Proprotein Convertase Subtilisin/Kexin Type 9 and Atherosclerosis

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is the last discovered member of the family of proprotein convertases (PCs), mainly synthetized in hepatic cells. This serine protease plays a pivotal role in the reduction of the number of low-density lipoprotein receptors (LDLRs) on the surface of hepatocytes, which leads to an increase in the level of cholesterol in the blood. The main anti-atherosclerotic effect of PCSK9 inhibitors results from their lipid-lowering efficiency.

Keywords: Proprotein convertase subtilisin/kexin type 9 ; Atherosclerosis ; Inflammation

1. Introduction

Proprotein convertases (PCs) are a family of nine serine proteases, which also includes proprotein convertase subtilisin/kexin type 9 (PCSK9). Each of those proteases plays a key role in post-translational modifications of propeptides leading to the formation of mature particles e.g., growth factors, enzymes, hormones, and transcriptional factors. Taking into consideration an ability for the activation of many substrates, to date, there seem to be a lot of physiological and pathophysiological processes that PCs take part in [1][2][3].

2. PCSK9 and Atherosclerosis

One of the main causes for the development of atherosclerosis is the deposition of excess LDL-C within the subendothelial matrix of selected arteries. Then transformed in the processes of oxidation, lipolysis and proteolysis into more reactive form—oxidized low-density lipoprotein (ox-LDL), it plays a key role in the initiation of atherogenesis [4]. Studies from the last few years have confirmed that PCSK9 accelerates the development of atherosclerosis due to the mechanism associated with increasing plasma concentration of LDL-C, but also by direct influence on the cells which build the arterial walls and atherosclerotic plaques [5][6][7]. Analysis of the metabolic pathways and PCSK9 functions in the vascular walls allows to predict the potentially beneficial anti-atherosclerotic effects associated with the therapeutical use of PCSK9 inhibitors [8].

The main anti-atherosclerotic effect of PCSK9 inhibitors results from their lipid-lowering efficiency. Data from the OSLER study indicate a significant reduction of LDL-C in the group of patients using evolocumab [9]. Similar results were observed in patients included in the ODYSSEY LONG TERM study, whose plasma LDL-C levels, after using alirocumab, were reduced by up to 62% compared with the placebo group [10].

Previous experiments revealed that PCSK9 inhibitors have a positive effect on the stabilization and morphology of atherosclerotic plaques, making them less vulnerable [11][12]. They also improve the function of platelets by reducing their thrombogenic potential [13][14][15]. Studies in which intravascular ultrasound (IVUS) was used suggest that the concentration of PCSK9 affects the size of the necrotic core within the atherosclerotic plaque, regardless of the concentration of LDL-C [5]. More detailed research carried out in 2020 showed that the use of PCSK9 inhibitors in therapy does not affect the size of the entire atherosclerotic plaque, but significantly improves its stabilization by reducing the lipid core burden index [16].

Arterial stiffness is acknowledged as one of the early predictors of cardiovascular disease [17]. For its indirect noninvasive assessment, pulse wave velocity (PWV) is widely used [18]. Positive correlation between circulating PCSK9 levels and arterial stiffness suggests another way (beyond lipid mechanism) for PCSK9 to affect cardiovascular risk [19]. Studies confirm improvements in arterial stiffness during therapy with PCSK9 inhibitors in patients with familial hypercholesterolemia [20][21][22].

3. Inflammation

Inflammatory processes play an important role in the pathophysiology of atherosclerosis [23]. Their intensification is associated with the development of atherogenesis. PCSK9 is capable of inducing the expression of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β) or interleukin-6 (IL-6). Moreover, it enhances the translocations of transcriptional factors for pro-inflammatory cytokine genes into the cell nucleus and reduces the formation of anti-inflammatory cytokines in macrophages [24][25]. Furthermore, it was shown that PCSK9 regulates the concentration of sirtuins—a family of proteins involved in histone deacetylation, playing a key role in metabolic driver inflammation [26][27].

