Pharmacological Research 73 (2013) 27–34

Contents lists available at SciVerse ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Review

The next generation of novel low-density lipoprotein cholesterol-lowering agents: Proprotein convertase subtilisin/kexin 9 inhibitors

Li Shen[a], Hongchun Peng[b], Danyan Xu[a,*], Shuiping Zhao[a,*]

[a] Department of Cardiology, Second Xiangya Hospital, Central South University, Changsha 410011, PR China
[b] Changsha Central Hospital, Changsha 410011, PR China

ARTICLE INFO

Article history:
Received 17 December 2012
Received in revised form 1 April 2013
Accepted 2 April 2013

Keywords:
Proprotein convertase subtilisin/kexin 9
LDL receptor
LDL cholesterol
Gain-of-function mutation
PCSK9 inhibitor
Dyslipidemia

ABSTRACT

Proprotein convertase subtilisin/kexin 9 (PCSK9) has been shown to degrade hepatic low-density lipoprotein receptors (LDLR). Gain-of-function mutations promote the development of familial hypercholesterolemia, whereas loss-of-function mutations are associated with lower levels of circulating low-density lipoprotein cholesterol (LDL-C) and significant protection against coronary heart disease. The major classes of commonly prescribed lipid-lowering medications, such as statins, increase serum PCSK9 levels, thus PCSK9 inhibition would increase the efficacy of statins on LDL-C lowering. Therefore, PCSK9 is an attractive therapeutic target for the new generation of cholesterol-lowering drugs. Here, we present a brief overview of the development of PCSK9 inhibitors and highlight the effect of currently prescribed LDL-C-lowering drugs on PCSK9, and the strategies that are being explored for its therapeutic inhibition. Current research and clinical trial results indicate that a PCSK9 inhibitor may be an exciting new therapeutic drug for the treatment of dyslipidemia and relevant cardiovascular diseases.

Contents

1. Introduction

Many data support the concept that an elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) is a cardinal risk factor in the pathogenesis of coronary heart disease (CHD) [1]. Compelling evidence from population-based data and clinical trials demonstrates that the causal relation between LDL-C reduction and CHD prevention is well established [1,2]. Statins, powerful first-line agents for lowering LDL-C, lead effectively to a 25–30% reduction in CHD risk [3]. Even though statins have been used successfully to treat dyslipidemia and reduce cardiovascular events in humans, residual risks remain: many patients fail to achieve the recommended LDL-C target in clinical practice that either are the highest cardiovascular risk or familial hypercholesterolemia (FH) who require larger reductions of LDL-C due to their high baseline LDL-C levels [3]. In addition, because of

* Corresponding author. Fax: +86 731 85295406.
E-mail address: zhaosp@medmail.com.cn (S. Zhao).

1043-6618/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.phrs.2013.04.001

its tolerability and/or efficacy, 5-years intensive statins therapy reduces the incidence of heart attacks by only 40%, even when LDL-C levels are reduced by 80 mg/dL [4]. Therefore, the impetus for the development of novel pharmacologic agents designed to lower LDL-C levels and CHD risk has been strong.

Proprotein convertase subtilisin/kexin 9 (PCSK9) was identified in 2003 [5] and was first characterized by Abifadel et al. [6] as the third locus involved in autosomal dominant hypercholesterolemia. Subsequently, the importance of PCSK9 was amplified by the observation that *PCSK9* loss-of-function mutations (LOF) were related with a 29% LDL-C decrease and an 88% reduction risk for CHD [7]. Owing to these landmark studies, PCSK9 has emerged as a promising therapeutic target, fostering a large number of research initiatives. Thus, inhibiting or decreasing PCSK9 levels may represent a safe and effective strategy for treating hypercholesterolemia.

2. The structure and physiology of PCSK9

PCSK9, a 72-kDa protease, is predominately synthesized in the liver and small intestine. It contains an N-terminal signal sequence, a prodomain, a catalytic domain, and a cysteine-rich C-terminal domain [8]. Following its synthesis, PCSK9 undergoes autocatalytic cleavage in the endoplasmic reticulum to form a mature enzyme [8]. Once secreted, PCSK9 binds to the epidermal growth factor A extracellular domain of low density lipoprotein receptor (LDLR) on the surface of hepatocytes, triggering LDLR degradation in lysosomes [8], thereby increasing circulating LDL-C levels.

A series of definitive studies rapidly validated the role of PCSK9 in LDL-C metabolism. In vitro, the addition of conditioned media containing recombinant PCSK9 to hepatocytes led to a reduction in the numbers of surface LDLRs [9]. In vivo, numerous overexpression and knockout animal studies demonstrated clearly that PCSK9 leads to LDLR degradation [10,11]. The most persuasive of these were the parabiosis experiments demonstrating that plasma PCSK9, present in the shared circulatory system of parabiosed mice, could be transferred from transgenic to wide-type mice, leading to a dramatic reduction in hepatic LDLR [12]. However, the exact mechanism has not been fully elucidated.

