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The Impact of PCSK9 on Risk Factors for Ischemic Stroke and Potential Mechanisms

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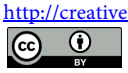

Abstract

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a member of the proprotein convertase (PCs) family, which facilitates the degradation of low-density lipoprotein receptors (LDL-R) via intracellular and cell surface pathways, consequently elevating serum LDL-C levels. PCSK9 is implicated in various processes such as lipid metabolism, atherosclerosis, oxidative stress, inflammatory responses, thrombosis, and apoptosis. It is closely linked to ischemic stroke through its role in inducing and advancing atherosclerosis. PCSK9 inhibitors play a useful role in both acute and secondary prevention of ischemic stroke and can reduce the risk of ischemic stroke. This review examines the influence of PCSK9 on the risk factors associated with ischemic stroke and explores its potential mechanisms, and briefly describes the application of PCSK9 inhibitors in ischemic stroke.

Keywords

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9), Ischemic Stroke, Atherosclerosis, Inflammation

1. Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9), also referred to as neural apoptosis-regulated convertase, is a member of the proprotein convertase subtilisin/kexin family (PCs) [1]. The PCSK9 gene, located on chromosome 1, encodes this protein, which is ubiquitously expressed across various tissues and cells [2]. Structurally, PCSK9 comprises a prodomain (residues 31-152), a signal peptide (residues 1-30), a C-terminal domain (residues 153-451), and a catalytic domain (residues 153-451) [3]. In the bloodstream, PCSK9 is synthesized in the liver's

endoplasmic reticulum, undergoes autocatalytic cleavage in the Golgi apparatus to form pro-PCSK9 (a soluble zymogen), and is subsequently converted into its mature secretory form, which is then secreted and stored in the plasma [4]. Additionally, PCSK9 expression is observed in renal interstitial cells, intestinal epithelial cells, embryonic cells, neural cells, cerebrospinal fluid, and atherosclerotic plaques. The median serum level of PCSK9 in healthy individuals ranges from 11 to 2988 ng/mL and shows a positive correlation with LDL-C levels [5]-[7]. Ischemic stroke (IS) is characterized by acute damage or death of brain cells due to reduced cerebral blood flow, primarily resulting from cerebral artery stenosis, thrombosis, or embolism from other regions of the cerebral artery, accounting for 70% - 80% of all stroke cases. Globally, stroke remains the second leading cause of death and the foremost cause of acquired disability. In China, despite advancements in medical care stabilizing the mortality rate of ischemic stroke, it continues to be a leading cause of death and disability [8]-[11]. The pathogenesis of ischemic stroke involves multiple risk factors, including dyslipidemia, diabetes, and atherosclerosis [8] [12]-[14]. PCSK9 is correlated with ischemic stroke to some extent, influencing its occurrence and progression through pathways associated with stroke risk factors.

2. Effect of PCSK9 on Risk Factors of Ischemic Stroke

2.1. The Role of PCSK9 in Modulating LDL Levels

In normal physiological conditions, the liver is responsible for clearing 70% - 80% of cholesterol in the human body. Within the bloodstream, LDL-C binds to LDL-R on the surface of liver cell membranes, facilitating its transport into the liver cells. Inside the cells, in the acidic environment of lysosomes, LDL-R and LDL-C dissociate. The dissociated LDL-C is then degraded in the lysosome, hydrolyzed into free cholesterol, and stored within the liver cells. Meanwhile, LDL-R recycles back to the hepatocyte surface to continue its role in binding LDL-C [15]. PCSK9 in the bloodstream modulates LDL-R on hepatocyte surfaces by binding its cysteine-rich C-terminal to the epidermal growth factor-like repeat A domain (EGF-A) of LDL-R, forming a complex that is transported into the cell. The acidic lysosomal environment induces a conformational change [16]-[20], enhancing the affinity between PCSK9 and LDL-R, which complicates the dissociation of the complex, leading to its degradation within lysosomes. This process impedes the release and recycling of LDL-R, thereby elevating blood LDL-C levels and contributing to hypercholesterolemia [21] [22]. Research indicates that cyclase-associated protein-1 (CAP-1) is crucial for PCSK9-mediated LDL-R degradation. PCSK9 interacts with CAP-1, resulting in the LDLR/PCSK9/CAP-1 complex undergoing lysosomal degradation via a caveolin-dependent mechanism, reducing the presence of LDLR on the cell surface and diminishing the capacity to clear LDL-C [23]. Furthermore, serum PCSK9 levels are linked to triglyceride and ApoB levels, indicating a strong connection with lipid metabolism [24] [25]. PCSK9 enhances the synthesis of ApoB in the liver and intestines, and as the primary structural

