 doi:10.20944/preprints202205.0290.v1

Review

PCSK9 Inhibitors: Pharmacology and Therapeutic Potential

Jeetendra Kumar Gupta[1], Shivendra Kumar[2], Krishanveer Singh[3], Kuldeep Singh[4*], Km. Meenakshi[5] and Karan Kumar[6]

Institute of Pharmaceutical Research, GLA University Mathura, Uttar Pradesh, india;*kuldeep.singh_mph20@gla.ac.in

* Correspondence: **author:**kuldeep.singh_mph20@gla.ac.in

Abstract

Proprotein convertase subtilisin/Kexin type 9 (PCSK9) is a proteolytic enzyme that indirectly regulates serum LDL cholesterol by destroying LDL receptors. In clinical studies, the main role of the proprotein convertase subtilisin/Kexin type9 (PCSK9) inhibitor in cholesterol regulation was elucidated. It is produced in the liver but is also present in the kidney and intestine. It prevents HMGCo from synthesizing cholesterol. SREBP-2 is a reductase that is induced by statins. In a dose-dependent manner, increasing SREBP-2 levels enhanced LDL-R and PCSK9 gene expression. At the minimum, two procedures have been developed to overcome the plasma level of PCSK9. This is the LDLR test, polyclonal antibodies, and sentience oligonucleotide. Lower dosage statin treatment with a proprotein convertase subtilisin/Kexin type9 inhibitor will be most efficient in lowering LDL and avoiding statin adverse effects. In multiple long-term trials, statins have been found to reduce cardiovascular mortality by 30% and stroke incidence by 20%. In this way, we conclude the role of PCSK9 in hypercholesterolemia.

Keywords: Cholesterol; PCSK9 inhibitors; HMG-CoA; LDL receptor; statins

Introduction:

Proprotein convertase subtilisin/Kexin type 9 is a proteolytic enzyme that binds to the LDL receptor in order to control serum LDL cholesterol levels by inducing LDL receptor degradation [1]. A pair of benefit-of-function mutations in the PCSK9 gene in a French family were shown to be responsible for autosomal dominant familial hypercholesterolemia (FH), a condition linked to premature cardiovascular disease (CVD) and death. It was the third part of an autosomal dominant mutation for familial hypercholesterolemia that increased mutations in the LDL-R (receptor) and apolipoprotein B genes [2]. Clinical research has highlighted the main role of the proprotein convertase subtilisin/Kexin type9 (PCSK9) inhibitor in cholesterol regulation [3]. PCSK9 (proprotein convertase subtilisin/Kexin type 9) is a serine protease of the subtilisin family that is excreted. It's made in the liver, but it's also found in the kidneys and intestine. In the pro-domain, c-terminal domain, catalytic domain, and signal sequence, PCSK9-pro, 692-AA (amino acid), and 75 KDa precursor of

 doi:10.20944/preprints202205.0290.v1

PCSK9 combine. It is produced in the endoplasmic reticulum (ER) and improved in the Golgi apparatus, where it is autocatalytically cleaved to enter the secretory pathway and then released into circulation [4]. SREBP-2 (sterol regulatory element bound protein-2) regulates hepatic LDL-R activity during transcription, while PCSK9 suppresses the LDL/LDL-R (receptor) complex. The maximal complex is internalized and attacked by the lysosome, resulting in a decrease in LDL-R and, as a result, a decrease in LDL-C (clearing), increasing LDL plasma levels. At the transcriptional level, both LDL-R and PCSK9 inhibitors are influenced by intracellular cholesterol levels via SREBP-2 (sterol regulatory element bound protein-2) [5]. It prevents HMGCo from synthesizing cholesterol. SREBP-2 is a reductase that is induced by statins. In addition, SREBP-2 elevated the expression of LDL-R and PCSK9 genes in a dose-dependent manner, with the former gene being more significantly regulated11. Increased PCSK-9 levels in fibrates via SREBP-2, as well as higher modulation of PCSK-9 levels by fibrates and statins, imply that PCSK-9 inhibition may improve the lipid-lowering efficacy of similar medications [6].

Inhibitors of PCSK9:

Inhibitors of PCSK9 lead to a new class of drugs that lower LDL levels, or bad cholesterol. At present, very few drugs have been approved by the United States Food and Drug Administration (US-FDA), such as alirocumab and evolocumab [7]. These have characteristics that

a. Those with atherosclerotic cardiovascular disease (CVD) who can't get enough LDL with current treatment (statins) have it.
b. Adult patients with familial hypercholesterolemia
c. In the case of an intolerable patient

Alirocumab:

The human monoclonal antibody, i.e., alirocumab. That is, by working with the liver, we were able to reduce the amount of "bad" cholesterol LDL- (low-density lipoprotein) circulating in our blood [8]. It is used in individuals having heart disease to reduce the risk of heart attack, a certain type of chest pain (unstable/angina), and stork conditions that require hospitalization [9]. It is used together with a free low diet, alone or together with other cholesterol-lowering medicines, in adults with high blood cholesterol levels, also called primary hyperlipidaemia (which includes high cholesterol called heterozygous) [10]. FH (Familial hypercholesterolemia) or inherited types of high cholesterol. This situation can cause higher blood levels of LDL cholesterol and also cause plaque inside our arteries [11]. Praluent is mainly used along with other LDL-lowering treatments in adults with high cholesterol called homozygous familial hypercholesterolemia who need additional lowering of LDL-C (low-density lipoprotein-c) [12].

 doi:10.20944/preprints202205.0290.v1

Mechanism of action:

Alirocumab inhibits the PCSK9 protein, which binds to the LDL-R (low-density lipoprotein receptor), causing cholesterol to be removed from circulation and the receptor to be destroyed, decreasing LDL cholesterol that is removed from circulation [13] .

