J Atheroscler Thromb, 2017; 24: 895-907.
http://doi.org/10.5551/jat.RV17012

Review

Lipid Lowering Therapy and Circulating PCSK9 Concentration

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Hypercholesterolemia, particularly an increase in low-density lipoprotein cholesterol (LDL-C) levels, contributes substantially to the development of coronary artery disease and the risk for cardiovascular events. As the first-line pharmacotherapy, statins have been shown to reduce both LDL-C levels and cardiovascular events. However, despite intensive statin therapy, a sizable proportion of statin-treated patients are unable to achieve the recommended target LDL-C levels, and not all patients can avoid future cardiovascular events. Proprotein convertase subtilisin/kexin type 9 (PCSK9) plays a key role in cholesterol homeostasis by enhancing the degradation of hepatic low-density lipoprotein receptor (LDLR). Owing to its importance in lipid metabolism, PCSK9 has emerged as a novel pharmacological target for lowering LDL-C levels. In this review, the potential role of circulating PCSK9 as a new biomarker of lipid metabolism is described. Next, previous studies evaluating the effects of lipid-modifying pharmacological agents, particularly statins, on circulating PCSK9 concentrations are summarized. Statins decrease hepatic intracellular cholesterol, resulting in increased LDLRs as well as increased PCSK9 protein. There is a clear dose-response effect of statin treatment on PCSK9 level, as increasing doses of statins also increase the level of circulating PCSK9. Finally, the available therapeutic strategies to inhibit PCSK9 are present. Monoclonal antibodies against PCSK9, in combination with statins, are one of the most promising and novel approaches to achieve further reduction of LDL-C levels and reduce the risk of cardiovascular events.

Key words: Familial hypercholesterolemia (FH), Low-density lipoprotein cholesterol (LDL-C), Low-density lipoprotein receptor (LDLR), Proprotein convertase subtilisin/kexin type 9 (PCSK9), Statin

Introduction

Hypercholesterolemia, particularly an increase in low-density lipoprotein cholesterol (LDL-C) levels, contributes substantially to the development of coronary artery disease (CAD) and the risk for cardiovascular events. Statins represent the first-line pharmacotherapy for hypercholesterolemia, having been shown to reduce both LDL-C levels and cardiovascular events[1]. However, a considerable number of statin-treated patients do not achieve the recommended target LDL-C levels, despite intensive statin therapy, and some go on to develop cardiovascular events and atherosclerosis progression[2].

Proprotein convertase subtilisin/kexin type 9 (PCSK9), also known as neural apoptosis-regulated convertase 1 (NARC-1), was first described in 2003[3]. The human *PCSK9* gene is located on the small arm of chromosome 1p32 and contains 12 exons that encode 692 amino acids[4]. PCSK9 is mainly secreted by the liver, but it is also highly expressed in the intestine and kidney. PCSK9 comprises a signal peptide (1−30 amino acids), a prodomain (amino acids 31−152), a catalytic domain (amino acids 153−421) and a C-terminal domain (amino acids 422−692) (**Fig. 1**). ProPCSK9 is a protein of 75 kDa, and following autocatalytic cleavage in the endoplasmic reticulum, the prodomain is separated from the 62 kDa mature PCSK9. Mature PCSK9 is secreted together with the prodomain, thus forming a prosegment-PCSK9 complex that forces the PCSK9 catalytic domain into an inactive conformation[4]. PCSK9 enhances the endosomal and lysosomal degradation of hepatic low-density lipoprotein receptor (LDLR), resulting in increased

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Received: July 11, 2017
Accepted for publication: July 20, 2017

