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REVIEW

Antisense oligonucleotides in the treatment of lipid disorders: Pitfalls and promises

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ABSTRACT

Dyslipidaemia is one of the pivotal risk factors for cardiovascular disease (CVD), and lipid-lowering therapy is therefore the cornerstone in cardiovascular risk management. With the currently available treatment options the relative risk reduction in CVD is approximately 30%, leaving a large residual risk. This calls for the development of additional therapeutic moieties and antisense oligonucleotides (ASOs) have proven to be such a new and effective treatment. ASOs are short single strands of DNA that intracellularly bind mRNA of specific proteins. This induces the degradation of the mRNA through which the protein cannot be produced. Based on knowledge of lipid metabolism several targets of ASO therapy can be identified. This review offers a summary of current developments in ASO therapy regarding lipid disorders.

KEYWORDS

Antisense oligonucleotides, mipomersen, lipid disorders, PCSK9

INTRODUCTION

Cardiovascular disease (CVD) is an important cause of mortality and morbidity in Western countries. Hypercholesterolaemia is an important risk factor for CVD, which is exemplified by the finding that patients with familial hypercholesterolaemia (FH) are at highly increased risk of CVD. In line, firm reduction of low-density lipoprotein cholesterol (LDL-C) results in reduction of CVD risk. Statins (HMG CoA reductase inhibitors), which strongly diminish LDL-C, are therefore widely accepted as the first-line treatment in patients with increased CVD risk. Despite optimal statin therapy, however, a substantial number of these patients will eventually experience a cardiovascular event (e.g. myocardial infarction or stroke). Apparently, the 30-50% reduction of LDL-C by statins is not able to abolish the risk of CVD. This is partly due to the fact that maximal dosages of statins, whether or not in combination with other lipid-lowering drugs, are not capable of lowering LDL-C to target levels in some of the patients. Moreover, increasing the dose often results in cumulative side effects which hinder dose augmentation.

Hence, there is a need for the identification of novel therapeutic moieties that lower LDL-C on top of statin therapy. Antisense oligonucleotides (ASOs) are considered a potential novel lipid-lowering therapy, which acts via a different mechanism than in statins. In this review we describe the mechanism of action of ASOs and provide an overview of ASOs with potential therapeutic value in the treatment of lipid disorders.

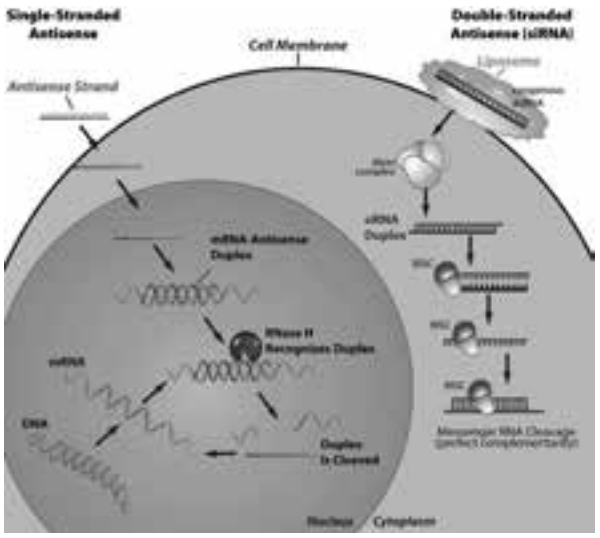
CURRENT TREATMENT OPTIONS IN LIPID DISORDERS

A decrease of LDL-C by 1 mmol/l (38.67 mg/dl) in patients with increased CVD risk reduces CVD risk by 22%.[1] In past decades the effect of statins on LDL-C and subsequent CVD risk has been extensively investigated. Statins lower LDL-C by 30-60% and CVD risk by 25-30%.[2] Unfortunately statin therapy is accompanied by side effects in a minority of subjects; 10% of treated patients develop muscle pain, which leads to discontinuation of treatment in a third of those patients.[3] In addition, a considerable number of

patients do not attain the LDL-C treatment goal despite optimal statin therapy.[4] Additional lipid-lowering drugs such as ezetimibe and fibrates only result in modest additional reductions of LDL-C. Moreover, CVD risk reduction by these drugs remains to be demonstrated.