One of the first meta-analyses carried out on the influence of PCSK9 inhibitors on the inflammatory process did not show any correlation between these drugs and the concentration of C-reactive protein (CRP), a basic marker of the ongoing inflammatory process assessed in clinical practice [28]. Nevertheless, in the assessment of any single biomarker (such as CRP), a certain percentage of subjects with false positive and false negative results should be taken into consideration. To avoid these biases and improve diagnostic sensitivity, biomarker panels and index scores have been introduced in research for several years [29]. On the other hand, some studies reveal the beneficial effects of inhibition of PCSK9 using siRNA on lowering the concentrations of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6 and TNF-α [25][30].

Neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-HDL-cholesterol ratio (MHR) are the novel, widely available markers of inflammation in cardiovascular diseases [31]. High NLR or MHR ratio increases cardiovascular risk [22][32]. Studies show that PCSK9 inhibitors may improve the inflammatory status of patients with familial hypercholesterolemia (FH) described with the use of the above-mentioned parameters [22][32].

4. Monocytes, Macrophages and Foam Cells

The cells responsible for the secretion of PCSK9 in the vessels are smooth muscle cells (SMCs) and the endothelial cells [33]. Fully functional protein is also detected in atherosclerotic macrophages [34].

The process initiating the development of atherosclerotic plaques is the transformation of monocytes and macrophages into foam cells due to the accumulation of ox-LDL inside them. The influx of lipoproteins through the cell membrane takes place with the participation of many proteins, such as scavenger receptors (SRs), CD36, CD68, lectin-like ox-LDL receptor-1 (LOX-1) [35]. Macrophages excrete the excess of toxic cholesterol into the extracellular space and HDL-C by using membrane transporters, e.g., Adenosine Triphosphate Binding Cassette A1 (ABCA1). PCSK9 shifts the balance of cholesterol transport towards the interior of macrophages by regulating the expression of appropriate membrane proteins, contributing to the formation of foam cells and intensification of atherogenesis [24][36][37].

Apolipoprotein E (apoE), produced in macrophages and smooth muscle cells, is an anti-atherosclerotic protein. It acts via apolipoprotein E receptor-2 (apoER2), which reduces intracellular lipoprotein accumulation and inhibits the formation of foam cells and promotes the anti-inflammatory phenotype of macrophages. [38]. PCSK9 reduces apoER2 expression, attenuating the protective effect of apoE [39].

A very important mechanism of action of PCSK9 inhibitors, which reduces the diapedesis of monocytes into atherosclerotic plaques, is associated with the elevation of the concentration of anti-inflammatory interleukin-10 (IL-10). The increase in its concentration leads to the drop of the expression of TNF-α and C-C chemokine receptor type 2 (CCR2), which are responsible for the influx of monocytes into the atherosclerotic plaque [40][41][42].

5. Endothelial Cells

Endothelial cell apoptosis promoted by ox-LDL increases its dysfunction and creates favorable conditions for the development of atherosclerosis [35]. Experiments on human endothelial cells obtained from umbilical cord blood indicate that PCSK9 is involved in the enhancement of apoptosis caused by ox-LDL via the Bcl/Bax–caspase-9–caspase-3 pathway [43].

In response to the disturbed balance between the accumulation and removal of excess ox-LDL from the foam cells of the subendothelial matrix, the endothelial cells that are lining them receive a signal to produce pro-inflammatory and adhesive cytokines. One of the signal components is the presence of PCSK9 [44].

Reactive oxygen species (ROS) produced in excess in mitochondria, e.g., in the course of inflammation, a cornerstone in the pathogenesis of atherosclerosis [23], are capable of inducing endothelial cells damage, as well as activating

inflammatory cells and thus intensifying the inflammatory process within the arterial wall [35]. Cells with silenced *PCSK9* genes produce fewer ROS in their mitochondria [33], which may lead to the conclusion that inhibition of PCSK9 might reduce the risk of endothelial damage. To date, there are no clinical trials that would confirm such an effect of PCSK9 inhibitors.

