REVIEW

PERSPECTIVES IN LIPID LOWERING THERAPY. NEW THERAPIES TARGETING LDL – CHOLESTEROL

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Abstract

New lipid-lowering drugs are needed, because current treatment options, in which statins play a central role, are limited by suboptimal control of low-density lipoprotein cholesterol (LDL-C), the ineffectiveness of treatment in some forms of dyslipidaemia, and residual cardiovascular risks. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, among which human monoclonal antibodies are currently recommended by guidelines, increase the number of LDL receptors, lowering serum LDL-C levels. Monoclonal antibodies are indicated, in addition to diet and statins in highest tolerated doses, in heterozygous familial hypercholesterolemia, or in patients with high cardiovascular risk, when a lower level of LDL-C is required. Bempedoic acid (ETC 1002) is an inhibitor of adenosine triphosphate citrate lyase, reducing cholesterol synthesis in hepatocytes, with hypocholesterolemic and anti-inflammatory effects. In familial hypercholesterolemia, apoB-100 inhibitors (the antisense oligonucleotide mipomersen) or microsomal triglyceride transfer protein inhibitors (lopitamid) are niche drugs because of adverse reactions, being only indicated in homozygous forms. LDL-C apheresis is an alternative in forms of familial hypercholesterolemia unresponsive to pharmacological treatment.

Rezumat

Noi medicamente hipolipemiante sunt necesare deoarece tratamentul actual, în care statinele au un rol central, este limitat de controlul suboptimal al colesterolului din lipoproteinele cu densitate joasă (LDL-C), de ineficienţa tratamentului în unele forme de dislipidemie, precum şi de prezenţa riscului cardiovascular rezidual. Inhibitorii de subtilisin/kexin proprotein convertaza tipul 9 (PCSK9), dintre care anticorpii monoclonali umani sunt în prezent recomandaţi de ghidurile terapeutice, cresc numărul de receptori LDL, scăzând nivelul LDL-C seric. Anticorpii monoclonali sunt indicaţi în asociere cu dietă şi statine în doze maxim tolerate, în hipercolesterolemiile familiale heterozigote sau la bolnavii cu risc cardiovascular crescut, atunci când este necesară atingerea unui nivel mai scăzut al LDL-C. Acidul bempedoic (ETC 1002) este un inhibitor al adenozin trifosfat citrat liazei, scăzând sinteza colesterolului în hepatocite, cu efecte hipocolesterolemiante şi antiinflamatoare. În hipercolesterolemia familială, inhibitorii de apoB-100 (oligonucleotidul antisens mipomersen) sau inhibitorii de proteină de transfer microzomal al trigliceridelor (lopitamid) sunt medicamente de nişă, datorită reacţiilor adverse, fiind indicate numai în formele homozigote. Afereza LDL-C este o alternativă terapeutică în formele de hipercolesterolemii familiale non-responsive la tratamentul farmacologic.

Keywords: LDL – cholesterol, new cholesterol-lowering drugs

Introduction

The treatment of dyslipidaemias stands as an essential strategy for primary and secondary cardiovascular (CV) risk prevention. The main therapeutic target remains low-density lipoprotein cholesterol (LDL-C), since observational studies demonstrated a linear correlation between LDL-C levels and coronary artery disease risk [33]. Lowering LDL-C by 1 mmol (40 mg/dL) is associated with a reduction by 10% in all-cause mortality, 20% in ischemic heart disease related death, and 22% in CV risk [14]. There is a dose-dependent reduction in cardiovascular disease (CVD) with LDL-C lowering, and epidemiologic data have not documented any threshold for low LDL-C level associated with harm [15, 16].