protein of LDL, elevated ApoB can further increase serum LDL-C levels. Thus, PCSK9 is positively correlated with ApoB levels in LDL-C and negatively correlated with ApoB metabolism. Studies have demonstrated that increased ApoB content can lead to a higher incidence of atherosclerosis, even if LDL-C levels are normal [26], thereby raising the risk of ischemic stroke events. PCSK9 also interacts with other receptors, such as LDL-R-related protein 1 (LRP1), apolipoprotein E receptor 2 (ApoER2), and CD36 [27] [28], influencing blood lipid levels by not only reducing lipoprotein clearance in liver cells but also by increasing hepatic fat production [29].

2.2. The Influence of PCSK9 on Atherosclerosis

Atherosclerosis (AS) develops through a complex and gradual process. The early stage of atherosclerosis is characterized by the deposition of lipids within the blood vessel walls, forming lipid streaks, which subsequently initiate inflammatory responses and the proliferation and migration of vascular smooth muscle cells. Apoptosis of vascular endothelial cells plays a crucial role in the progression of this condition [30].

In hepatocytes, cholesterol regulatory element binding protein (sterol regulatory element binding protein, SREBP) and hepatocyte nuclear factor 1 α (hepatocyte nuclear factor-1 α, HNF-1 α) can regulate PCSK9 synthesis at the transcriptional level. It has been found that the HNF-1 α binding site is located in the proximal promoter of PCSK9 gene, which can further regulate PCSK9 by activating miRNA-122-dependent SREBP2 [31]. PCSK9 was synthesized in endoplasmic reticulum of hepatocytes and transferred to plasma after catalytic activation. Soluble PCSK9 mainly binds to the epidermal growth factor homologous domain A (EGF-A) of LDL-R on the surface of hepatocytes, making it impossible for LDL-R to bind to LDL-C. With the increase of LDLC level, LDLC accumulates and oxidizes, producing ox-LDL, recruiting monocytes into intima to differentiate into macrophages, and macrophages phagocytosis and differentiate into foam cells. At the same time, the C-terminal of PCSK9 can bind and activate Toll-like receptor 4 (Toll-like re ceptor-4 TLR4) and cyclase-related protein 1 to cause pro-inflammatory response, and activate TLR4/nuclear factor-κB (nuclear factor kappa B-NF-κB) pathway to further regulate inflammatory factors [32]. PCSK9 stimulates macrophages to produce pro-inflammatory factors in intima, which promotes vascular smooth muscle cells to transform into macrophages, and then into foam cells, resulting in intimal fat deposition [33]. Deposited macrophages and vascular smooth muscle cells can also secrete PCSK9, which further promotes the deposition of LDLC in blood vessels and inflammation, and finally forms atherosclerotic plaques. PCSK9 is prominently expressed in atherosclerotic plaques and is specifically distributed within the vascular intima [34]. Research has shown that PCSK9 can worsen plaque instability, thereby increasing the risk of plaque rupture and leading to various clinical cardiovascular and cerebrovascular events [35]. PCSK9 inhibitors can target PCSK9 via the MAPK signaling pathway to prevent endothelial cell apoptosis, indicating that PCSK9 may

contribute to the formation of atherosclerotic plaques by modulating inflammatory responses and inhibiting cell apoptosis [36].

