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PCSK9 inhibitors: Novel therapeutic agents for the treatment of hypercholesterolemia

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ABSTRACT

Reducing plasma levels of low-density lipoprotein cholesterol (LDL-c) remains the cornerstone in the primary and secondary prevention of cardiovascular disease. However, a substantial proportion of patients fail to achieve acceptable LDL-c levels with currently available lipid-lowering drugs. Over the last decade, inhibition of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) has emerged as a promising target to reduce residual cardiovascular disease risk. Binding of PCSK9 to the LDL receptor targets the LDL receptor for lysosomal degradation. Inhibition of PCSK9 increases expression of the LDL receptor. This observation has led to the development of a number of approaches to directly target PCSK9. Three monoclonal antibodies against PCSK9 are currently being evaluated in phase 3 trials involving various patient categories on different background lipid lowering therapies. Current evidence shows reductions in LDL cholesterol levels of up to 70%, independent of background statin therapy. The results of phase 3 trials will demonstrate the long-term efficacy and safety of PCSK9 inhibition, and will indicate whether LDL-c lowering induced by this novel approach translates into beneficial effects on CVD outcome.

1. Introduction

Despite advances in prevention and treatment, cardiovascular disease (CVD) remains the number one cause of death globally (WHO, 2011). Numerous intervention studies have shown that lowering Low-Density Lipoprotein cholesterol (LDL-c) effectively Q3 reduces the risk of subsequent CVD (CCTC et al., 2010). Clinical guidelines recommend statins as first choice lipid-lowering agents because of their efficacy in lowering LDL-c and CVD risk and their favorable safety profile and cost-effectiveness. (NCGC, 2014; Stone et al., 2014; Lazar et al. 2011) However, a significant proportion of patients is either unable to tolerate statins at adequate doses or fails to achieve acceptable lipid control on statin therapy (CCTC et al., 2010; Baigent et al., 2005; Bruckert et al., 2005; Zhang et al. 2013). Consequently, novel targets to lower LDL-c are eagerly sought. Several recent clinical trials have shown promising results for Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) inhibitors.

2. PCSK9

2.1. Discovery

The crucial role of PCSK9 in cholesterol metabolism was first recognized when gain-of-function mutations were identified in two French families with the clinical phenotype of familial hypercholesterolemia (FH). FH is an autosomal dominant disorder characterized by severely elevated LDL-c levels and increased cardiovascular risk. In these families, Abifadel and coworkers did not identify mutations in the genes that were known to be associated with FH at that time (*LDLR*, coding for the LDL receptor or *APOB*, coding for apoB). However, linkage analysis did show mutations in *PCSK9*-a gene without a known direct biological link with cholesterol metabolism until that moment (Abifadel et al., 2003).

2.2. Endogenous function

PCSK9, originally discovered as neural apoptosis regulated convertase-1 (NARC-1), is primarily synthesized in the small intestine and the liver (Benjannet et al., 2004). After intracellular autocatalytic cleavage of its prodomain, mature PCSK9 is secreted from the liver cells (McNutt et al., 2007). Animal studies have shown that binding of PCSK9 to the LDL receptor targets the receptor for lysosomal degradation, thereby providing a possible

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mechanism through which PCSK9 may affect cholesterol metabolism (Lo Surdo et al., 2011). In the absence of PCSK9, the hepatic LDL receptor is shuttled back to the plasma membrane after delivering cholesterol to the lysosome for degradation. Binding of PCSK9, however, prevents this shuttling of the LDL receptor and instead targets it for degradation (Lagace et al., 2006). PCSK9 primarily acts on the LDL receptor as a circulating plasma protein and several small-scaled studies have shown a positive relationship between circulating PCSK9 and levels of LDL-c (Lambert et al., 2012).

PCSK9 and the LDL receptor expressions are both primarily regulated by intracellular levels of cholesterol through the transcription factor sterol-responsive element binding protein-2 (SREBP-2). Consequently, by reducing hepatic intracellular cholesterol levels, the beneficial effect of statins on LDL receptor expression is partially counteracted by increased expression of PCSK9. Indeed, several studies have demonstrated a positive relationship between statin treatment and circulating PCSK9 levels (Lambert et al., 2012). Consequently, inhibition of PCSK9 could potentially exert a synergistic effects on statins.

2.3. *In-vitro and animal studies*

The mechanisms by which PCSK9 is involved in cholesterol metabolism were first elucidated when *in vitro* studies revealed that adding PCSK9 to liver cells reduced LDL receptor levels (Lagace et al., 2006). It was subsequently shown that carriers of a gain-of function mutation in the *PCSK9* gene were characterized by severe hypercholesterolemia and had a ten-fold reduction in LDL receptor levels as compared to non-carriers. Furthermore, *in vivo* models demonstrated that overexpression of PCSK9 reduced LDL receptor expression and caused elevated plasma LDL-c. PCSK9-knockout mice, on the other hand, were characterized by increased LDL receptor expression and reduced circulating LDL-c (Maxwell and Breslow, 2004; Park et al., 2004; Rashid et al., 2005).