Statins are the cornerstone therapy for lowering LDL-C. By inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in the hepatocyte, statins reduce cholesterol synthesis and promote an increase in the number of LDL–C receptors (LDL-R) on the hepatocytes surface. As a consequence, the clearance of LDL-C particles from the blood is increased, lowering serum LDL-C and apoB-containing lipoproteins levels. However, the efficacy of statin therapy in lowering the LDL-C serum level is related to the specific statin and dosage used. Nevertheless, there are individual responses to the same statin dosage, caused by genetic variations

in cholesterol or statin metabolism [6, 13, 50]. In familial hypercholesterolemia (FH), there is a LDL-receptor (LDR-R) loss-of-function, thus statins BEING far less efficient in reducing LDL-C. Last but not least, adverse reactions and statin intolerance are associated with less than optimal control over LDL-C levels. Major statin trials prove that, despite benefit, substantial residual CV risk remains and coronary heart disease (CHD) events do occur in patients treated with statins. The percentage of patients experiencing major CHD events ranges from 5.8% in "WOSCOPS" to 19.4% in "4S" study [1, 61].

The need for new lipid-lowering therapies is based on the suboptimal control over LDL-C levels using the existing therapeutic options and on the fact that the benefits related to LDL-C reduction are not specific for statin therapy [10].

Progresses in the field of biologic agents and decoding genetic involvement in lipid metabolism have generated new lipid lowering drugs classes.

Proprotein convertase subtilisin/kexin type 9 inhibition

The proprotein convertase subtilisin/kexin type 9 (PCSK9) is one of the new targets for LDL-C lowering therapy.

PCSK9 is a 692–amino acid serine protease with a major regulatory role in cholesterol metabolism, by adjusting the intracellular level of LDL-R. PCSK9 is synthesized mainly by the liver and is secreted into the circulation, where it can bind to the LDL-R, modifying its mechanism of action [68]. It has been extensively studied ever since its discovery in 2003 and has revolutionized the field of LDL-C regulation [59, 60].

The LDL-R has an important role in the clearance of plasma LDL. The complex formed between LDL and LDL-R is endocytosed into the hepatocytes. After dissociation from these complexes, LDL particles are degraded inside the lysosomes, while, LDL-R recirculate from the endosome back to the plasma membrane [37]. This is the major pathway for plasma LDL removal, and a single LDL-R recycles about 150 times [30].

The mechanism by which PCSK9 works is by preventing LDL-R recycling. PCSK9 binds to the receptor, generating a complex consisting of PCSK9, an LDL-R, and an LDL particle. The complex enters the cell, where PCSK9 prevents dissociation, so the entire complex (including the LDL-R) is headed toward lysosomal degradation, so the receptor is not recycled [47]. As a consequence, there is a decreased removal rate of LDL from plasma due to a lower number of LDL-R, and the plasma concentration of LDL-C increases.

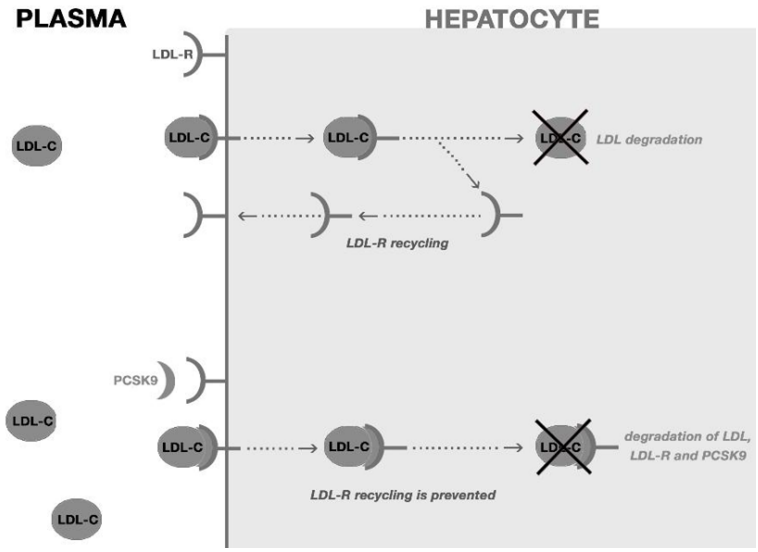

Figure 1.
PSCK9 effects on hepatocyte LDL-receptors (LDL-R = low-density lipoprotein receptor, LDL-C = low-density lipoprotein cholesterol, PCSK9 = proprotein convertase subtilisin/kexin type 9) [modified from 5]

PCSK9 regulation is performed by sterol regulatory element-binding proteins (SREBPs), transcription factors that regulate transcription of enzymes involved in sterol biosynthesis [36]. There is a statin-induced rise in PCSK9 expression which counterbalances the statin-induced increase in LDL-R expression and restricts the LDL reduction. Therefore, inhibitors of PCSK9 not only lower LDL, but could also enhance the efficacy of statins, making this strategy very attractive, either as monotherapy, or as an add-on therapy for patients on statins [65].