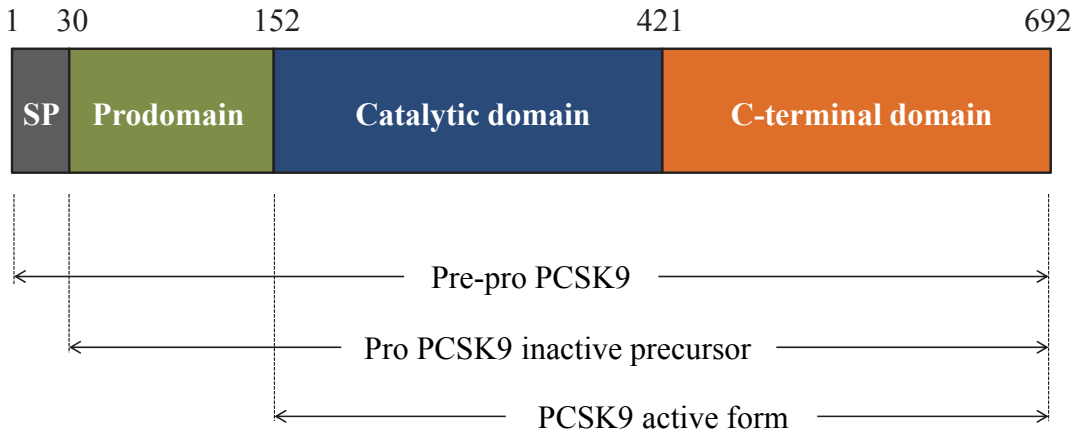

Fig. 1. PCSK9 protein structure

PCSK9 comprises a signal peptide (1−30 amino acids), a prodomain (amino acids 31−152), a catalytic domain (amino acids 153−421) and a C-terminal domain (amino acids 422−692). The molecular weight of proPCSK9 is 75 kDa, and the mature form is 62 kDa. Following autocatalytic cleavage in the endoplasmic reticulum, the prodomain is separated from the 62 kDa mature PCSK9 protein and both are secreted following the formation of a prosegment-PCSK9 complex.
SP, signal peptide; PCSK9, proprotein convertase subtilisin/kexin type 9.

serum LDL-C concentrations[5, 6]. Thus, PCSK9 is a key regulator of serum LDL-C levels[7]. Genetic variants of PCSK9 affect circulating PCSK9 concentrations[8] as well as plasma LDL-C levels[9]. Furthermore, gain-of-function mutations in PCSK9 result in familial hypercholesterolemia (FH), a genetic disease characterized by greatly increased levels of LDL-C[10, 11], whereas loss-of-function mutations of PCSK9 are associated with significantly decreased serum LDL-C levels[12] and an approximately 80−90% reduction in cardiovascular disease[13]. To date, several ELISA-based methods have been developed to measure circulating PCSK9 concentrations[14-20].

In this review, the potential role of PCSK9 as a new biomarker of lipid metabolism is described. Next, previous studies evaluating the effects of lipid-modifying pharmacological agents, particularly statins, on circulating PCSK9 concentrations are summarized, and finally, data are presented on PCSK9 inhibition as a novel approach to the treatment of hypercholesterolemia.

PCSK9 Concentrations and LDL-C Levels

Several groups have reported a correlation between circulating PCSK9 concentration and LDL-C level[14-16, 21, 22]. Furthermore, plasma PCSK9 concentration has been shown to positively correlate with the LDL-apolipoprotein (apo) B100 fractional catabolic rate, suggesting that PCSK9 is a marker of LDL catabolism[23]. However, the correlation between PCSK9 and LDL-C level has shown to be less significant than expected, with several factors potentially associated with this observation. First, the serum PCSK9 level does not reflect total hepatic PCSK9 secretion, as the high levels of PCSK9 are cleared from circulation by binding to hepatic LDLRs. However, the mechanism by which PCSK9 is cleared from the circulation is not fully understood, as Cameron *et al.* have reported that plasma PCSK9 is cleared by an LDLR-independent mechanism[24]. Second, circulating PCSK9 is present not only in its free form, but is also as a complex with apoB-containing lipoproteins[25]. Furthermore, PCSK9 directly increases hepatic production of apoB-containing lipoproteins[7]. Third, among several ELISAs that have been developed to measure PCSK9 concentration[14-20], it remains unclear which form of PCSK9 is detected by each assay, except for two reports of the detection of both mature and furin-cleaved PCSK9[14, 17]. Moreover, PCSK9 concentrations varied widely between different assays (80−4000 ng/mL). Finally, PCSK9 concentrations are reduced with fasting (up to 58% lower following 36 h of fasting[26, 27]. Despite wide fluctuations in plasma PCSK9 concentrations over the course of a day, however, little diurnal variation in plasma LDL-C levels has been reported. Thus, additional factors may contribute to the relationship between circulating PCSK9 and LDL-C levels.