Dosing information:

Adult dose for hyperlipidemia patients:

The usual dose of alirocumab is 75 mg subcutaneously every 2 weeks or 300 mg subcutaneously once every 4 weeks [1]. For inadequate LDL-(low-density lipoprotein) response, may adjust to 150 mg subcutaneously every 2 weeks [12]. Patient with heterozygous familial hypercholesterolemia (HFH) undergoing LDL aphaeresis-150mg subcutaneously once every 2 weeks [14].

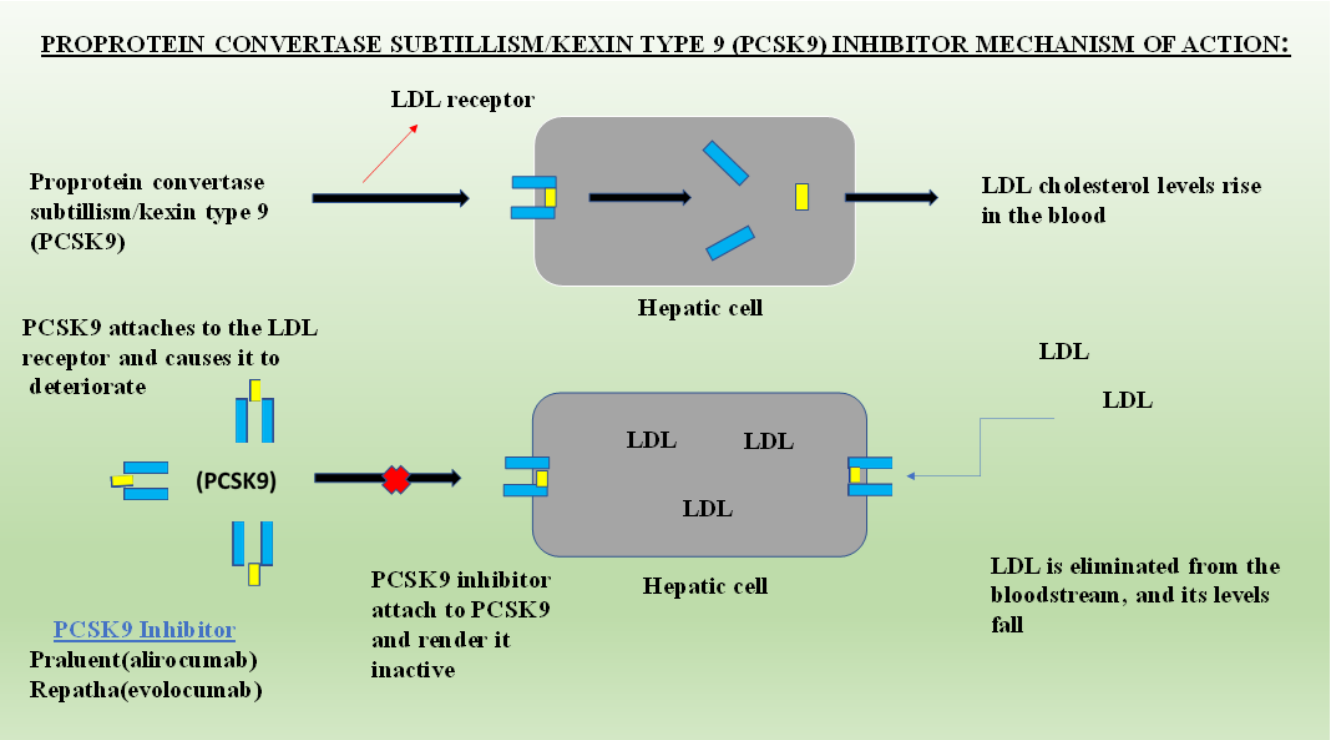
Evolocumab:

Evolocumab (Repatha) is a human monoclonal antibody. That helps the liver reduce the level of "bad" cholesterol (LDL) circulating in our blood [15]. It is used in combination with a low-fat diet and other cholesterol-lowering medications in people with homozygous or heterozygous familial hypercholesterolemia (FH) [16]. It is mainly used to help reduce the risk of stroke, heart attack, or other heart problems in people who have or have had vessel problems caused by plague in the arteries [17].

Mechanism of action:

A monoclonal antibody (PCSK9) was used in the study. The LDL-R pathway is particularly important in clearing LDL-C from circulation. PCSK9 is a serine protease that breaks down LDL-R in the liver, lowering LDL-C clearance and increasing plasma LDL-C. PCSK9 inhibitor inhibits PCSK9 degradation of LDL-R, improving LDL-C clearance and lowering plasma LDL-C levels [18].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 May 2022 doi:10.20944/preprints202205.0290.v1



Absorption:

Evolocumab is a drug that is used to treat a variety of conditions. The peak plasma concentration is 18.6 mcg/ml and lasts 3–4 days. As a result, it has a bioavailability of 72 percent in humans. has an AUC (least) of 188 days mcq/ml (140 mg) and a Vd of 3.3 L (420 mg).