6. Smooth Muscle Cells (SMCs)

Under the influence of PCSK9, smooth muscle cells acquire the ability to proliferate, migrate, synthesize collagen and uptake lipoproteins [35]. This cumulatively accelerates the formation of atherosclerotic plaques [45]. Moreover, under the influence of PCSK9, within SMCs, there is an increase in the production of vascular cell adhesion molecule 1 (VCAM-1), facilitating the process of macrophage infiltration into the atherosclerotic plaque [46]. So far, no studies have been carried out to assess the effect of PCSK9 inhibition on the concentration of adhesive factors in SMCs.

7. Coagulation and Platelet Aggregation

After several clinical trials, such as JUPITER, showed that the use of lipid-lowering therapy with statins reduces the cardiovascular risk much more strongly than it would result just from the decrease in plasma lipids, scientists began to consider other beneficial mechanisms responsible for this phenomenon [47]. Similar observations regarding cardiovascular risk were also made in the case of PCSK9 inhibitors [48].

One of the possible explanations indicates that cardiovascular risk might be related to thrombotic processes caused by inflammation in the vascular endothelium. CD46 and LOX-1 are involved in them [49][50] and, accompanied by ox-LDL binding protein, play a key role in the formation of blood clots [51]. A separate mechanism is associated with the toll-like receptor 2 (TLR-2) stimulation, which activates the process of platelet aggregation through lipid-peroxide-modified phospholipids in the transport of Lp(a) [52][53][54].

Due to the mechanisms described above, the use of PCSK9 inhibitors may limit the process of platelet aggregation in several ways, thus reducing the cardiovascular risk. The first one is by lowering the cholesterol level in the cell membrane of platelets, which results in the drop of their activity [55]. The second one is by decline in the LOX-1 and ox-LDL concentration [33][56]. The ultimate is by reduction of Lp(a) plasma level which decreases the activity of platelets via peroxide-modified phospholipids [57]. The above-mentioned ways of weakening the activity of platelets by inhibiting PCSK9 were unequivocally confirmed in a clinical trial from 2017, with the use of alirocumab and evolocumab, and associated with the reduction of cardiovascular risk [55].

Noteworthy is the influence of PCSK9 inhibitors on the incidence of venous thromboembolism, which is related to the inflammatory process in the endothelium and the atherogenesis [58][59]. The studies conducted so far have not shown a correlation between the concentration of LDL-C and the occurrence of venous thromboembolism [60], however, such a correlation was observed when Lp(a) levels were taken into account [61]. It is crucial in case of PCSK9 inhibitors, which in contrast to statins reduce both, to consider the plasma concentration of LDL-C, and Lp(a) [62]. For this reason, clinical trials have been conducted to assess the impact of alirocumab on the incidence of venous thromboembolism. The results clearly confirmed the beneficial effect of alirocumab therapy on the reduction of the risk of venous thromboembolism incidents, which was associated with a significant reduction in Lp(a) concentration [10]. The second plausible antithrombotic mechanism of PCSK9 inhibitor action, which requires further experimental studies, is associated with their ability to increase the clearance of blood-clotting factor VIII (FVIII)—the essential protein in coagulation processes [63].

References

1. Seidah, N.G.; Prat, A. The biology and therapeutic targeting of the proprotein convertases. Nat. Rev. Drug Discov. 201 2, 11, 367–383.
2. Małuch, I.; Walewska, A.; Sikorska, E.; Prahl, A. Konwertazy probiałkowe—Rodzina proteaz serynowych o szerokim sp ektrum funkcji fizjologicznych. Post. Bioch. 2016, 62, 472–481.
3. Seidah, N.G.; Prat, A. The multifaceted biology of PCSK9. Endocr. Rev. 2021, bnab035.
4. D'Ardes, D.; Santilli, F.; Guagnano, M.T.; Bucci, M.; Cipollone, F. From endothelium to lipids, through microRNAs and P CSK9: A fascinating travel across atherosclerosis. High Blood Press. Cardiovasc. Prev. 2020, 27, 1–8.
5. Cheng, J.M.; Oemrawsingh, R.M.; Garcia-Garcia, H.M.; Boersma, E.; van Geuns, R.J.; Serruys, P.W.; Kardys, I.; Akker huis, K.M. PCSK9 in relation to coronary plaque inflammation: Results of the ATHEROREMOIVUS study. Atheroscleros

is 2016, 248, 117–122.