In addition to the well-established effect of PCSK9 on LDL-C, an increasing number of studies have observed a close correlation between PCSK9 and triglyceride (TG)-rich lipoprotein has been noticed in increasing studies [13,14]. Two clinical studies initially found the highest correlations between plasma levels of PCSK9 and TG concentration in the blood [15,16]. Subsequently, it was found in animal models that PCSK9-deficient mice exhibited dramatically decreased postprandial triglyceridemia compared with their wild-type littermates, whereas the output of very low-density lipoprotein in PCSK9-overexpression mice was increased [17]. In addition, a new study demonstrated that D374Y-PCSK9 transgenic mice secrete more TG into the circulation than WT mice [18]. Thus, in addition to its role in modulating plasma LDL-C, PCSK9 may also increase postprandial TG levels. More importantly, pharmacological inhibition of PCSK9 may therefore benefit the management of LDL-C and postprandial TG levels, two important risk factors for cardiovascular diseases.

3. *PCSK9* mutations

PCSK9 gain-of-function (GOF) mutation is related with the significant elevation of plasma PCSK9 concentration. Carriers of the D374Y-PCSK9 was found to have a 5- to 30-fold higher affinity for binding to LDLR compared with the wild-type, and was predisposed to the onset of premature CHD earlier for 10 years [19]. It resulted in extremely severe FH and a decreased sensitivity to statin administration [20]. In some subjects, Cohen et al. [7] identified two nonsense PCSK9 variants, Y142X and C679X, that lowered plasma LDL-C by about 1.0 mmol/L and were associated with an 88% reduction in the incidence of CHD. Another variant R46 L, mainly found in Caucasian subjects, was associated with an LDL-C reduction of 0.5 mmol/L, accompanying a 47% reduction of CHD risk [7]; whereas a recent statin treatment trial produced an LDL-C reduction of 1.0 mmol/L and only reduced CHD risk by 36% [1]. Therefore, at a similar magnitude of LDL-C reduction, the extent of cardiovascular protection achieved in subjects with the *PCSK9* LOF mutation greatly exceeds that obtained in 5-year statin clinical trials.

4. LDL-C lowering agents and their effects on PCSK9

Statin treatment enhances PCSK9 expression in cultured cells, mouse liver, and human plasma by increasing the activity of sterol regulatory element binding protein 2 (SREBP-2), which various studies have confirmed [21,22]. The impact of statins on PCSK9 levels at different degrees mainly depends on the type and dose of statins used, as well as the duration of treatment. Consequently, statins enhance plasma PCSK9 levels dose-dependently [23,24]. PCSK9 upregulation, which promotes LDLR degradation, serves as a counter-regulatory molecular brake on LDL-C lowering. This regulation may limit the effectiveness of statins and provides insight into the mechanism of the nonlinear statin dose–response relationship.

Unlike statin treatment, the effect of fibrates on PCSK9 levels remains controversial. Kourimate et al. [25] have reported that fibrate treatment leads to the reduction of PCSK9 expression in hepatocytes; however, several clinical studies have observed that fenofibrate administration significantly increased circulating PCSK9 levels in patients [21,26,27]. These inconsistent findings have led to much speculation, with one possibility being the difference in plasma PCSK9 level measurement despite all cited studies using the enzyme-linked immunosorbent assay method. Another possible reason for the discrepancies was the different durations of drug administration.

Ezetimibe blocks dietary cholesterol absorption in the intestine by inhibiting the activity of the sterol transporter Niemann-Pick C1-Like 1. Treatment with ezetimibe alone did not exert a significant effect on circulating PCSK9 levels. However, the combination of ezetimibe and statins further increased plasma PCSK9 levels compared to the elevations observed with statins alone [22,28].

The compound berberine (BBR), which is extracted from a plant, has been considered a new cholesterol-lowering drug [29]. BBR decreased PCSK9 expression in HepG2 cells, and the combination of berberine and mevastatin suppressed the PCSK9 mRNA upregulation induced by mevastatin alone [29].

As summarized in Table 1, the major classes of commonly prescribed lipid-lowering medications increase serum PCSK9 levels. These observations come close to explaining why these agents cannot lower LDL-C effectively. Therefore, there is an urgent need to explore methods or novel compounds that decrease or inhibit PCSK9 to improve the efficiency of these agents, something that multiple research laboratories worldwide should consider a top priority.

5. Potential strategies for PCSK9 inhibition

PCSK9 has been considered a highly desirable therapeutic target for a novel cholesterol-lowering drug based on the following observations. First, secreted PCSK9 in the circulation is mainly responsible for the degradation of hepatic LDLRs, and plasma LDL-C is reduced in PCSK9$^{-/-}$ mice [11]. Second, gene inactivation of PCSK9 reduced the areas of atherosclerotic lesions in