Lipid-Modifying Pharmacological Agents and PCSK9 Concentrations

Statins

Statins, HMG-CoA reductase inhibitors, are the

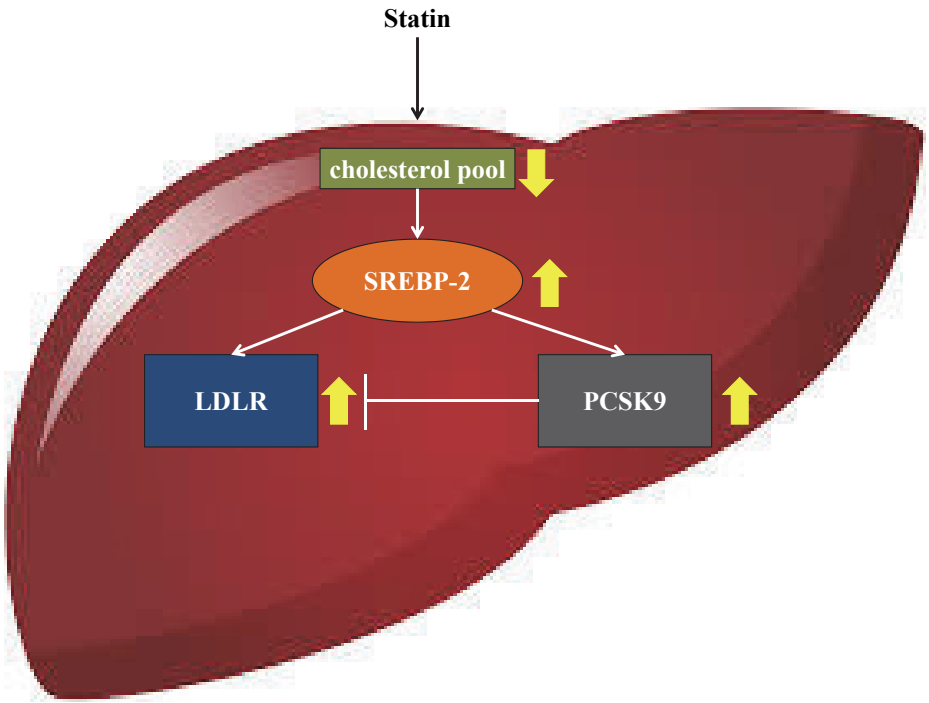

Fig. 2. SREBP-2-mediated co-expression of LDLR and PCSK9

Statin-induced reduction of the cholesterol pool in hepatocyte activates SREBP-2-medicated LDLR expression, thereby increasing hepatic LDL-C uptake. Concurrently, SREBP-2 up-regulates the expression of PCSK9 and enhances hepatic LDLR degradation. Thus, SREBP-2-mediated co-expression of LDLR and PCSK9 prevent excessive cholesterol uptake in hepatocytes in order to preserve cholesterol homeostasis.
SREBP-2, sterol-regulatory element binding protein-2; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9.

most commonly prescribed class of LDL-C-lowering drugs, although they have several limitations. One limitation is that a linear dose-dependent LDL-C lowering effect is not seen with statin use[28], and another is termed the "stain escape phenomenon," whereby LDL-C levels are reported to increase following prolonged statin therapy[29]. Statins decrease hepatic intracellular cholesterol resulting in increased nuclear translocation of sterol-regulatory element binding protein-2 (SREBP-2), which activates LDLRs as well as PCSK9 gene expression[30-32] and increases circulating PCSK9 levels[14-16, 33] (**Fig. 2**). As might be expected, both PCSK9 and LDLR levels are increased by statin therapy[34]. The increased PCSK9 binds to LDLR and directs it toward lysosomal degradation rather than to regular recycling[4, 35], and thus has the potential to limit the efficacy of statin-induced LDL-C reduction[30, 36]. These observations explain the "rule of 6%" for statins, whereby each doubling of the statin dose results only in an approximately 6% additional reduction in LDL-C level. Given this limitation of statin therapy, PCSK9 inhibition represents a logical strategy to enhance statin-induced LDL-C reduction[34].

The effect of statin therapy on circulating PCSK9 concentration is summarized in **Table 1**. Mayne *et al.* reported that 6 weeks of treatment with 10 mg of atorvastatin significantly increased plasma PCSK9 by 7.4%[37]. Costet *et al.* also reported that 10 mg of atorvastatin treatment increased PCSK9 by 14% at 6 weeks[38]. Guo *et al.* reported that atorvastatin 10 mg slightly increased serum PCSK9 by 5−7%, although the effect was not significant, while atorvastatin 20 mg significantly increased serum PCSK9 by 35% at 8 weeks[39]. Careskey *et al.* reported that 16 weeks of treatment with 40 mg of atorvastatin reduced LDL-C levels by 42%, with a significant 34% increase in serum PCSK9 levels[15]. Welder *et al.* reported that 80 mg of atorvastatin increased PCSK9 levels by 47% and decreased LDL-C levels by 55%[33]. Finally, Khera *et al.* reported that 16 weeks of treatment with 10 mg and 80 mg atorvastatin increased circulating PCSK9 levels by 19% and 27%, respectively[40]. These results suggest a clear dose-response effect of atorvastatin on PCSK9 levels, with higher doses of atorvastatin causing a greater increase in circulating PCSK9 concentrations. However, the effects of other types of statins on