6. Denis, M.; Marcinkiewicz, J.; Zaid, A.; Gauthier, D.; Poirier, S.; Lazure, C.; Seidah, N.G.; Prat, A. Gene inactivation of pr oprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice. Circulation 2012, 125, 894–901.
7. Tavori, H.; Giunzioni, I.; Predazzi, I.M.; Plubell, D.; Shivinsky, A.; Miles, J.; Devay, R.M.; Liang, H.; Rashid, S.; Linton, M.F.; et al. Human PCSK9 promotes hepatic lipogenesis and atherosclerosis development via apoE- and LDLR-mediat ed mechanisms. Cardiovasc. Res. 2016, 110, 268–278.
8. Karagiannis, A.D.; Liu, M.; Toth, P.P.; Zhao, S.; Agrawal, D.K.; Libby, P.; Chatzizisis, Y.S. Pleiotropic anti-atherosclerotic effects of PCSK9 inhibitors from molecular biology to clinical translation. Curr. Atheroscler. Rep. 2018, 20, 20.
9. Sabatine, M.S.; Giugliano, R.P.; Wiviott, S.D.; Raal, F.J.; Blom, D.J.; Robinson, J.; Ballantyne, C.M.; Somaratne, R.; Le gg, J.; Wasserman, S.M.; et al. Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investiga tors. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. N. Engl. J. Med. 2015, 372, 1500–1509.
10. Schwartz, G.G.; Steg, P.G.; Szarek, M.; Bittner, V.A.; Diaz, R.; Goodman, S.G.; Kim, Y.U.; Jukema, J.W.; Pordy, R.; Ro e, M.T.; et al. Peripheral Artery Disease and Venous Thromboembolic Events after Acute Coronary Syndrome: Role of Lipoprotein(a) and Modification by Alirocumab: Prespecified Analysis of the ODYSSEY OUTCOMES Randomized Clini cal Trial. Circulation 2020, 141, 1608–1617.
11. Kühnast, S.; Van Der Hoorn, J.W.; Pieterman, E.J.; van den Hoek, A.M.; Sasiela, W.J.; Gusarova, V.; Peyman, A.; Schä fer, H.L.; Schwahn, U.; Jukema, J.W.; et al. Alirocumab inhibits atherosclerosis, improves the plaque morphology, and e nhances the effects of a statin. J. Lipid Res. 2014, 55, 2103–2112.
12. Yano, H.; Horinaka, S.; Ishimitsu, T. Effect of evolocumab therapy on coronary fibrous cap thickness assessed by optic al coherence tomography in patients with acute coronary syndrome. J. Cardiol. 2020, 75, 289–295.
13. Barale, C.; Bonomo, K.; Frascaroli, C.; Morotti, A.; Guerrasio, A.; Cavalot, F.; Russo, I. Platelet function and activation markers in primary hypercholesterolemia treated with anti-PCSK9 monoclonal antibody: A 12-month follow-up. Nutr. Me tab. Cardiovasc. Dis. 2020, 30, 282–291.
14. Koskinas, K.C.; Windecker, S.; Buhayer, A.; Gencer, B.; Pedrazzini, G.; Mueller, C.; Cook, S.; Muller, O.; Matter, C.M.; Räber, L.; et al. Design of the randomized, placebo-controlled evolocumab for early reduction of LDL-cholesterol levels in patients with acute coronary syndromes (EVOPACS) trial. Clin. Cardiol. 2018, 41, 1513–1520.