Metabolism:

Lower concentration: saturable bound to the PCSK9 target is the primary mode of elimination. Higher concentration: evolocumab is mostly eliminated by a non-saturable proteolytic route. The half-life of evolocumab is 11–17 days [19].

Dosing information:

In the intervention arm, high-cardiovascular-risk haemodialyzed statin-intolerant patients with hypercholesterolemia will receive evolocumab 140 mg subcutaneous every 2 weeks for 24 weeks.

Naturally occurring inhibitors of PCSK9:

PCSK9 stands for proprotein appropriate subtilisin/Kexin 9 inhibitor. (PSCK), a polar regular of the poor solidity lipoprotein receptor (LDL), and thus the LDL cholesterol level. The inhibitor is principally symphonized through the hepatocytes [20]. It suffers from a mechanical cleft in the endoplasmic reticulum at the Golgi apparatus. PCSK9 is one of 33 genes organized through the sterol regulatory elements (SRE) protein

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 May 2022 doi:10.20944/preprints202205.0290.v1

binding (SREBP) family of copying factors. When the PCSK9 promoter rapidity is inhibited, cell cholesterol transcription or cell synthesis are inhibited [21]. to enhance transcription. The PCSK9 inhibitor of nuclear 1 (HNF1) is the second factor of transcription implicated in the arrangement within the hepatocyte factor. Already secreted, PCSK9 cleaves epidermal growth factor identical homology domain A (EGFA) by that catalytic domain to the receptor of (LDLR). This promotes the objection of LDLR to the lysosome, intended to accept it for recycling within the surface cell [22]. Their degeneration response suppresses the LDLR number on hepatocytes but not by the liver uptake of circulating LDL particles. For this purpose, gain-of-function of PCSK9 genetic (GOF) variations is equivalent to hypercholesterolemia consideration. Although its prohibition of pharmaceuticals has been judged as an intervention of a new line to inhibit cardiovascular disease. At a minimum, two procedures have been developed to overcome the plasma level of PCSK9[23]. This is the LDLR test, polyclonal antibodies, and sentience oligonucleotide. However, a pharmacological strategy to prohibit PCSK9 can be done immersed. The development and identification of orally absorbed tinny molecules within the activity of PCSK9 Pharmacological history has provided enthralling evidence of the significance of recognized naturally-arising chemical moieties to therapeutic effects [24]. Because, for this reason, in the current review, our own knowledge was epitomized within the essential combination obtained that saw important activity of PCSK9 inhibitory. Naturally occurring inhibitors of PCSK9 are quite common in phytomedicines [25].

Berberine:

Berberine is a moiety of chemicals rescued from different plants, along with European barberry, goldenseal, Oregon grape, Goldthread, greater celandine, philodendron, and tree turmeric [26]. This is mostly done for diabetes, high levels of cholesterol or other fat lipids in the bloodstream (hyperlipidaemia), and high blood pressure [27]. Its berberine has specified that it cures and treats many metabolic health conditions [28].

- polygenic disorder
- stoutness
- cardiac problems [29]

Benefits:

Bacterial infection: It inhibits the growth of Staphylococcus Aureus and cures and fights many problems like sepsis, pneumonia, and meningitis. It also helps with skin conditions and problems [30].

Diabetes: Its benefits are found on the basis of a positive effect on blood sugar, insulin, and triglycerides [31].

 doi:10.20944/preprints202205.0290.v1

High blood pressure: It's a new drug that leads to blood pressure lowering cures of heart disease in berberine. In a meta-analysis, the BP-reducing drug was much more efficacious than single. Berberine could detain the high blood pressure that developed at that moment, and Berberine helped reduce its severity [32].

Obesity: It is a common problem. It can increase the risk of Type 2 Diabetes, Cardiac problems, high blood pressure, and cholesterol risk [33].

Dose: In the case of obesity, people took 750 mg of Berberine twice a day for 90 days [34].

High cholesterol: high levels of poor-solidity lipoprotein (LDL) triglycerides and cholesterol. It may increase the risk of heart attacks and other problems [35]. People took 750mg of barberry two times a day for 90 days. decrease in body weight. Take 200mg of berberine three times a day. experimented with people's decrease in body mass index reading [36].

Polycystic ovary syndrome: PCO5 Syndrome is feminine has a high level of certain masculine hormones [37].

These concerns are as follows:

- Increase insulin
- High levels of cholesterol
- Heavy body weight
- Boost Blood Pressure

Cancer: Berberine, as a cell/tissue 42 molecule, can cause significant differentiation [38]. It also benefits another potential cancer fighter [39].

The following points on cancer are also mentioned as inhibitory effects.