Table 1. Effect of statins on circulating PCSK9 levels

Types of statin	Dose (mg/day)	Duration of treatment	% change in PCSK9	Author (reference)
Atorvastatin	10	1 day	13%	Guo (39)
	10	1 day	24%	Costet (38)
	10	4 weeks	5.7%	Guo (39)
	10	6 weeks	7.4%	Mayne (37)
	10	6 weeks	14%	Costet (38)
	10	8 weeks	7.2%	Guo (39)
	10	16 weeks	19%	Khera (40)
	20	4 weeks	30%	Guo (39)
	20	8 weeks	35%	Guo (39)
	20	8 weeks	39%	Guo (98)
	40	12 weeks	38%	Tremblay (99)
	40	16 weeks	34%	Careskey (15)
	80	1 day	27%	Guo (39)
	80	4 weeks	37%	Raal (42)
	80	16 weeks	27%	Khera (40)
	80	16 weeks	47%	Welder (33)
Simvastatin	10	6 weeks	no change	Lakoski (21)
	20	16 weeks	13%	Khera (40)
	40	14 days	68%	Berthold (41)
	80	16 weeks	41%	Khera (40)
Rosuvastatin	20	1 year	28-35%	Awan (22)
	40	4 weeks	37%	Raal (42)
Pitavastatin	4	8 months	31%	Nozue (43)
Pravastatin	20	8 months	34%	Nozue (43)

PCSK9; proprotein convertase subtilisin/kexin type 9.

circulating PCSK9 levels have been less evaluated. Lakoski *et al*. demonstrated that 10 mg of simvastatin for 6 weeks did not increase circulating PSCK9 levels[21], while Berthold *et al*. reported that 40 mg of simvastatin treatment for 14 days increased circulating PCSK9 concentrations by 68%[41]. In addition, Khera *et al*. reported that 16 weeks of treatment with simvastatin 20 mg increased circulating PCSK9 levels by 13% (not statistically significant), whereas simvastatin 80 mg significantly increased PCSK9 levels by 41% in the same period[40]. Awan *et al*. reported that 20 mg of rosuvastatin increased PCSK9 by 35% in women and 28% in men after 1 year of treatment[22], while Raal *et al*. reported that 40 mg of rosuvastatin for 4 weeks increased PCSK9 levels by 37% in heterozygous FH[42]. Recently, we reported that 4 mg of pitavastatin and 20 mg of pravastatin significantly increased serum PCSK9 levels by 31% and 34%, respectively[43]. A recent meta-analysis demonstrated that a greater PCSK9 elevation was observed with lipophilic statins (atorvastatin, simvastatin, pitavastatin, and fluvastatin) compared with hydrophilic statins (rosuvastatin and pravastatin)[44]. Taken together, each type of statins appears to cause an increase in plasma PCSK9 concentration, although the extent of the increase in circulating PCSK9 may be dependent on the dose and the liphophilic or hydrophilic natures of the statin.

It is important to understand the rapidity with which statins increase PCSK9 levels, or whether this effect is sustained over time. Welder *et al*. reported that serum PCSK9 levels increased by 47% after 4 weeks of 80 mg atorvastatin, and this increase was sustained at 8, 12, and 16 weeks. They concluded that high-dose atorvastatin caused a rapid, sustained increase in serum PCSK9[33]. Guo *et al*. also reported that atorvastatin 10 mg showed a tendency to increase PCSK9 levels but this effect was not statistically significant, although atorvastatin 20 mg significantly increased serum PCSK9 by 30% at 4 weeks and by 35% at 8 weeks[39]. They further investigated the rapidity of atorvastatin treatment on PCSK9 level, and found that serum PCSK9 rapidly increased by 13% following 24 h treatment with atorvastatin 10 mg and by 27% with atorvastatin 80 mg[39]. Thus, they concluded that the short-term impact of low-dose atorvastatin on PCSK9 level was time and dose dependent, with a

rapid increase in PCSK9 level observed within 24 h. This indicates that, although atorvastatin may up-regulate both the LDLR and PCSK9 genes by activating SREBP-2, the PCSK9 gene response to atorvastatin might be faster or more sensitive − or more dose-dependent − than the LDLR gene. By comparison, Okada *et al*. reported that plasma PCSK9 levels were significantly increased at 12 weeks of statin therapy, although this increase was not sustained and was lower at 52 weeks[45]. Therefore, increases in serum PCSK9 levels caused by statin therapy appear to differ over short-, middle-, and long-term periods. Statins likely increase the expression and secretion of the PCSK9 protein to such an extent that circulating PCSK9 levels exceed LDLR binding capacity, resulting in increased PCSK9 protein being detected in the serum at an early stage of treatment[46]. However, the increased PCSK9 protein subsequently binds to LDLR and this complex is cleared from the circulation. Thus, circulating PCSK9 may reach a plateau concentration in the long term.