15. Basiak, M.; Kosowski, M.; Cyrnek, M.; Bułdak, Ł.; Maligłówka, M.; Machnik, G.; Okopień, B. Pleiotropic Effects of PCSK -9 Inhibitors. Int. J. Mol. Sci. 2021, 22, 3144.
16. Omori, H.; Ota, H.; Hara, M.; Kawase, Y.; Tanigaki, T.; Hirata, T.; Sobue, Y.; Okubo, M.; Kamiya, H.; Matsuo, H.; et al. Ef fect of PCSK-9 Inhibitors on Lipid-Rich Vulnerable Coronary Plaque Assessed by Near-Infrared Spectroscopy. JACC C ardiovasc. Imaging 2020, 13, 1639–1641.
17. Kim, H.L.; Lim, W.H.; Seo, J.B.; Kim, S.H.; Zo, J.H.; Kim, M.A. Prognostic value of arterial stiffness according to the car diovascular risk profiles. J. Hum. Hypertens. 2021, 35, 978–984.
18. Mikael, L.R.; Paiva, A.; Gomes, M.M.; Sousa, A.; Jardim, P.; Vitorino, P.; Euzébio, M.B.; Sousa, W.M.; Barroso, W. Vasc ular Aging and Arterial Stiffness. Arq. Bras. Cardiol. 2017, 109, 253–258.
19. Ruscica, M.; Ferri, N.; Fogacci, F.; Rosticci, M.; Botta, M.; Marchiano, S.; Magni, P.; D'Addato, S.; Giovannini, M.; Borg hi, C.; et al. Circulating Levels of Proprotein Convertase Subtilisin/Kexin Type 9 and Arterial Stiffness in a Large Popula tion Sample: Data From the Brisighella Heart Study. J. Am. Heart Assoc. 2017, 6, e005764.
20. Scicali, R.; Russo, G.I.; Di Mauro, M.; Manuele, F.; Di Marco, G.; Di Pino, A.; Ferrara, V.; Rabuazzo, A.M.; Piro, S.; Mor gia, G.; et al. Analysis of Arterial Stiffness and Sexual Function after Adding on PCSK9 Inhibitor Treatment in Male Pati ents with Familial Hypercholesterolemia: A Single Lipid Center Real-World Experience. J. Clin. Med. 2020, 9, 3597.
21. Mandraffino, G.; Scicali, R.; Rodríguez-Carrio, J.; Savarino, F.; Mamone, F.; Scuruchi, M.; Cinquegrani, M.; Imbalzano, E.; Di Pino, A.; Piro, S.; et al. Arterial stiffness improvement after adding on PCSK9 inhibitors or ezetimibe to high-inten sity statins in patients with familial hypercholesterolemia: A Two-Lipid Center Real-World Experience. J. Clin. Lipidol. 20 20, 14, 231–240.
22. Scicali, R.; Di Pino, A.; Ferrara, V.; Rabuazzo, A.M.; Purrello, F.; Piro, S. Effect of PCSK9 inhibitors on pulse wave veloc ity and monocyte-to-HDL-cholesterol ratio in familial hypercholesterolemia subjects: Results from a single-lipid-unit real -life setting. Acta Diabetol. 2021, 58, 949–957.
23. Geovanini, G.R.; Libby, P. Atherosclerosis and inflammation: Overview and updates. Clin. Sci. 2018, 132, 1243–1252.
24. Ricci, C.; Ruscica, M.; Camera, M.; Rossetti, L.; Macchi, C.; Colciago, A.; Zanotti, I.; Lupo, M.G.; Adorni, M.P.; Cicero, A.F.; et al. PCSK9 induces a pro-inflammatory response in macrophages. Sci. Rep. 2018, 8, 2267.