- Colorectal cancer
- Lung cancer can be fatal
- Cervical cancer
- The prostate cancer
- Ovarian cancer

Dose: Barbering capsules formulations dose took some people, but there is no set dosage, with most people taking 1000 to 1500mg per day [40].

 doi:10.20944/preprints202205.0290.v1

Vegetable Proteins and Sterol /Stanols:

There is an easily-obtainable dietary supplement substitute for cholesterol reduction. Plant sterols and stanols are obtained in one broad category of use. There is no clear data on these given substances, requiring this inhibitor to show no activity at the PCSK9 level and findings in any case [41]. Two groups have analysed the PCSK9 layer under the influence of plant stanol inlet in LDL-C's poor condition. In a randomly imperturbable double-blind trial in rational and hypercholesteraemic subjects, they evacuated the effect of six months of depletion of vegetable oil (20g/day) [42]. Improve the control group or not, the stanol group by taking 3g of stanol per day as an ester. The long-time/duration of consumption of plant stanol ester decreased LDL-C by 7–10% in the absence of influencing either PCSK9 plasma congregation or the liver disease levels of LDLR [43]. Plant stanol esters without those interested in PCSK9 metabolism may impair LDL-C by inhibition of cholesterol absorption. Blotted soy-lupine peptide mix: This reduced the activity of HMG-CoA by 50% at the 0.5 mg/mL level, which is half the nanomolar IC of known statins. Poor initiation activity was also reduced [44].

Lupin protein:

The probed lupine peptides are derived from protein-rich grain legumes, i.e., Lupinus albus (white lupin), L. luteus (yellow lupin), L. mutabilis (pearl lupin) and L. angustifolius (sweet leaf lupin; Fabaceae) [45]. Lupin proteins have been investigated for several years, primarily for their ability to lower plasma cholesterol levels, which is due in part to an LDLR-activating mechanism. Lupin proteins have been shown to have hypolipidemic and antiatherosclerotic properties in animal models [46]. The amount of sequence homology present within the display template allows obtaining models of a reasonable quality that is supported by MD simulation. The last stage of stimulation the short peptide has a small amount of energy in the form of a minimum level of structure. were to blame for the prohibition. T1, T2, and T16 showed the helix to be correct [47].

Soy Proteins:

Soy protein has been demonstrated to have decreasing properties in different populations, from children (Laurie et al. 1991) to renal patients (D'Amico 1992). The ultimate mechanism was found to be reliable for the plasma cholesterol disease [48]. Direct execution of the major isoflavones in soybeans Alternatively, the protein constituent is mainly 7S globulin from soybeans and their fragments. There is now a health claim that soy protein can help lower the risk of coronary heart disease (FDA 1999) [49].

Dose: According to research, some people took 25 grams of soy protein per day. It’s mostly given its cholesterol-decreasing effect [50].

 doi:10.20944/preprints202205.0290.v1

Polyphenols:

Polyphones are derived from the secondary metabolites present in fruit, nuts, seeds, vegetables, stems, herbs, and flowers. It's also found in tea and red urine. This class undergoes the counting of different substances such as flavonoids, lignans, stilbenes, and condensed (flavan-3-ol polymers known as proanthocyanins) [51]. Many epidemiological studies, including clinical trials, have reported various cardiovascular benefits of polyphones. There are several mechanisms, including plasma LDL-poor (decreased) activity of cholesterol. Most substances (molecules) act through up-regulation of LDLR on the local hepatic surface [52]. This proof led researchers to analyse the potential influence of polyphenols on PCSK9. It also helps in reducing the chances and probability of inflammation in the body cells. It may be that polyphenols play a role in anti-inflammatory effects, especially by influencing the activity of enzymes that make arachidonic acid (phospholipase A2, COX) and arginine (NOS) metabolism. This is because polyphenols may be able to remove free radicals from the body [53].

Quercetin:

It is the source of flavonoids, particularly the flavonoid cluster of polyphenols. It is present in different fruits, leaves, seeds, vegetables, and grains. It's also found that red onions and kale are coarse foods containing an appreciable amount of quercetin. In vitro studies revealed that quercetin within its glycosylated form incubated with HepG-2 cells. The concentration ranged from 1 to microns of deceased PCSK9 mRNA points, 20–30% [54]. It seems to be authorized that the intracellular PCSK9 point in the culture medium is increased by 20–90% and PCSK9 secretion by 30–35%. The role of Quercetin is as an antioxidant and anti-inflammatory agent. It mostly helps in reducing inflammation, killing cancer cells, and regulating blood sugar. It also prevents heart disease [55].

Dose: this is taken as a supplement to your daily routine. A common dose is 500mg per day.

Eugenol:

It is the grated component of (4-allyl-2-methoxyphenyl) essential oil (Syzygium aromatic L). cloves. It is a phenolic nutraceutical with recognized hypocholesterolemia activities. It has a large human body-friendly daily intake of 25 mg/kg as a safe nutrient. Eugenol appears to reduce blood cholesterol levels and prevent lipogenesis in the liver in the animals investigated, implying a protective action against atherosclerosis and fatty liver disease [56]. Molecule docking determines whether hydrophobic interactions are detected within

 doi:10.20944/preprints202205.0290.v1

the ligand's eugenol and PCSK9. The ligands eugenol and PCSK9 are in the mixture. Eugenol was present to kill the PCSK9 aspect in Jurkat cells [57].