After the discovery of the gene encoding PCSK9 in 2003, several PCSK9 mutations have been identified: gain-of-function mutation in 2003 and loss-of-function

mutations in 2005. PCSK9 gain-of-function mutation has a very low prevalence, being the third cause of FH, after LDL-R and ApoB defects [43]. The consequences of gain-of-function mutations are hypercholesterolemia and an increased CV risk. Conversely, PCSK9 loss-of-function mutations were associated with significantly low LDL-C and a significant reduction in CHD risk both in Caucasians, and in African Americans [18].
These data suggest that PCSK9 inhibition is an important therapeutic target for LDL-C lowering and reducing CV risk. Whether PCSK9 inhibition should be reversible or permanent remains to be determined, but a permanent inhibition strategy should probably be used with caution [62].
There are several directions in the development of drugs for PCSK9 inhibition: anti-PCSK9 monoclonal antibodies that bind to and inactivate PCSK9 in the plasma, mimetic peptides and adnectins acting as competitive inhibitors of PCSK9 and gene silencing using a PCSK9 antisense oligonucleotide (ASO) that reduces gene transcription (Figure 2) [31]. Two permanent PCSK9 inhibition strategies have also been proposed: PCSK9 vaccination and CRISPR-Cas9 gene silencing of PCSK9.

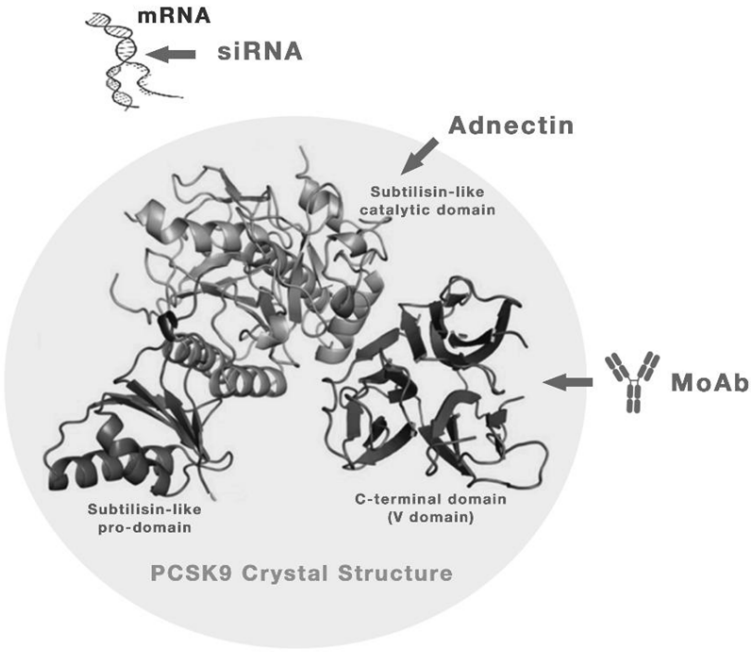

Figure 2.
PCSK9 inhibitors and their targets on PCSK9 structure (mRNA = messenger ribonucleic acid, siRNA = Small interfering RNA, MoAb = monoclonal antibodies, PCSK9 = proprotein convertase subtilisin/kexin type 9) [modified from 51]