More importantly, statin treatment completely abolishes the correlation between PCSK9 and plasma LDL-C level, restricting its putative usefulness as a biomarker of lipid metabolism in routine clinical practice[33, 47]. It is therefore relevant to consider whether baseline circulating PCSK9 levels can predict the efficacy of statins on LDL-C reduction. At least three studies have addressed this question, leading to discrepant results[14, 21, 33]. First, Dubuc *et al*. observed a significant positive correlation between baseline plasma PCSK9 level and the percentage reduction in LDL-C after statin therapy[14]. Welder *et al*. reported a modest relationship between baseline PCSK9 level and change in LDL-C, with a relatively higher baseline PCSK9 level tending to be associated with a numerically greater decrease in LDL-C; however, this correlation did not achieve statistical significance[33]. Finally, another study failed to show a significant relationship between baseline PCSK9 level and response to LDL-lowering therapy[21]. Additional randomized studies with higher doses and other types of statins are therefore needed to explicitly address this question.

Another question is whether the statin-induced increase in PCSK9 correlates with the magnitude of statin-induced reduction in LDL-C level. Awan *et al*. observed a significant relationship between LDL-C reduction and increased PCSK9 concentration in response to statin therapy[22]. Berthold *et al*. also reported that increased PCSK9 was inversely correlated with the percentage change in LDL-C concentration[41]. Welder *et al*. showed a trend toward an inverse correlation between percentage change in PCSK9 level and in LDL-C level, although this trend did not achieve statistical significance[33]. However, Careskey *et al*. observed no significant relationship between increased PCSK9 level and reduced LDL-C after 4 months of treatment with 40 mg atorvastatin[15]. Lakoski *et al*. also reported that changes in plasma PCSK9 level did not correlate with the magnitude of the LDL-C lowering response achieved with 10 mg of simvastatin[21]. Consistent with these reports, we previously showed that the percentage change in PCSK9 level did not correlate with the LDL-C reduction observed after statins therapy[48]. Furthermore, although the reduction in LDL-C was greater with pitavastatin than with pravastatin, the level of increase in PCSK9 did not differ between these statins[48]. Therefore, it should be noted that the serum PCSK9 level might not reflect the total statin-induced increase in hepatic PCSK9 secretion, as higher levels of PCSK9 bind to hepatic LDLRs and are subsequently removed from the circulation. This likely explains why statin-induced increases in serum PCSK9 levels might not reflect the full extent of the modulation of hepatic synthesis and secretion of the PCSK9 protein by statins.

Ezetimibe

Another commonly prescribed LDL-C-lowering drug is ezetimibe, a cholesterol absorption inhibitor that potently inhibits the absorption of biliary and dietary cholesterol by binding the cholesterol transport protein Niemann-Pick C1-Like 1[49]. Ezetimibe lowers hepatic cholesterol levels by decreasing the amount of cholesterol supplied to the liver through intestinal uptake, leading to increased hepatic LDLR expression, which results in increased LDL uptake from the plasma and thus decreased circulating LDL-C levels[49, 50]. LDL-C reductions of approximately 21% and 23% were achieved by ezetimibe monotherapy and combination therapy with statins, respectively[51]. Compared with statins, fewer studies have been reported on the effect of ezetimibe (alone or combined with statins) on PCSK9 levels. In a pre-clinical study, ezetimibe therapy alone for 7 months significantly increased plasma PCSK9 levels by 37% in dyslipidemic monkeys[52]. Davignon *et al*. showed that ezetimibe added to statin therapy further increased PCSK9 levels beyond the increases observed with a statin alone[53]. Dubuc *et al*. also reported that treatment with ezetimibe in combination with statins was associated with a significant increase in plasma PCSK9 levels compared with statin therapy alone[14]. Berthold *et al*. investigated the effect of ezetimibe and simvastatin, alone and in combination, on circulating PCSK9 concentrations in healthy men. They found that ezetimibe treatment alone for 2 weeks increased plasma PCSK9 con-