Nutrients:

The hepatic nuclear transcription representative, active hepatocyte nuclear proxy 1 (HNF1), which is known to ultimately involve pancreatic insulin secretion in the fasted state, decreased the hepatic HNF1 protein aspect [58]. Interestingly, the PCSK9 gene had HNF1. Sterol homology element site of the PCSK9 promoter, and reduced amounts of dead PCSK9 protein in the HNF1 protein. It may also bind to cell signalling pathways that use serine-threonine kinase to hinder its mechanistic targeting of rapamycin (mTOR) [59]. Research in mice with dysregulated mTOR complex 1 activity through the exit from the upstream inhibitory tuberous sclerosis complex resulted in concurrent upregulation of HNF1 and PCSK9 expression in hepatic LDLR protein concentration and a decrease in rapamycin (mTOR complex 1 dysregulated PCSK9 mRNA expression) [60]. The aggression response to decreased PCSK9 expression may therefore be involved in FA catabolism of fenofibrate (a PPAR agonist), which appears to reduce PCSK9 mRNA expression through repressed PCSK9 promoter activity in human hepatocytes. The aggression response to decreased PCSK9 expression may therefore be involved in FA catabolism of fenofibrate (a PPAR agonist), which appears to reduce PCSK9 mRNA expression through repressed PCSK9 promoter activity in human hepatocytes [61]. Hepatic PPAR agonist mRNA expression is spent in the chronic stage (48 h) of hamsters: Treatment with PPAR mRNA agonist treatment does not reduce the PCSK9 protein or mRNA aspect upon engraftment of primary hepatocytes. Collectively, it seems that SREBP2 is an ascending nuclear transcription factor that involves the coordination of PCSK9 in the feed-derived state. However, SREBP1C and HNF1 also cause the PCSK9 part to be cleaved when the body isn't getting enough food [62].

Curcumin:

Curcumin and its depreciation. The active constituents responsible for the major medicinal properties of turmeric are curcuminoids [63]. Turmeric is obtained from the rhizome of the long curcuma. The Ant It has also been used as a food ingredient in Asian cooking and in the practice of Chinese medicine. similar antioxidant and anti-inflammatory, anti-thrombotic activities for the prevention or treatment of inflammatory processes [64]. It also cures and helps with neurodegenerative disorders and heart disease problems. It has been reported that curcumin restricted lipid accumulation in peritoneal macrophages isolated from LDLRs from rats fed a high-fat diet [65]. Its curcumin attenuates oxLDL-induced CD36 and scavenger receptor-A (SR-A) aspects. This results in increased oxLDL uptake in PMA-differentiated THP-1 macrophages [66].

 doi:10.20944/preprints202205.0290.v1

Lycopene:

Lycopene is the most powerful and efficient antioxidant equivalent to the major carotenoid, which has been linked to an increased risk of cardiovascular disease (CVD). Endothelin-1 (ET-1) is an omnipotent vasopressor synthesized by the endothelial cell and plays an important role in the pathophysiology of CVD [67]. The effect of lycopene on vascular endothelial cells has not been adequately described. This study investigated the execution of lycopene on the ET-1 aspect induced by cyclic stress in human umbilical cord endothelial cells (HUVECs) and identified the pathways involved in this process [68]. Cultured HUVECs were susceptible to cycle stress and stress-induced manipulation of the ET-1 aspect in the lycopene aspect or defect [69]. Oxidative stress, external person Phosphorylation of ERK and, as a result, oxygenase-1 (HO-1) were induced. Lycopene inhibited cyclic stress-induced ET-1 factor and ERK phosphorylation. Lycopene also reduced cyclic stress-induced p22Fox mRNA levels via the NAD (P) H oxidase response and reactive oxygen splicing [70]. Furthermore, HO-1 silencing almost completely eliminated lycopene's repressive effect on the stress-induced ET-1 aspect [71]. This study reports for the first time that lycopene prevents cyclic stress-induced ET-1 secretion by suppression of p22 and induction of HO-1 in HUVECs. This study is very effective at giving new information about the molecular pathways that could make lycopene more beneficial to the cardiovascular system [72].

Clinically available PCSK9 inhibitors:

In 2013, at the American College of Cardiology/American Heart Association cholesterol management centre, the first patient with a decreased risk of CVD event was identified [73]. Treating the risk, those who take medication (statins) have been shown to have fewer CVD incidents in the future [74]. Patients with FH (familial hypercholesterolemia) who are statin resistant or have increased LDL-C levels despite being on maximum tolerated statin medication benefit from PCSK9 inhibitors. PCSK9 inhibitors combined with low-dose statin therapy will be the most successful in lowering LDL while avoiding statin side effects. In multiple long-term studies, statins have been proven to reduce cardiovascular mortality by 30% and stroke incidence by 20% [75]. If the LDL-C is less than 70 mg/dl, the patient will be given 75 mg of alirocumab every two weeks, and subsequently 150 mg every two weeks. In addition to statin therapy or in patients who were statin intolerant, the maximum dose of alirocumab in these trials was 150mg every 2 weeks, which decreased plasma LDL-C levels by approximately 60%, similar to what was seen in the evolocumab regimen of 300mg every 4 weeks, which decreased plasma LDL-C levels by 55-60 percent [76].

 doi:10.20944/preprints202205.0290.v1

PCSK9 inhibitors and adverse effects:

- Nasopharyngitis
- No increased signal for hepatotoxicity
- Injection site reactions are generally mild.
- Muscle-related symptoms did not rise, nor did muscle enzymes.
- There is no increase in the risk of cognitive impairment.
- Muscle toxicity
- Neurocognitive toxicity
- There were no clinically significant drug-drug interactions.