2.3. The Influence of PCSK9 on Diabetes

Recent research has identified type 2 diabetes mellitus (T2DM) as a low-grade chronic inflammatory condition [37], with inflammation serving as the pathological foundation for insulin resistance. PCSK9, a multifunctional enzyme, significantly influences glucose and lipid metabolism, insulin resistance, and obesity in diabetic patients [38]-[40]. Evidence indicates that serum PCSK9 levels are notably elevated in T2DM patients compared to individuals with normal glucose tolerance [41]-[44], and high circulating PCSK9 levels are associated with increased T2DM prevalence [38] [45]. Metabolic factors such as BMI and blood biochemical markers like HbA1c are independently linked to PCSK9 levels [46]-[48]. These findings imply a potential association between PCSK9 and disturbances in glucose metabolism, insulin resistance, and obesity, although the precise mechanisms remain unclear. Contrarily, some studies report no link between blood PCSK9 levels and the onset of new diabetes cases [49]. A recent cohort study based on population data revealed a positive correlation between circulating PCSK9 levels and the risk of developing type 2 diabetes in prediabetic female subjects, while no significant correlation was observed in prediabetic male subjects [50]. Currently, there is no widespread consensus regarding the relationship between PCSK9 and diabetes.

3. Mechanism of Effect of PCSK9 on Risk Factors of Ischemic Stroke

3.1. PCSK9 in Inflammatory Response and Oxidative Stress

Atherosclerosis is characterized by chronic inflammatory changes in the vascular wall [51]. Multi-regional positron emission tomography-magnetic resonance imaging has revealed varying degrees of arterial inflammation in middle-aged individuals with subclinical atherosclerosis [52], suggesting that inflammation is present in the vascular wall even at the early stages of atherosclerosis. As atherosclerosis progresses, plaques in the vascular wall often become infiltrated with numerous monocytes, macrophages, and T cells. If these plaques rupture and are accompanied by endothelial damage, platelet aggregation and other signs may occur [53]. Reactive oxygen species (ROS) generated by mitochondria can induce oxidative damage to DNA, RNA, proteins, and lipids, leading to mitochondrial dysfunction, cell death, and subsequent tissue damage, thereby promoting atherosclerosis [54]. Research indicates a strong correlation between serum PCSK9 levels and the extent of inflammation. Inflammatory responses can upregulate PCSK9 levels, and various inflammatory stimuli can enhance PCSK9 expression. For instance, during the induction of inflammation in macrophages by oxidized low-density lipoprotein (OX-LDL), increased PCSK9 expression is observed, which stimulates the secretion of inflammatory cytokines such as IL-1a, IL-6, and TNF-

α [55]. In animal studies, following the injection of inflammatory stimuli, increased PCSK9 mRNA expression in hepatocytes is detectable at various time points. Transfection with PCSK9 siRNA can inhibit the secretion of inflammatory cytokines like IL-1a, IL-6, and TNF-α by macrophages to varying extents [56]. These findings suggest that PCSK9 contributes to and exacerbates the inflammatory response by modulating the secretion of cytokines such as IL-1a, IL-6, and TNF-α, while oxidative stress and inflammation further induce PCSK9 expression [57] [58].

3.2. PCSK9 and Thrombosis

Thrombosis plays a pivotal role in the development of ischemic stroke. The rupture of arterial plaques exposes sites of vascular injury, triggering platelet aggregation and activation of the coagulation cascade, ultimately leading to thrombus formation and subsequent ischemic events [59]. Research indicates that PCSK9 influences the coagulation system [60]. In the bloodstream, PCSK9 can engage in the coagulation process by binding to the platelet CD36 receptor, thereby directly promoting platelet activation and subsequent thrombus formation. Studies using CD36 knockout mice models have demonstrated that the enhancement of platelet activation by PCSK9 is dependent on CD36 [61]. Furthermore, PCSK9 can increase platelet reactivity by elevating ox-LDL levels, which, through the CD36-PKC signaling pathway, augments ROS production, further promoting platelet activation and enhancing the body's coagulation response [62]. During thrombosis, activated platelets have the capacity to release PCSK9 [63], suggesting that platelets can modulate PCSK9 levels in local atherosclerotic plaques, with the released PCSK9 further contributing to thrombus formation.