25. Tang, Z.H.; Peng, J.; Ren, Z.; Yang, J.; Li, T.T.; Li, T.H.; Wang, Z.; Wei, D.H.; Liu, L.S.; Zheng, X.L.; et al. New role of PCSK9 in atherosclerotic inflammation promotion involving the TLR4/NF-κB pathway. Atherosclerosis 2017, 262, 113–122.

26. Hovland, A.; Retterstøl, K.; Mollnes, T.E.; Halvorsen, B.; Aukrust, P.; Lappegård, K.T. Anti-inflammatory effects of non-statin low-density lipoprotein cholesterol-lowering drugs: An unused potential? Scand. Cardiovasc. J. 2020, 54, 274–279.

27. D'Onofrio, N.; Vitiello, M.; Casale, R.; Servillo, L.; Giovane, A.; Balestrieri, M.L. Sirtuins in vascular diseases: Emerging roles and therapeutic potential. Biochim. Biophys. Acta 2015, 1852, 1311–1322.

28. Cao, Y.X.; Li, S.; Liu, H.H.; Li, J.J. Impact of PCSK-9 monoclonal antibodies on circulating hs-CRP levels: A systematic review and meta-analysis of randomised controlled trials. BMJ Open 2018, 8, e022348.

29. Fung, E.T.; Wilson, A.M.; Zhang, F.; Harris, N.; Edwards, K.A.; Olin, J.W.; Cooke, J.P. A biomarker panel for peripheral arterial disease. Vasc. Med. 2008, 13, 217–224.

30. Tang, Z.; Jiang, L.; Peng, J.; Ren, Z.; Wei, D.; Wu, C.; Pan, L.; Jiang, Z.; Liu, L. PCSK-9 siRNA suppresses the inflammatory response induced by oxLDL through inhibition of NF-κB activation in THP-1-derived P-1-derived macrophages. Int. J. Mol. Med. 2012, 30, 931–938.

31. Bhat, T.; Teli, S.; Rijal, J.; Bhat, H.; Raza, M.; Khoueiry, G.; Meghani, M.; Akhtar, M.; Costantino, T. Neutrophil to lymphocyte ratio and cardiovascular diseases: A review. Expert Rev. Cardiovasc. Ther. 2013, 11, 55–59.

32. Scicali, R.; Mandraffino, G.; Di Pino, A.; Scuruchi, M.; Ferrara, V.; Squadrito, G.; Purrello, F.; Piro, S. Impact of high neutrophil-to-lymphocyte ratio on the cardiovascular benefit of PCSK9 inhibitors in familial hypercholesterolemia subjects with atherosclerotic cardiovascular disease: Real-world data from two lipid units. Nutr. Metab. Cardiovasc. Dis. 2021, 31, 3401–3406.

33. Ding, Z.; Liu, S.; Wang, X.; Deng, X.; Fan, Y.; Shahanawaz, J.; Shmookler-Reis, R.J.; Varughese, K.I.; Sawamura, T.; Mehta, J.L. Cross-talk between LOX-1 and PCSK9 in vascular tissues. Cardiovasc. Res. 2015, 107, 556–567.

34. Ferri, N.; Tibolla, G.; Pirillo, A.; Cipollone, F.; Mezzetti, A.; Pacia, S.; Corsini, A.; Catapano, A.L. Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by cultured smooth muscle cells reduces macrophages LDLR levels. Atherosclerosis 2012, 220, 381–386.

35. Yurtseven, E.; Ural, D.; Baysal, K.; Tokgözoğlu, L. An update on the role of PCSK9 in atherosclerosis. J. Atheroscler. Thromb. 2020, 27, 909–918.

36. Adorni, M.P.; Cipollari, E.; Favari, E.; Zanotti, I.; Zimetti, F.; Corsini, A.; Ricci, C.; Bernini, F.; Ferri, N. Inhibitory effect of PCSK9 on Abca1 protein expression and cholesterol efflux in macrophages. Atherosclerosis 2017, 256, 1–6.

37. Ding, Z.; Liu, S.; Wang, X.; Theus, S.; Deng, X.; Fan, Y.; Zhou, S.; Mehta, J.L. PCSK9 regulates expression of scavenger receptors and ox-LDL uptake in macrophages. Cardiovasc. Res. 2018, 114, 1145–1153.