Side effects:

- Cold and flu-like symptoms
- Pain or swelling at the injection site
- Allergic skin reaction
- Muscle pain
- Diarrhea
- Back pain
- Redness
- Cough

Conclusion

PCSK9 (Proprotein convertase subtilisin/Kexin type 9) is a proteolytic enzyme that destroys LDL receptors and hence indirectly modulates serum LDL cholesterol. Clinical investigations have revealed the primary involvement of the proprotein convertase subtilisin/Kexin type9 (PCSK9) inhibitor in cholesterol control. The liver produces it, although it's also found in the kidneys and gut. Because of this inhibitor, HMGCo is unable to synthesize cholesterol. Statins activate SREBP-2, a reductase enzyme. In a dose-dependent manner, increasing SREBP-2 levels enhanced LDL-R and PCSK9 gene expression. PCSK9's relevance as a novel molecular target for treating hypercholesterolemia and related cardiovascular illnesses has been demonstrated by the clinical success of two FDA/EMA-approved monoclonal antibodies, alirocumab and evolocumab. As a result, small-molecule medications that are less expensive and may be used orally are desperately needed. The discovery of natural substances with lipid-lowering activity linked to an anti-PCSK9 inhibitory effect might be a solution to this problem. Many substances with efficient anti-PCSK9 inhibitory activity were discovered

 doi:10.20944/preprints202205.0290.v1

in this study, mostly through acting at the transcriptional level, with just a few examples of the autocatalytic secretion phase or PCSK9 interaction with the LDL receptor. Finally, proof must be founded on in vitro mechanisms of action of active ingredients, preclinical investigations in experimental animals, and finally, human safety and efficacy. All of these properties were not always available for the natural compounds mentioned in this review. As a result, the compounds chosen can only be considered a starting point for future oral PCSK9 inhibitor development. Finally, proof must be founded on in vitro mechanisms of action of active ingredients, preclinical investigations in experimental animals, and finally, human safety and efficacy. All of these properties were not always available for the natural compounds mentioned in this review. As a result, the compounds chosen can only be considered a starting point for future oral PCSK9 inhibitor development.

Conflict of interest:
The authors declare no conflict of interests.

Financial support statement:
The authors received no financial support to produce this manuscript.

Authors' contributions:
All authors contributed equally.

Reference

1. Chaudhary R et al. 2017. PCSK9 inhibitors: A new era of lipid lowering therapy. World J Cardiol Baishideng Publishing Group Inc. 9:76.

2. Youngblom E et al. 2016. Familial Hypercholesterolemia. GeneReviews® University of Washington, Seattle.

3. Chaudhary R et al. 2017. PCSK9 inhibitors: A new era of lipid lowering therapy. World J Cardiol Baishideng Publishing Group Inc. 9:76.

4. Horton JD et al. 2007. Molecular biology of PCSK9: its role in LDL metabolism. Trends Biochem Sci NIH Public Access. 32:71.

5. Lagace TA. 2014. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. Curr Opin Lipidol Wolters Kluwer Health. 25:387.

6. Jia YJ et al. 2014. Enhanced circulating PCSK9 concentration by berberine through SREBP-2 pathway in high fat diet-fed rats. J Transl Med BioMed Central Ltd. 12:1–8.
7. **7** A M. 2015. Alirocumab: First Global Approval. Drugs Drugs. 75:1699–1705.

8. Huffman JC et al. 2018. Collaborative care and related interventions in patients with heart disease: an update and new directions. Psychosomatics NIH Public Access. 59:1.

9. Schaiff RAB et al. 2008. An Overview of Cholesterol Management. Am Heal Drug Benefits Engage Healthcare Communications, LLC. 1:39.

 doi:10.20944/preprints202205.0290.v1

10. Castro-Orós I De et al. 2010. The genetic basis of familial hypercholesterolemia: inheritance, linkage, and mutations. Appl Clin Genet Dove Press. 3:53.

11. Dong B et al. 2010. Strong induction of PCSK9 gene expression through HNF1α and SREBP2: mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. J Lipid Res American Society for Biochemistry and Molecular Biology. 51:1486.

12. Feingold KR. 2021. Cholesterol Lowering Drugs. Feingold KR et al., editors. NCBI Bookshelf MDText.com, Inc.

13. Page MM, Watts GF. 2016. PCSK9 inhibitors – mechanisms of action. Aust Prescr NPS MedicineWise. 39:164.

14. Latimer J et al. 2016. PCSK9 inhibitors in the prevention of cardiovascular disease. J Thromb Thrombolysis Springer. 42:405.

15. A M. 2015. Evolocumab: First Global Approval. Drugs Drugs. 75:1567–1573.

16. Pokhrel B et al. 2021. PCSK9 Inhibitors. StatPearls StatPearls Publishing.

17. Dishart KL et al. 2003. Gene Therapy for Cardiovascular Disease. J Biomed Biotechnol Hindawi Limited. 2003:138.

18. Shapiro MD et al. 2018. PCSK9: From Basic Science Discoveries to Clinical Trials. Circ Res NIH Public Access. 122:1420.