centrations by 10%, although this increase was not significant[41]. Furthermore, when ezetimibe was added to 40 mg of simvastatin, an incremental increase in PCSK9 concentration was not observed. Lakoski *et al*. reported 6 weeks of ezetimibe monotherapy had no significant effect on PCSK9 concentration, although LDL-C levels were lowered by 20%[21]. Miyoshi *et al*. also reported that PCSK9 concentrations were unchanged following ezetimibe monotherapy or in combination with strong statins for 24 weeks[54]. Thus, although there are some discrepancies regarding the effect of ezetimibe on circulating PCSK9 levels, a recent meta-analysis reported that there was no significant elevation in plasma PCSK9 levels with statin/ezetimibe combination therapy compared with statin monotherapy[44]. There are a number of potential explanations for these discrepancies. First, these differences may be related to the study period, as increases in serum PCSK9 levels caused by statin therapy differ according to treatment duration. Indeed, Okada *et al*. reported that ezetimibe as add-on to stain therapy had no effect on plasma PCSK9 levels at 12 weeks, but that PCSK9 was significantly increased at 52 weeks[45]. Second, statin treatment maximally increases circulating PCSK9 to a plateau concentration, after which no further increase is possible by further lowering of LDL-C using ezetimibe. Third, statins upregulate peroxisome proliferator-activated receptor (PPAR)-α, β, γ, and δ, which are involved in the regulation of PCSK9 expression in the liver[55, 56]. Finally, ezetimibe might be unable to increase PCSK9 concentrations because of its weak LDL-C-lowering effect.

Fibrates

Activators of PPAR-α, fibrates are the next most commonly prescribed class of lipid-lowering drugs. Following its activation by fibrates, PPAR-α alters the transcription of multiple target genes that play a key role in lipid metabolism. Fibrates thus reduce plasma levels of triglycerides by approximately 30−50% and increase high-density lipoprotein cholesterol levels by 5−15%. Unlike statin treatment, the effect of fibrate treatment on LDL-C and PCSK9 is less clear. Kourimate *et al*. demonstrated that fibrate treatment reduced PCSK9 mRNA levels and PCSK9 protein expression in hepatocytes[57]. Post-hoc analysis of a subgroup of the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study indicated that 6-week treatment with 200 mg of fenofibrate moderately decreased plasma PCSK9 concentrations by 8.5% in patients with type 2 diabetes[19]. Chan *et al*. also reported that 12-week treatment with fenofibrate 145 mg decreased serum PCSK9 concentrations by 13% in patients with type 2 diabetes who were treated with statins[58]. However, in contrast to these results, Mayne *et al*. reported that 24-week treatment with fibrates increased serum PCSK9 levels by 17%[37], while Costet *et al*. reported that 6-week treatment with fenofibrate 160 mg increased PCSK9 by 26%[38]. Troutt *et al*. reported that fenofibrate (200 mg for 12 weeks) significantly increased circulating PCSK9 levels by 25%[59]. The precise reasons for these discrepancies in the effect of fibrate on circulating PCSK9 concentrations and the mechanisms of fibrate-induced changes in PCSK9 remain unclear. Noguchi *et al*. compared the effects of bezafibrate (400 mg), a pan-agonist for PPAR-α, β, and δ with fenofibrate (200 mg), a more selective ligand for PPAR-α, on plasma PCSK9 concentrations[60]. They reported that plasma PCSK9 concentrations at 8 weeks were significantly increased by 39.7% for bezafibrate and 66.8% for fenofibrate. Thus, the effect of fibrate on circulating PCSK9 may differ according to type of fibrate. Additional studies are therefore required to better understand the mechanism by which fibrate induces changes in PCSK9 concentrations.

Nicotinic Acid (Niacin)

Nicotinic acid (niacin) has been used clinically as an LDL-C lowering drug for more than 50 years, although the mechanism behind its LDL-C lowering effect remains unclear. Khera *et al*. reported that one year of treatment with a combination of simvastatin 20 mg and niacin decreased PCSK9 levels by 13%, suggesting that niacin offsets the statin-mediated increase in PCSK9[40]. Furthermore, niacin decreased PCSK9 levels by 17% in patients treated with both atorvastatin and fenofibrate. This reduction was significantly positively correlated with LDL-C reduction ($r=0.62$, $p=0.006$). Thus, the LDL-C reduction observed with niacin therapy may be due in part to a reduction in PCSK9.

Inhibitors of PCSK9

Several therapeutic approaches to inhibit PCSK9 are summarized in **Table 2**. These approaches include blocking the binding of PCSK9 to LDLR using antibodies, adnectins, or mimetic peptides; inhibiting PCSK9 expression with CRISPR/Cas9 genome-editing technology, antisense oligonucleotides (ASOs), or small interfering RNA (siRNA); and interfering with PCSK9 secretion from the endoplasmic reticulum.