3.3. PCSK9 and Cellular Apoptosis

The apoptosis of vascular endothelial cells and macrophages plays a multifaceted role in the pathogenesis and progression of atherosclerosis [30]. Excessive apoptosis of endothelial cells compromises the integrity of the vascular endothelium, increasing vascular wall permeability, while excessive transformation and deposition of macrophages contribute to atherosclerosis formation [64]. During the progression of atherosclerosis, PCSK9 is expressed in macrophages, promoting the production of OX-LDL, which induces apoptosis in THP-1-derived macrophages, thereby exacerbating inflammatory responses and plaque instability [65]. Treatment with PCSK9 inhibitors can mitigate inflammation and apoptosis within atherosclerotic plaques [66], indicating a correlation between PCSK9 and cellular apoptosis in atherosclerosis. Research further indicates that PCSK9 can induce endothelial cell apoptosis via the intracellular Bcl2/Bax-Caspase9-Caspase3 mitochondrial pathway and the p38/c-Jun N-terminal kinase (JNK)-mitogen-activated protein kinase (MAPK) signaling pathway [67]. Initially identified in the metabolic products of neural cells, PCSK9 is a gene associated with cholesterol metabolism and the differentiation and apoptosis of neural cells. During neural cell

apoptosis, PCSK9 expression can increase. In vitro studies also demonstrate that PCSK9 can promote neural cell apoptosis, although the underlying mechanisms remain unclear [68].

4. Application of PCSK9 Inhibitor in Ischemic Stroke

PCSK9 inhibitors affect the function and quantity of PCSK9 in vivo through different ways. There are three mechanisms of PCSK9 inhibitors: 1) blocking the binding of PCSK9 and LDLR; 2) interfering with the secretion of PCSK9; 3) inhibiting the expression of PCSK9. Around these three mechanisms, researchers explored PSCK9 inhibitors acting on different links and targets, including monoclonal antibodies, peptide mimics, antisense oligodeoxynucleotides, small interference RNA (siRNA), vaccines, CRISPR therapy, anti-phantom, and low-dose molecules.

A new lipid-lowering drug PCSK9 inhibitor can selectively bind to PCSK9 to increase the number of LDL-R, thereby reducing the level of LDL-C in the blood [69]. At present, there is evidence that PCSK9 inhibitors have good efficacy and safety in reducing LDL-C levels, improving cardiovascular disease outcomes and increasing clinical benefits in patients with atherosclerotic cardiovascular disease [70] [71]. As the pathogenesis of atherosclerotic stroke is similar to that of coronary atherosclerotic heart disease, clinical studies have confirmed that PCSK9 inhibitors can delay the progression of atherosclerosis and reduce the mortality, disability and recurrence rate of ischemic stroke [72]. Studies have analyzed and compared the incidence of stroke events in patients with a history of stroke and those without a history of stroke in one year. Eiluzumab can significantly reduce the risk of end-point events in patients with non-hemorrhagic stroke. A post-mortem analysis of the study found that iloizumab could reduce the incidence of various types of stroke [73]. Another meta-analysis showed that PCSK9 inhibitors significantly reduced the relative risk of any type of stroke by up to 25% [74]. In addition, in the report of the clinical practice guidelines of the American Heart Association/American Heart Association working group, high-level recommendations are given for the application of PCSK9 inhibitors in the secondary prevention of ischemic stroke [75] [76]. However, there are no large-scale intervention studies to explore the efficacy and safety of PCSK9 inhibitors for lipid reduction and long-term blood lipid management in patients with ischemic stroke.

5. Summary and Prospect

PCSK9 is crucial in regulating cholesterol homeostasis by promoting the degradation of LDL receptors on the liver surface. It also influences the occurrence and progression of ischemic stroke through various mechanisms, significantly impacting atherosclerosis, inflammation, thrombosis, and glucose metabolism. As PCSK9 is likely to be a key factor in understanding atherosclerosis and lipid metabolism, further research is needed to elucidate its role and mechanisms in ischemic stroke. In addition, PCSK9 inhibitors play a useful role in both acute phase

and secondary prevention of ischemic stroke, which can reduce the risk of ischemic stroke and provide new options for lipid-lowering therapy.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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