19. U K et al. 2007. Clinical pharmacokinetics and use of infliximab. Clin Pharmacokinet Clin Pharmacokinet. 46:645–660.

20. Adorni MP et al. 2020. Naturally Occurring PCSK9 Inhibitors. Nutrients Multidisciplinary Digital Publishing Institute (MDPI). 12.

21. H L et al. 2009. Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine. J Biol Chem J Biol Chem. 284:28885–28895.

22. Nguyen MA et al. 2014. Internalized PCSK9 dissociates from recycling LDL receptors in PCSK9-resistant SV-589 fibroblasts. J Lipid Res American Society for Biochemistry and Molecular Biology. 55:266.

23. Ito MK, Santos RD. 2017. PCSK9 Inhibition With Monoclonal Antibodies: Modern Management of Hypercholesterolemia. J Clin Pharmacol J Clin Pharmacol. 57:7–32.

24. Gu HM et al. 2013. Characterization of the role of EGF-A of low density lipoprotein receptor in PCSK9 binding. J Lipid Res Elsevier. 54:3345–3357.

25. Fernandes JPS. 2018. The Importance of Medicinal Chemistry Knowledge in the Clinical Pharmacist's Education. Am J Pharm Educ American Association of Colleges of Pharmacy. 82:106–114.

 doi:10.20944/preprints202205.0290.v1

26. M I, H H. 2019. Berberine and barberry (Berberis vulgaris): A clinical review. Phytother Res Phytother Res. 33:504–523.

27. Goldenseal - PubMed.

28. High-cholesterol foods: Foods to avoid and include.

29. Yin J et al. 2008. Efficacy of Berberine in Patients with Type 2 Diabetes. Metabolism NIH Public Access. 57:712.

30. Tong SYC et al. 2015. Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin Microbiol Rev American Society for Microbiology (ASM). 28:603.

31. Asif M. 2014. The prevention and control the type-2 diabetes by changing lifestyle and dietary pattern. J Educ Health Promot Wolters Kluwer -- Medknow Publications. 3:1.

32. Zhang M et al. 2016. Therapeutic Potential and Mechanisms of Berberine in Cardiovascular Disease. Curr Pharmacol Reports 2016 26 Springer. 2:281–292.

33. Martín-Timón I et al. 2014. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? World J Diabetes Baishideng Publishing Group Inc. 5:444.

34. Tabeshpour J et al. 2017. A review of the effects of Berberis vulgaris and its major component, berberine, in metabolic syndrome. Iran J Basic Med Sci Mashhad University of Medical Sciences. 20:557.

35. Hajar R. 2017. Risk Factors for Coronary Artery Disease: Historical Perspectives. Heart Views Wolters Kluwer -- Medknow Publications. 18:109.

36. Hill JO et al. 2012. Energy Balance and Obesity. Circulation NIH Public Access. 126:126.

37. Johansson J, Stener-Victorin E. 2013. Polycystic Ovary Syndrome: Effect and Mechanisms of Acupuncture for Ovulation Induction. Evid Based Complement Alternat Med Hindawi Limited. 2013:16.

38. Och A et al. 2020. Biological Activity of Berberine—A Summary Update. Toxins (Basel) Multidisciplinary Digital Publishing Institute (MDPI). 12.

39. Greenwell M, Rahman PKSM. 2015. Medicinal Plants: Their Use in Anticancer Treatment. Int J Pharm Sci Res Europe PMC Funders. 6:4103.

40. Naumov DY et al. 1998. The monoclinic form of acetaminophen at 150 K. Acta Crystallogr Sect C Cryst Struct Commun Blackwell Munksgaard. 54:653–655.

41. Silbernagel G et al. 2019. The interrelations between PCSK9 metabolism and cholesterol synthesis and absorption. J Lipid Res American Society for Biochemistry and Molecular Biology. 60:161.

42. Peterson AS et al. 2008. PCSK9 function and physiology. J Lipid Res American Society for

Biochemistry and Molecular Biology. 49:1152.

43. DO L et al. 2011. A randomized, double-blind, placebo-controlled study to assess the efficacy and tolerability of gabapentin enacarbil in subjects with restless legs syndrome. J Clin Sleep Med J Clin Sleep Med. 7:282–292.

44. Feingold KR. 2021. Cholesterol Lowering Drugs. Feingold KR et al., editors. NCBI Bookshelf MDText.com, Inc.

45. Labaree R V. Research Guides: Organizing Your Social Sciences Research Paper: 6. The Methodology.

46. Harno E et al. 2018. POMC: The Physiological Power of Hormone Processing. Physiol Rev American Physiological Society. 98:2381.

47. Burdick DJ et al. 2020. Design of Organo-Peptides As Bipartite PCSK9 Antagonists. ACS Chem Biol ACS Chem Biol. 15:425–436.

48. EA K et al. 1998. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. J Nutr J Nutr. 128:954–959.

49. Messina M. 2016. Soy and Health Update: Evaluation of the Clinical and Epidemiologic Literature. Nutrients Multidisciplinary Digital Publishing Institute (MDPI). 8.