Monoclonal Antibodies

Monoclonal antibodies to PCSK9 are the most common method of PCSK9 inhibition since this approach was first described in 2009[61]. These antibodies bind to a specific region of PCSK9 to inhibit the

Table 2. A new approach to PCSK9-targeting drugs

Mechanisms	Representative agents
Blocking the combination of PCSK9 and LDLR	Mimetic peptides Adnectins Monoclonal antibodies (alirocumab, evolocumab, bococizumab)
Inhibiting PCSK9 expression	CRISPR/Cas9 technology Small molecule (berberine, oleanoic acid) Antisense oligonucleotides Small interfering RNA
Interfering with PCSK9 secretion	Sortilin Sec24a

PCSK9; proprotein convertase subtilisin/kexin type 9, LDLR; low-density lipoprotein receptor.

interaction between PCSK9 and LDLR. The first neutralizing anti-PCSK9 antibody was shown to increase LDLR expression in hepatocytes and reduce LDL-C concentrations by 30%[61]. Several other monoclonal antibodies have since been described, with dose-dependent reductions of 20−50% in LDL-C levels[62, 63]. Many clinical trials of monoclonal antibodies against PCSK9, including evolocumab and alirocumab, have been reported[64-73]. In phase Ⅱ studies lasting 8−12 weeks, alirocumab lowered LDL-C levels by 40−70% when added to statin therapy[64-66]. Evolocumab has been shown to reduce LDL-C levels by approximately 60% in phase Ⅲ trials[67-71]. Thus, significant reductions in LDL-C levels have been reported for each PCSK9 antibody. Furthermore, the effects of PCSK9 antibodies on LDL-C levels are not affected by age, gender, body mass index, LDL-C concentration, background statin intensity, or dose of statins[64-73]. Thus, PCSK9 antibody therapy represents one of the most promising and novel approaches to reducing LDL-C levels. However, unlike evolocumab and alirocumab, which are fully humanized monoclonal antibodies, bococizumab is a humanized monoclonal antibody with 3% murine sequence remaining in the antigen-binding complementarity-determining regions[74]. Bococizumab lowered LDL-C by 54.2% at 12 weeks, but this reduction was attenuated to 40.4% at 52 weeks. After 1 year of bococizumab treatment, 48% of patients had detectable antidrug antibodies, and a titer-dependent attenuation in LDL-C reduction was noted. Thus, the development of bococizumab was discontinued in November 2016.

It remains questionable whether the reduction of LDL-C using PCSK9 antibodies might reduce the risk of cardiovascular events. Sabatine *et al*. reported that evolocumab reduced LDL-C levels by 61%, and the rate of cardiovascular events following 1 year of treatment was reduced to 0.95% from 2.18% in the control group (hazard ratio, 0.47; $p=0.003$)[75]. In a post-hoc analysis of the ODYSSEY LONG TERM (Long-term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy) trial, the rate of major adverse cardiovascular events (death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization) was lower with alirocumab than with placebo (hazard ratio, 0.52; $p=0.02$)[76]. A systematic review and meta-analysis of 24 randomized control trials showed that use of PCSK9 antibodies was associated with a lower rate of all-cause mortality (odds ratio, 0.45; 95% confidence interval [CI], 0.23−0.86; $p=0.015$) and myocardial infarction (odds ratio, 0.49; 95% CI, 0.26−0.93; $p=0.03$) and no statistically significant reduction in cardiovascular mortality (odds ratio, 0.50; 95% CI, 0.23−1.10; $p=0.084$) compared with no anti-PCSK9 treatment[77]. Furthermore, the more recent randomized, double-blind, placebo-controlled FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) trial demonstrated that evolocumab as add-on to statin therapy decreased LDL-C levels from 92 mg/dL to 30 mg/dL (−59%). Relative to placebo, evolocumab treatment significantly reduced the risk of the primary composite endpoint such as cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization (hazard ratio, 0.85; 95% CI, 0.79−0.92; $p<0.001$)[78]. However, this reduction in events was less than would be expected for such a significant reduction in LDL-C, and the reasons for this disconnect between LDL-C lowering effect and cardiovascular events remain unclear.

The next question is whether elevated circulating PCSK9 and inhibition of PCSK9 affect the progres-

sion/regression of atherosclerosis. A significant correlation between circulating PCSK9 and carotid intima-media wall thickness has been reported[79]. In addition, Li *et al*. reported that plasma PCSK9 levels were positively associated with platelet count in patients with CAD, suggesting a potential link between PCSK9 and platelets that may underlie the mechanisms involved in atherosclerosis[80]. Furthermore, Cheng *et al*. found that PCSK9 concentrations were linearly associated with a higher necrotic core fraction, as evaluated by intravascular ultrasound with virtual histology within coronary plaques[81]. Thus, the PCSK9 protein appears to be a key modulating factor in atherosclerosis[82]. However, Zhu *et al*. reported that there was no significant relationship between PCSK9 concentration and cardiovascular events. They concluded that although PCSK9 is an important therapeutic target for reducing LDL-C, it is unlikely to present a biomarker of atherosclerotic risk[83]. A recent meta-analysis also reported that circulating PCSK9 concentration was not significantly associated with the risk of cardiovascular events[84]. To date, no trial has evaluated whether LDL-C lowering with a PCSK9 antibody has favorable effects on coronary atherosclerosis, and no data have assessed whether the very low LDL-C levels achieved with a PCSK9 antibody lead to an incremental benefit in reducing disease progression compared with statins alone. The GLAGOV (Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound) trial was designed to evaluate the effects of evolocumab on the progression of atherosclerosis using intravascular ultrasound[85]. This trial demonstrated that 420 mg of evolocumab in addition to statins for 76 weeks reduced LDL-C levels from 92.6 mg/dL to 36.6 mg/dL (−56.3%), and significantly decreased the percent atheroma volume by 0.95% compared with placebo[86]. Thus, a PCSK9 antibody in combination with statin therapy had a favorable effect on progression of coronary artery plaques. However, no trial to date has examined whether PCSK9 inhibitors can stabilize coronary artery plaques, as reported with statins.