38. Bai, X.Q.; Peng, J.; Wang, M.M.; Xiao, J.; Xiang, Q.; Ren, Z.; Wen, H.Y.; Jiang, Z.S.; Tang, Z.H.; Liu, L.S. PCSK9: A potential regulator of apoE/apoER2 against inflammation in atherosclerosis? Clin. Chim. Acta 2018, 483, 192–196.

39. Poirier, S.; Mayer, G.; Benjannet, S.; Bergeron, E.; Marcinkiewicz, J.; Nassoury, N.; Mayer, H.; Nimpf, J.; Prat, A.; Seidah, N.G. The proprotein convertase PCSK9 induces the degradation of low-density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. J. Biol. Chem. 2008, 283, 2363–2372.

40. Bernelot Moens, S.J.; Neele, A.E.; Kroon, J.; van der Valk, F.M.; van den Bossche, J.; Hoeksema, M.A.; Hoogeveen, R.M.; Schnitzler, J.G.; Baccara-Dinet, M.T.; Manvelian, G.; et al. PCSK-9 monoclonal antibodies reverse the pro-inflammatory profile of monocytes in familial hypercholesterolaemia. Eur. Heart J. 2017, 38, 1584–1593.

41. Grune, J.; Meyborg, H.; Bezhaeva, T.; Kappert, K.; Hillmeister, P.; Kintscher, U.; Pieske, B.; Stawowy, P. PCSK-9 regulates the chemokine receptor CCR2 on monocytes. Biochem. Biophys. Res. Commun. 2017, 485, 312–318.

42. Nahrendorf, M.; Swirski, F.K. Cholesterol, CCR2, and monocyte phenotypes in atherosclerosis. Eur. Heart J. 2017, 38, 1594–1596.

43. Wu, C.Y.; Tang, Z.H.; Jiang, L.; Li, X.F.; Jiang, Z.S.; Liu, L.S. PCSK9 siRNA inhibits HUVEC apoptosis induced by ox-LDL via Bcl/Bax-caspase9-caspase3 pathway. Mol. Cell. Biochem. 2012, 359, 347–358.

44. Leiva, E.; Wehinger, S.R.; Guzmán, L.; Orrego, R. Role of oxidized LDL in atherosclerosis. In Hypercholesterolemia; Kumar, S.A., Ed.; IntechOpen Limited: London, UK, 2015; pp. 55–77.

45. Campbell, J.H.; Popadynec, L.; Nestel, P.J.; Campbell, G.R. Lipid accumulation in arterial smooth muscle cells. Influence of phenotype. Atherosclerosis 1983, 47, 279–295.

46. Diedrich, G. How does hepatitis C virus enter cells? FEBS J. 2006, 273, 3871–3885.

47. Fruchart, J.C.; Sacks, F.; Hermans, M.P.; Assmann, G.; Brown, W.V.; Ceska, R.; Chapman, M.J.; Dodson, P.M.; Fioretto, P.; Ginsberg, H.N.; et al. The Residual Risk Reduction Initiative: A call to action to reduce residual vascular risk in pat

ients with dyslipidemia. Am. J. Cardiol. 2008, 102 (Suppl. 10), 1K–34K.