Monoclonal antibodies
The most important advance in the cholesterol lowering therapies since the discovery of statins are anti-PCSK9 monoclonal antibodies (MoAbs) that bind to and neutralize PCSK9 in plasma. There are two fully human monoclonal antibodies, alirocumab and evolucumab, and a humanized monoclonal antibody, bococizumab. Novel MoAbs are still under development.
MoAbs act by bounding to the region on PCSK9 required for interaction with LDL-R, reducing the plasmatic levels of PCSK9 and preventing the association between PCSK9 and LDL-R. As a consequence, the higher expression of LDL-R on the hepatocytes increases LDL-C uptake, reducing the plasmatic level of LDL-C [3, 11, 41]. The effects of PCSK9 MoAb inhibitors on the lipid profile consist in a significant decrease in LDL-C levels along with other atherogenic lipoproteins, such as total cholesterol, non-HDL cholesterol, apolipoprotein B and lipoprotein(a). The LDL-C decreases by about 60% either in monotherapy, or with background statin therapy, the PCSK9 monoclonal antibody inhibitors having a dose dependent effect on serum LDL-C level.
PCSK9 MoAb inhibitors also reduce lipoprotein(a) concentration by 30%, in contrast to statins, which do not significantly influence lipoprotein(a) levels [3, 11]. The mechanism has not been clarified yet, lipoprotein(a) being mainly regulated by hepatic secretion and not cleared by LDL-R [41]. Thus, MoAbs could also reduce the independent residual risk associated with elevated lipoprotein(a) levels.

Evidence and recommendations

The effects of evolocumab on coronary atherosclerotic plaque volume were assessed by serial coronary intravascular ultrasound in the trial „Global Assessment of Plaque Regression with a PCSK9 Antibody as

Measured by Intravascular Ultrasound (GLAGOV)" [53]. In patients treated with evolucumab together with statin therapy, the LDL cholesterol levels were significantly lower than in the *placebo* group (36.6 mg/dL *versus* 93.0 mg/dL, $p < 0.001$). Patients on evolucumab also had a greater change in the atheroma volume (nominal change -0.95% *versus* 0.05% in the *placebo* group), and a significantly higher proportion of patients had plaque regression (64.3% with evolocumab *versus* 47.3% with *placebo*; $p < 0.001$) [48].

It is shown that continued CV benefit can be accrued even when LDL cholesterol levels are reduced to 20 - 25 mg *per* deciliter (0.52 - 0.65 mmol/L), a range well below current targets [35, 42, 70]. The reduction of CV events according to the magnitude of LDL-C lowering was suggested by the preliminary data of large phase III trials regarding CV outcomes (ODYSSEY Outcomes for alirocumab, FOURIER for evolocumab and SPIRE for bococizumab). These large outcome studies demonstrated the safety and benefit of PCSK9 inhibition in achieving very low LDL-C [27, 49, 63]. Dose-dependent reduction in LDL-C (up to 70%), timing of nadir LDL-C (between 4 and 14 days), and delay in return to baseline LDL-C (2 to 8 weeks) were observed with each of these MoAbs [29]. Both evolocumab and alirocumab have been studied as monotherapies *versus* ezetimib [40, 57]. In 614 patients with baseline LDL-C 100 to 190 mg/dL, evolocumab reduced LDL-C at 12 weeks by 55% - 57% (80 mg/dL) on average compared with *placebo*, whereas ezetimib only generated an 18% - 19% (26 mg/dL) reduction [40]. Similarly, in a study of alirocumab monotherapy *versus* ezetimib in 103 patients with a 1% - 5% 10-year risk of fatal CV events, alirocumab reduced LDL-C by 47% (66 mg/dL) at 24 weeks, compared with only 16% (22 mg/dL) for ezetimib [57]. No neutralizing antibodies to fully human monoclonal antibodies have been reported in the trials to date. Phase II/III trials have demonstrated good tolerability without clear drug-related toxicity, although the number and duration of patients treated to date is modest. The safety and tolerability profile of the 2 most extensively studied MoAbs (alirocumab, evolocumab) for periods of up to 2 years appears promising. However, longer exptreatment periods are necessary to more completely evaluate potentially delayed adverse effects, such as neurocognitive impairment and cancer.

PCSK9 MoAbs are currently approved for lowering of LDL-C levels in patients having as background statin therapy. They have a target patient population represented by heterozygous familial hypercholesterol-aemia (80% of treated patients do not achieve the LDL-C goal despite intensive therapy), patients at high CV risk under maximally tolerated statin dose who need a lower LDL-C, and those with statin intolerance.