It has been reported that PCSK9 antibodies reduce lipoprotein (Lp)(a) levels[66-68, 77, 87]. However, the mechanisms of this effect are poorly understood. Serum Lp(a) levels in FH with LDLR mutations have been shown to be elevated, suggesting that Lp(a) is catabolized via the LDLR pathway[88]. In addition, serum Lp(a) was elevated in patients with FH as a result of PCSK9 gain-of-function mutations to the same extent as that caused by LDLR mutations in FH[89]. This suggests that LDLR plays an important role in Lp(a) catabolism. However, statins, whose main mechanism of action involves the up-regulation of LDLR, are incapable of reducing Lp(a) levels[90]. Furthermore, apoB in Lp(a) does not interact with LDLR, suggesting that LDLR does not play a role in Lp(a) kinetics[91]. Recently, we have reported that serum PCSK9 levels were positively correlated with serum levels of Lp(a), small, dense LDL, and oxidized LDL in patients with CAD[48]. Thus, the interaction between serum PCSK9 and apoB-containing lipoproteins plays a role in atherosclerosis[7]. Although there are no reports about the effects of PCSK9 antibodies on small, dense LDL or oxidized LDL, our findings suggest that PCSK9 antibodies might reduce Lp(a) to the same extent that they reduce small, dense LDL and oxidized LDL levels. Indeed, only one study to data has reported that PCSK9 antibodies reduced LDL-C levels and particle numbers[92]. These effects of PCSK9 antibodies on apoB-containing atherogenic lipoproteins might be responsible for the associated reduction in risk for cardiovascular events.

Antisense Oligonucleotides (ASO) and Small Interfering RNA (siRNA)

ASO and siRNA induce PCSK9 mRNA degradation. Thus, both strategies ultimately result in the silencing of the gene. Graham *et al*. demonstrated that an ASO for PCSK9 reduced total cholesterol by 53% in mice fed a high-fat diet, while also causing a two-fold increase in hepatic LDLR protein levels[93]. Similarly, Gupta *et al*. reported that an ASO lowered PCSK9 mRNA expression by 60% in mice and increased hepatic LDLR protein expression almost threefold[94]. Furthermore, Yamamoto *et al*. reported that the strong inhibition of PCSK9 by twice weekly ASO administration for 6 weeks reduced LDL-C levels by 43% in atherogenic diet-fed mice[95]. Administration of siRNA against PCSK9 mRNA in rats reduced LDL-C concentrations by 30%[96], while in monkeys, LDL-C was reduced by 56−70% and the effects lasted for a week[96]. Fitzgerald *et al*. reported the results of a phase I trial which investigated the safety and efficacy of an administration of siRNA in healthy volunteers. siRNA produced a 70% reduction in circulating PCSK9 protein levels and a 40% reduction in LDL-C from baseline[97]. This study was the first to demonstrate the use of an siRNA therapy to modulate a clinically validated endpoint in humans. These results support the further assessment of siRNA therapy in patients with hypercholesterolemia, including those treated with statins. However, relative to antibody-based therapies, siRNA represents a novel treatment strategy and their long-term safety remains unknown. Therefore, further trials are necessary to evaluate the efficacy and safety of siRNA for reducing LDL-C levels and risk of cardiovascular events.

Conclusions

PCSK9 has been identified as a key regulator of serum cholesterol levels and represent a novel pharmacological target for hypercholesterolemia. The major classes of commonly prescribed lipid-lowering agents, particularly statins, clearly increase circulating PCSK9 levels and which likely diminishes the effect of these drugs on the reduction of LDL-C concentrations. Thus, PCSK9 inhibitors, particularly monoclonal antibodies against PCSK9, in combination with statins, are one of the most promising and effective approaches to achieving very low LDL-C levels and reducing the risk of cardiovascular events.

Disclosure

None.

Conflict of Interest

None.

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