50. Ramdath DD et al. 2017. Beyond the Cholesterol-Lowering Effect of Soy Protein: A Review of the Effects of Dietary Soy and Its Constituents on Risk Factors for Cardiovascular Disease. Nutrients Multidisciplinary Digital Publishing Institute (MDPI). 9.

51. Gorzynik-Debicka M et al. 2018. Potential health benefits of olive oil and plant polyphenols. Int J Mol Sci MDPI AG. 19.

52. Khurana S et al. 2013. Polyphenols: Benefits to the Cardiovascular System in Health and in Aging. Nutrients Multidisciplinary Digital Publishing Institute (MDPI). 5:3779.

53. Yahfoufi N et al. 2018. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. Nutrients Multidisciplinary Digital Publishing Institute (MDPI). 10.

54. Janabi AHW et al. 2020. Flavonoid-rich foods (FRF): A promising nutraceutical approach against lifespan-shortening diseases. Iran J Basic Med Sci Mashhad University of Medical Sciences. 23:140.

55. Pan Y et al. 2018. Effect of quercetin glucosides from Allium extracts on HepG2, PC-3 and HT-29 cancer cell lines. Oncol Lett Spandidos Publications. 15:4657.

56. Lee JE et al. 2018. Severity of nonalcoholic fatty liver disease is associated with subclinical cerebro-cardiovascular atherosclerosis risk in Korean men. PLoS One Public Library of Science. 13.

57. Kamatou GP et al. 2012. Eugenol—From the Remote Maluku Islands to the International Market Place: A Review of a Remarkable and Versatile Molecule. Molecules Multidisciplinary Digital Publishing Institute (MDPI). 17:6953.

 doi:10.20944/preprints202205.0290.v1

58. Li H et al. 2009. Hepatocyte Nuclear Factor 1α Plays a Critical Role in PCSK9 Gene Transcription and Regulation by the Natural Hypocholesterolemic Compound Berberine. J Biol Chem American Society for Biochemistry and Molecular Biology. 284:28885.

59. Ballou LM, Lin RZ. 2008. Rapamycin and mTOR kinase inhibitors. J Chem Biol Springer. 1:27.

60. Ai D et al. 2012. Regulation of hepatic LDL receptors by mTORC1 and PCSK9 in mice. J Clin Invest J Clin Invest. 122:1262–1270.

61. Cameron J et al. 2008. Berberine decreases PCSK9 expression in HepG2 cells. Atherosclerosis Atherosclerosis. 201:266–273.

62. Ip E et al. 2004. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. Hepatology Hepatology. 39:1286–1296.

63. Kocaadam B, Şanlier N. 2017. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Crit Rev Food Sci Nutr Crit Rev Food Sci Nutr. 57:2889–2895.

64. Aggarwal BB et al. 2011. Identification of Novel Anti-inflammatory Agents from Ayurvedic Medicine for Prevention of Chronic Diseases: “Reverse Pharmacology” and “Bedside to Bench” Approach. Curr Drug Targets NIH Public Access. 12:1595.

65. Al-Saud NBS. 2020. Impact of curcumin treatment on diabetic albino rats. Saudi J Biol Sci Elsevier. 27:689.

66. Min KJ et al. 2013. Curcumin inhibits oxLDL-induced CD36 expression and foam cell formation through the inhibition of p38 MAPK phosphorylation. Food Chem Toxicol Food Chem Toxicol. 58:77–85.

67. Yamagata K. 2017. Carotenoids Regulate Endothelial Functions and Reduce the Risk of Cardiovascular Disease. Carotenoids IntechOpen.

68. Di Pietro N et al. 2020. Role of Polyphenols and Carotenoids in Endothelial Dysfunction: An Overview from Classic to Innovative Biomarkers. Oxid Med Cell Longev Hindawi Limited. 2020.

69. Sung LC et al. 2015. Lycopene inhibits cyclic strain-induced endothelin-1 expression through the suppression of reactive oxygen species generation and induction of heme oxygenase-1 in human umbilical vein endothelial cells. Clin Exp Pharmacol Physiol 42:632–639.

70. SE L et al. 2011. Fisetin induces Nrf2-mediated HO-1 expression through PKC-δ and p38 in human umbilical vein endothelial cells. J Cell Biochem J Cell Biochem. 112:2352–2360.

71. Lee J et al. 2021. Lycopene Inhibits Oxidative Stress-Mediated Inflammatory Responses in Ethanol/Palmitoleic Acid-Stimulated Pancreatic Acinar AR42J Cells. Int J Mol Sci Int J Mol Sci. 22:1–18.

72. Tang FY et al. 2008. Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. Mol Nutr Food Res 52:646–654.

 doi:10.20944/preprints202205.0290.v1

73. 2018 Guideline on the Management of Blood Cholesterol GUIDELINES MADE SIMPLE.

74. Fiévet C, Staels B. 2009. Combination therapy of statins and fibrates in the management of cardiovascular risk. Curr Opin Lipidol Inserm. 20:505.

75. Chaudhary R et al. 2017. PCSK9 inhibitors: A new era of lipid lowering therapy. World J Cardiol Baishideng Publishing Group Inc. 9:76.

76. Katzmann JL et al. 2020. PCSK9 Inhibition: Insights From Clinical Trials and Future Prospects. Front Physiol Frontiers Media S.A. 11:1391.