreased risk of cardiovascular incidents. Even though with Alirocumab and Evolocumab the decrease in LDL-C is considerable, conclusive proof that the rate of occurrence of cardiovascular incidents has lowered is indispensable. A few clinical trials are specifically being conducted to gather data that can be used to illuminate the clinical advantages of these drugs as well as potential adverse effects. The possible advantages from the remarkable decrease in LDL-C levels achieved by these medications should be cautiously evaluated in contrast to their long-term safety profile which is as yet inconclusive.

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