Emerging PCSK9 inhibition therapies

Adnectins

Adnectins are a family of binding proteins derived from the 10th type III domain of human fibronectin (10Fn3), which is part of the immunoglobulin superfamily and normally binds integrin. A high-affinity PCSK9 binder, called BMS-962476, has been engineered as a potential alternative to monoclonal antibodies. This binds to the catalytic subunit of PCSK9, inhibiting its interaction with the LDL-R. The progress of this type of inhibitor in clinical trials is still awaited [46].

Epidermal growth factor precursor homology domain A (EGF-A) mimetics

PCSK9 interacts with the epidermal growth factor precursor homology domain A (EGF-A) domain of the LDL-R, so a competitive EGF-A mimetic LDL-R is plausible to act as an entrap to block the activity of extracellular PCSK9 on the LDLR [58]. Screens of phage-displayed peptide libraries have identified a 13-amino acid linear peptide (Pep2-8) as the smallest known PCSK9 inhibitor, but further research is needed before these structures may be applicable *in vivo* [69].

Inhibition of proprotein convertase subtilisin/kexin type 9 synthesis by gene silencing

Inclisiran. One strategy to reversibly lower PCSK9 expression would be decreasing the mRNA levels. Small interfering RNA (siRNA) molecules alter the pathway of RNA interference (RNAi) by binding to the RNA-induced silencing complex (RISC) inside the cell, cleaving mRNA molecules encoding PCSK9, making it unavailable for protein translation, so the levels of PCSK9 drop. Inclisiran (ALN-PCSsc) is a long-acting, subcutaneously delivered, synthetic siRNA directed against PCSK9, conjugated to triantennary N-acetylgalactosamine carbohydrates. In a phase 1 trial, reductions in the PCSK9 level went up to 83.8%, and up to 59.7% in the LDL-C level, as well as when inclisiran was administered to patients on stable doses of statins [26]. A phase 2, multicentre, double-blind, *placebo*-controlled, multiple ascending-dose trial of inclisiran administered subcutaneously in patients at high risk of CV disease who had elevated LDL-C, inclisiran was found to lower both PCSK9 and LDL-C levels. The adverse events rates were similar to those for monoclonal antibodies to PCSK9. Symptoms of immune activation, a common concern with therapies targeting RNA, were rare [56]. Inclisiran has very potent and durable effects: a single subcutaneous injection can reduce a patient's LDL-C for 6 months, and this makes it an important alternative to statins [38].

Inhibiting proPCSK9 zymogen autocatalytic cleavage or secretion from cells

Another plausible research direction would be to identify a small molecule that would prevent PCSK9 from exiting the endoplasmic reticulum, either by

inhibiting its autocatalysis, or by increasing oligomerization [17].

PCSK9 vaccination. A recent report suggested the use of peptide-based anti-PCSK9 vaccines, isolated *via* polyclonal high affinity and persistent antibodies that stay functional for up to one year [28]. In an experimental study, the AT04A anti-PCSK9 vaccine was recently tested on mice commonly used as a model for atherosclerosis development [44]. After vaccination, there were significantly lower levels of plasma lipids, reduced vascular and systemic inflammation, associated with regression of aortic atherosclerotic lesions. If successful, the vaccine administered annually by injection, could represent an innovative therapy for improving or even preventing coronary atherosclerosis.

CRISPR-Cas9 gene silencing of PCSK9. A recent proof-of-principle study suggested the possibility of permanent alteration of PCSK9 with *in vivo* use of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9 genome editing [22]. The study used adenoviral-induced silencing technology, not yet suitable for human use, but the concept has been proven in mice. The long term benefits and safety have to be documented before it becomes clinically available.

Inhibition of apolipoprotein synthesis

Mipomersen

Apolipoprotein B (ApoB) is an essential component of both VLDL and LDL, therefore the inhibition of ApoB synthesis will decrease the plasma concentration of all ApoB-containing lipoproteins. Familial hypobetalipoproteinemia (FHBL) is a rare disorder caused by an autosomal, codominant mutation in the ApoB gene, resulting in a truncated form of ApoB. Homozygotes for this condition have very low concentrations of LDL and ApoB, while heterozygotes have decreased levels and are mostly asymptomatic [5]. In patients with homozygous familial hypercholesterolemia (hoFH), who lack functional LDL-R and do not respond to statins and PCSK9 inhibitors, ApoB synthesis inhibition may be a therapeutic option.