Subsequent animal studies demonstrated a positive relationship between PCSK9 and atherosclerosis. Overexpression of PCSK9 in mice fed a Western Diet resulted in a 230% increase in aortic cholesteryl ester content compared to wild-type mice. Conversely, PCSK9-knockout mice were characterized by a 74% reduction in aortic cholesteryl ester content (Denis et al., 2012).

2.4. *PCSK9 mutations in humans*

Several large cohort studies have demonstrated an association between mutations in the *PCSK9* gene and LDL-c levels and CVD risk (Cohen et al., 2005, 2006; Benn et al., 2010). The prevalence of specific PCSK9 mutations differs amongst various ethnicities. Specifically, nonsense mutations Y142X and C679X are rare in white individuals, whereas these mutations were present in 2.6% of black individuals. In black individuals, these mutations were associated with a 28% reduction in LDL-c levels and a 88% reduction in the risk of coronary heart disease (CHD). The R46L variation was present in 3.2% of the white subjects compared to 0.6% of the black subjects. This mutation was associated with a 15% reduction in circulating LDL-c and a 47% reduction in CHD risk in the white subjects (Cohen et al., 2006). Three Danish cohort studies demonstrated an 11–16% reduction in LDL-c levels and a 6–46% reduction in CHD risk among patients with a null-allele (Benn et al., 2010). In addition, individuals with FH and a D374Y mutation in the *PCSK9* gene were characterized by severe hypercholesterolemia and premature CHD (Naoumova et al., 2005). The effect of single nucleotide polymorphisms (SNPs) in the *PCSK9* gene on LDL-c levels and CVD has been further substantiated in several Mendelian randomization studies (Benn et al., 2010; Cohen et al., 2006). Notably, Cohen et al. (2006) showed that patients with a 46L allele were characterized by a greater reduction in CVD risk than would be expected solely on the reduction of LDL-c levels (Ference et al., 2012).

Importantly, there is paucity of data on the association between circulating PCSK9 plasma levels and CVD events. In an observational study, Li et al. (2014) showed that plasma levels of PCSK9 correlated with the severity of coronary atherosclerosis in a cohort of 243 patients with established coronary lesions who were not treated with lipid-lowering drugs. The association persisted after adjusting for lipid parameters. Moreover, in a prospective analysis performed in patients with stable CHD on statin treatment, PCSK9 levels were shown to predict future cardiovascular events (Werner et al., 2014; Leander et al., 2014).

3. PCSK9 inhibition

A number of pharmacologic approaches to inhibit PCSK9 function are currently in development. One approach is the development of agents that interfere with LDL receptor binding by targeting PCSK9 in the circulation, including several monoclonal antibodies (mAb), small peptides and adnectines. A second approach is to reduce hepatic PCSK9 synthesis through gene silencing with small interfering RNA (siRNA) or antisense oligonucleotides. The third approach, which has not yet reached clinical development, involves inhibition of PCSK9 production by targeting its intracellular processing (Lambert et al., 2012).

3.1. *Gene silencing*

Several animal studies have demonstrated the LDL-c lowering potential of *PCSK9* gene silencing.

Twice-weekly administration of the antisense oligonucleotide ISIS394814, produced by ISIS Pharmaceuticals (Carlsbad, CA, USA), induced a 2-fold increase in hepatic LDL receptor levels in mice on a high-fat diet, and a 38% reduction in LDL-c levels (Graham et al., 2007). The potential of *PCSK9* gene silencing to induce LDL receptor up-regulation was subsequently confirmed in mice using locked nucleic acid (LNA) oligonucleotides produced by Santaris Pharma A/S (Copenhagen, Denmark, currently Roche) (Gupta et al., 2010). Intravenously administered LNA oligonucleotides induced a 60% reduction in PCSK9 mRNA for >16 days, which was accompanied by a 2.5 to 3-fold increase in hepatic LDL receptor levels without hepatotoxicity. These results were subsequently corroborated in monkeys. A loading dose of LNA oligonucleotides (20 mg/kg) followed by 4 weekly maintenance doses of 5 mg/kg decreased LDL-c by 50% while HDL-c levels remained unaffected. Furthermore, no changes were observed in laboratory parameters of hepatic and renal function (Lindholm et al., 2012).

In addition, the siRNA molecules LNP-PCS-A2 and LNP-PCS-B2 developed by Alnylam Pharmaceuticals (Cambridge, MA, USA), administered using lipidoid nanoparticles (LNP) for efficient hepatic delivery at 5 mg/kg, induced an average 56% reduction in LDL-c after a single administration in nonhuman primates, without affecting HDL-c levels. Importantly, the beneficial effects on LDL-c levels persisted 3 weeks after a single intravenous dose (Frank-Kamenetsky et al., 2008).

The efficacy and safety of ALN-PCS developed by Alnylam (Cambridge, MA, USA), a siRNA comparable to LNP-PCS, was subsequently investigated in a phase I placebo-controlled dose-escalation study in healthy adults with an LDL-c >116 mg/dl who were not receiving lipid-lowering therapy. In total, 24 participants were randomized to receive a single intravenous bolus of one of 6 doses of ALN-PCS, varying between 0.015 and 0.400 mg/kg, and 8 patients received placebo. Between groups, the adverse event rates were similar and there were no serious drug-related adverse

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