48. Ridker, P.M. Mortality differences associated with treatment responses in CANTOS and FOURIER: Insight and implicati ons. Circulation 2018, 137, 1763–1766.
49. Chen, K.; Febbraio, M.; Li, W.; Silverstein, R.L. A specific CD36-dependent signaling pathway is required for platelet act ivation by oxidized low-density lipoprotein. Circ. Res. 2008, 102, 1512–1519.
50. Hofmann, A.; Brunssen, C.; Morawietz, H. Contribution of lectin-like oxidized low-density lipoprotein receptor-1 and LO X-1 modulating compounds to vascular diseases. Vascul. Pharmacol. 2017, S1537–S1891, 30171–30174.
51. Magwenzi, S.; Woodward, C.; Wraith, K.S.; Aburima, A.; Raslan, Z.; Jones, H.; McNeil, C.; Wheatcroft, S.; Yuldasheva, N.; Febbriao, M.; et al. Oxidized LDL activates blood platelets through CD36/NOX2-mediated inhibition of the cGMP/pr otein kinase G signaling cascade. Blood 2015, 125, 2693–2703.
52. Biswas, S.; Xin, L.; Panigrahi, S.; Zimman, A.; Wang, H.; Yakubenko, V.P.; Byzova, T.V.; Salomon, R.G.; Podrez, E.A. N ovel phosphatidylethanolamine derivatives accumulate in circulation in hyperlipidemic ApoE-/- mice and activate platele ts via TLR2. Blood 2016, 127, 2618–2629.
53. Leibundgut, G.; Scipione, C.; Yin, H.; Schneider, M.; Boffa, M.B.; Green, S.; Yang, X.; Dennis, E.; Witztum, J.L.; Koschi nsky, M.L.; et al. Determinants of binding of oxidized phospholipids on apolipoprotein (a) and lipoprotein (a). J. Lipid Re s. 2013, 54, 2815–2830.
54. Pęczek, P.; Leśniewski, M.; Mazurek, T.; Szarpak, L.; Filipiak, K.J.; Gąsecka, A. Antiplatelet Effects of PCSK9 Inhibitors in Primary Hypercholesterolemia. Life 2021, 11, 466.
55. Barale, C.; Bonomo, K.; Frascaroli, C.; Morotti, A.; Guerrasio, A.; Cavalot, F.; Russo, I. Effects of PCSK-9 inhibitors on platelet function in adults with hypercholesterolemia. Atherosclerosis 2017, 263, 30–31.
56. Cammisotto, V.; Baratta, F.; Castellani, V.; Bartimoccia, S.; Nocella, C.; D'Erasmo, L.; Cocomello, N.; Barale, C.; Scical i, R.; Di Pino, A.; et al. Proprotein Convertase Subtilisin Kexin Type 9 Inhibitors Reduce Platelet Activation Modulating o x-LDL Pathways. Int. J. Mol. Sci. 2021, 22, 7193.
57. Kotani, K.; Banach, M. Lipoprotein(a) and inhibitors of proprotein convertase subtilisin/kexin type 9. J. Thorac. Dis. 201 7, 9, 78–82.
58. Folsom, A.R.; Lutsey, P.L.; Astor, B.C.; Cushman, M. C-reactive protein and venous thromboembolism. A prospective in vestigation in the ARIC cohort. Thromb. Haemost. 2009, 102, 615–619.
59. Prandoni, P.; Bilora, F.; Marchiori, A.; Bernardi, E.; Petrobelli, F.; Lensing, A.W.; Prins, M.H.; Girolami, A. An association between atherosclerosis and venous thrombosis. N. Engl. J. Med. 2003, 348, 1435–1441.
60. Glynn, R.J.; Danielson, E.; Fonseca, F.A.; Genest, J.; Gotto, A.M., Jr.; Kastelein, J.J.; Koenig, W.; Libby, P.; Lorenzatti, A.J.; MacFadyen, J.G.; et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N. Engl. J. Med. 2009, 360, 1851–1861.
61. Sofi, F.; Marcucci, R.; Abbate, R.; Gensini, G.F.; Prisco, D. Lipoprotein (a) and venous thromboembolism in adults: A m eta-analysis. Am. J. Med. 2007, 120, 728–733.
62. O'Donoghue, M.L.; Fazio, S.; Giugliano, R.P.; Stroes, E.S.G.; Kanevsky, E.; Gouni-Berthold, I.; Im, K.; Lira Pineda, A.; Wasserman, S.M.; Češka, R.; et al. Lipoprotein(a), PCSK-9Inhibition, and Cardiovascular Risk. Circulation 2019, 139, 1 483–1492.
63. Siegler, J.E.; Samai, A.; Albright, K.C.; Boehme, A.K.; Martin-Schild, S. Factoring in Factor VIII with Acute Ischemic Stro ke. Clin. Appl. Thromb. Hemost. 2015, 21, 597–602.