Mipomersen is a second generation anti-sense oligonucleotide (ASO) targeted to mRNA for ApoB-100, the principal apolipoprotein of LDL [2]. The inhibition of ApoB-100 translation decreases the ApoB synthesis in the liver, reducing hepatic VLDL production and decreasing LDL levels.

Mipomersen has frequent adverse effects, from severe injection site reactions to liver enzymes elevation (in 3% - 17% of cases) [54]. The potential risk of hepatotoxicity represents a major limitation, rendering mipomersen a backup treatment only for hoFH patients in which other hypolipemiant therapies failed to lower the LDL at the target tissue level [24]. The patients should be closely monitored for liver toxicity and liver function should be assessed prior to and after the initiation of the treatment. After discontinuation of the drug treatment, the liver function returns to normal [24].

Mipomersen was approved by FDA in 2013 for hoFH patients as a complementary treatment to diet and lipid-lowering medication [55]. In patients without hoFH, there are no clinical data related to the safety and efficacy of mipomersen. To date, there are no ongoing trials to demonstrate the impact of mipomersen on CV morbidity and mortality [45].

Inhibition of microsomal triglyceride transfer protein

Lomitapide

Microsomal Triglyceride Transfer Protein (MTP) is responsible for triglyceride transfer to ApoB in the liver and intestine, to generate atherogenic ApoB-containing lipoproteins.

Lomitapide is a selective inhibitor of MTP and also of cytochrome P450 3A4, and P-glycoprotein. Lopitamide directly binds and inhibits MTP in the endoplasmic reticulum of hepatocytes and enterocytes, thus preventing the assembly of ApoB-containing lipoproteins. The inhibition of the synthesis of chylomicrons and VLDL leads to a decrease in the plasmatic LDL-C levels by an LDL-R independent mechanism [5].

Lomitapide reduced LDL-C by 40% - 50% in a small study including patients with hoFH [20]. It has many adverse effects, including diarrhoea, vomiting, abdominal pain, elevations of liver enzymes and hepatic steatosis. The induced hepatic steatosis could be a risk factor for cirrhosis. Lopitamide also interacts with many drugs, increasing warfarin levels and increasing systemic exposure to atorvastatin and simvastatin, with a higher risk of statin – related myopathy [24]. In 2012, lomitapide was approved by the FDA only for patients with hoFH as an adjunct treatment to a low-fat diet and other lipid-lowering treatments, including LDL apheresis [45]. Until now there is no evidence related to the lopitamide efficacy on CV morbi-mortality.

Adenosine triphosphate citrate lyase inhibition

Bempedoic acid (ETC 1002)

Adenosine triphosphate citrate lyase (ACL) is a cytosolic enzyme highly expressed in hepatocytes and adipocytes. It represents a key enzyme in different metabolic pathways and constitutes a link in energy metabolism, from carbohydrates to fatty acids and cholesterol production, in liver and lipogenic tissues [4]. ACL supplies substrate for cholesterol and fatty acid synthesis in the liver [32]. ACL is involved in lipogenesis in the synthesis of acetyl-CoA, an important starting molecule for both fatty acid and cholesterol production, being positioned upstream

from HMG-CoA reductase, a key rate-limiting enzyme of the cholesterol biosynthetic pathway. ACL catalyses the conversion of citrate and HSCoA to oxaloacetate and acetyl-CoA, the latter being subsequently converted to 3-hydroxy-3methylglutaryl-CoA (HMG-CoA), then to mevalonic acid by HMG-CoA reductase and finally into cholesterol (Figure 3) [8].

Given its important role in lipid biosynthesis, ACL has been investigated as a potential target for lipid-lowering therapy. Bempedoic acid or ETC-1002 (8-hydroxy-2, 2, 14, 14 tetramethylpentadecanedioic acid), having an improved bioavailability and substrate affinity and specificity then the previous ACL inhibitors, is currently in the most advanced stage of clinical development among all ACL inhibitors [19]. Bempedoic acid is a small molecule, a prodrug converted by acyl-CoA synthetase to the active metabolite within the hepatocyte. This specific isozyme is absent in the skeletal muscle, potentially avoiding the adverse muscular effects of the drug [51].

Bempedoic acid, converted in the liver to a coenzyme A (CoA) derivative, or ETC-1002-CoA, directly inhibits ACL and reduces cytosolic acetyl-CoA, which in turn leads to decreased cholesterol production within the hepatocyte. Cholesterol biosynthesis is directly regulated by the intracellular cholesterol levels, the main regulatory mechanism being the sterol regulatory element-binding protein (SREBP), a transcription factor which, by sensing intracellular cholesterol in the endoplasmatic reticulum, stimulates the transcription of several genes, including the LDL-R gene [9]. LDL-R upregulation increases the uptake of LDL particles by hepatocytes, leading to a substantial reduction of plasmatic LDL-C levels [8].

In addition to lowering plasma LDL-C due to ACL inhibition, bempedoic acid activates AMP-activated protein kinase (AMPK), which inhibits HMG-CoA reductase (the rate limiting enzyme for cholesterol synthesis) and acetyl-CoA carboxylase (the rate-limiting enzyme for fatty acid synthesis) [7].

Bempedoic acid treatment has been shown to be effective in reducing plasmatic cholesterol, free fatty acids and triglycerides levels, in decreasing liver fat and also, in improving glycaemic control and insulin resistance in experimental animal models [19, 34]. Moreover, there was a decreased production of pro-inflammatory cytokines and chemokines and fewer vascular complications of metabolic syndrome under bempedoic acid treatment, proven effective in reducing atherosclerosis [25].

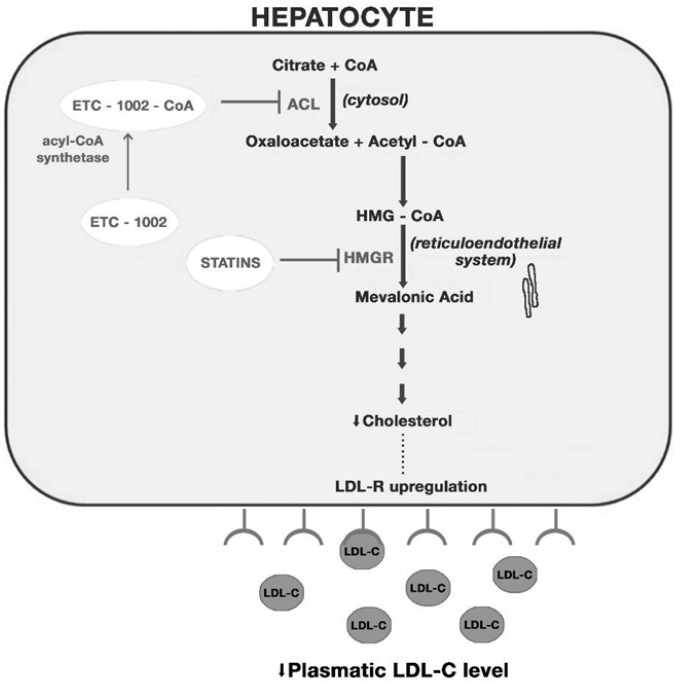

Figure 3.

The mechanism of LDL-C lowering by ETC 1002 (bempedoic acid) (LDL-R = low-density lipoprotein receptor, LDL-C = low-density lipoprotein cholesterol, ETC 1002 = bempedoic acid, CoA = coenzyme A, ACL = adenosine triphosphate citrate lyase, HMG = 3-hydroxy-3-methylglutaryl, HMGR = 3-hydroxy-3-methylglutaryl reductase) [modified from 25]

Bempedoic acid reduces LDL-C levels in a dose-dependent manner in eight phase II clinical trials, including over 1045 subjects. The dose of 180 mg/dL reduces LDL-C levels by 30% as monotherapy, and there is an incremental LDL-C reduction of 20 - 22% when added to statins and of approximately 50% when combined with ezetimib. The high sensitivity C reactive protein has also decreased by up to 40%

under bempedoic acid treatment. Though, in the clinical trials, bempedoic acid only reduced cholesterol synthesis, with no effect on fatty acid production. There was also a neutral effect on weight, glucose metabolism, insulin resistance and blood pressure, suggesting that the metabolic effect of AMPK activation by bempedoic acid in humans is not clinically relevant. Safety data from clinical trials showed that bempedoic acid is well tolerated without apparent adverse effects [8].

Bempedoic acid is a promising drug, significantly lowering LDL-C levels in patients with hypercholesterolemia and having convenient long-term safety and tolerability. Bempedoic acid efficacy, associated with statins or added to ezetimib in statin-intolerant patients, as well as its effects on reducing CV risk, are the rationale of a large phase III program that is currently in progress [12, 64].

LDL apheresis

LDL apheresis is a technique that has proven over time to increase life-expectancy in hoFH. Cascade filtration, immunoadsorption, heparin-induced LDL precipitation, LDL adsorption through dextran sulphate and LDL hemoperfusion system are several clinically relevant methods [39, 67].

Cascade filtration was the first semi-selective technique for the treatment of hypercholesterolemia, being superior to conventional plasmapheresis. It uses a secondary membrane which retains plasma LDL-C, reducing total cholesterol by 35 - 50% and LDL-C by 30 - 45% [67].

The immunoadsorption technique consists of the perfusion of patient plasma through columns coated with LDL antibodies, the LDL molecules from plasma thus being adsorbed. The LDL-apheresis technique is the most e□ective method for lowering lipoprotein (a) (Lp(a)) using immunoadsorption anti-Lp(a) polyclonal antibody columns [67].

Heparin-induced LDL precipitation consists of mixing the patient plasma with an acid buffer, then adding heparin. LDL-C precipitates together with fibrinogen and heparin in the acid environment, forming insoluble precipitates. After removing the precipitates from the plasma using a polycarbonate membrane, the plasma, whose pH is reverted to a physiological value, is returned to the patient [67].

LDL adsorption through dextran sulfate technique is based on the selective absorption of all substances containing apolipoprotein B by dextran sulfate. Low-molecular dextran sulfate has a direct interaction with positively charged surface of apolipoprotein B - containing lipoproteins (LDL, VLDL, and Lp(a)) and cholesterol levels are reduced by more than 60% [67].

LDL hemoperfusion technique involves direct adsorption of lipoproteins using a matrix of polyacrylate beads. The blood is pumped through the LDL adsorber which covalently binds with the cationic groups in the apolipoprotein B moiety of LDL and Lp(a) in a simple extracorporeal circuit [67].

LDL apheresis may reduce LDL-C levels to 50 - 60% of the pre-procedural levels when the procedure is performed weekly or biweekly. LDL-C increases after each session, but not to the pre-procedural level [39].

LDL apheresis is indicated in patients with FH, including children above the age of seven and pregnant women, unresponsive or intolerant to pharmacologic and dietary management. In hoFH, LDL apheresis has been reliable proven to increase life-expectancy. There are limited data showing that LDL apheresis has similar effects to those of maximal lipid-lowering drug therapy on the progression of CV disease in heterozygous FH. Apheresis may be considered in individual patients with family history of premature cardiac death whose coronary disease progresses and LDL-C cholesterol remains > 190 mg/dL or is decreased by less than 40% under maximal drug therapy, as well as in patients with rapid progressing coronary disease and whose LDL-C cholesterol remains > 120 mg/dL despite maximal drug therapy [12, 21, 45].

LDL apheresis achieves a robust reduction in LDL-C, although it is costly, time-consuming and not readily available as an extracorporeal technique.

Conclusions

In summary, new therapeutic options for LDL-C lowering are needed due to the strong relationship between LDL-C levels and atherosclerosis and the remanence of residual cardiovascular risk, despite current hypolipemiant treatment. Many new molecules targeting different stages in LDL-C metabolism are rapidly gaining ground, and the anti-PCSK9 MoAbs are the most advanced cholesterol lowering therapies, being already implemented in clinical practice.

These new LDL-C lowering drugs appear promising for the prevention of atherosclerosis; however, the beneficial effects on the long term still await clinical confirmation in the future.

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