Another lipid disorder which has gained attention is the presence of decreased high-density lipoprotein cholesterol (HDL-C).[5] HDL particles play an important role in the 'reverse cholesterol transport'. By this mechanism cholesterol is taken up from peripheral cells and transported to the liver where it is excreted via the faeces. There is a strong, inverse correlation between HDL-C and CVD which is independent of LDL-C concentration.[6] These epidemiological findings have directed the attention to HDL-increasing therapies as a promising target in CVD prevention.[5] Whether HDL increase reduces CVD risk, however, remains to be demonstrated. An example of an HDL-increasing drug is niacin which enhances HDL-C by 26% with an additional decrease in both LDL-C and triglycerides of 20%.[7] Its effect on cardiovascular disease was examined in the AIM HIGH study that was terminated prematurely, prompted by inefficacy and an unexpected high number of ischaemic strokes among treated patients.[8] More recently, the effect of niacin on cardiovascular events was addressed in the HPS2-Thrive study (ClinicalTrials.gov: NCT00461630). This study was halted by the US Food and Drug Administration (FDA) due to futility.[9] In the same year, the research on dalcetrapib, a selective HDL-C increasing drug that works by inhibiting CETP activity, was also terminated since there was no significant decrease in CVD risk.[10] Although other studies on CETP-increasing drugs, such as the CETP inhibitor anacetrapib, are still ongoing, it is safe to state that the bar for novel HDL-C raising agents in CVD prevention has been raised significantly.

Figure 1. *Steps of RNase H (A) and siRNA (B) antisense mechanisms*



The single-stranded DNA oligonucleotide passes through the cell membrane and enters into the cytoplasm of the cell. Although the RNase H enzymes are present in the cytoplasm and the nucleus of the cell, data suggest that RNase H oligonucleotides work predominantly in the nucleus. The oligonucleotide enters into the nucleus from the cytoplasm. Once in the cell nucleus, the oligonucleotide binds or hybridises to the target mRNA, resulting in the formation of a sense–antisense duplex. The formation of the duplex initiates the recruitment of the RNase H enzyme, an endogenous nuclease. RNase H degrades the target mRNA, which results in inhibition of target mRNA expression. The oligonucleotide moves on and then hybridises to another target mRNA.
The RNAi oligonucleotide (siRNA) passes through the cell membrane and enters into the cytoplasm of the cell. A helicase separates the sense and antisense strands of the oligonucleotide. RISC, an endogenous conglomerate of functional components, associates with the antisense oligonucleotide. The antisense strand of the oligonucleotide hybridises to the target mRNA, resulting in the formation of a sense–antisense duplex. The nuclease component of RISC is anendogenous nuclease that degrades the target mRNA. This results in the inhibition of target mRNA expression.

RISC: RNA-induced silencing complex; RNAi: RNA interference; siRNA: Small interfering RNA. From: Crooke RM. Antisense oligonucleotides as therapeutics for hyperlipidaemias. Expert opinion on biological therapy. 2005 Jul;5(7):907–17.

ANTISENSE OLIGONUCLEOTIDES

ASOs are short single strands of DNA comprising 12-20 nucleotides. This provides a highly specific complementary binding to mRNA. The binding prevents translation of mRNA, thereby preventing translation and the production of the respective proteins.[11] ASOs can exert their function in two ways (*figure 1*). A general advantage of ASOs in the treatment of lipid disorders is that they mainly accumulate in the liver where the majority of the proteins involved in lipid metabolism is also produced. Moreover, ASOs are not metabolised by cytochrome P450 as most other drugs, which reduces the risk of drug-drug interactions.[12] The binding of ASOs to mRNA of one specific protein is another valuable characteristic, which can be expected to decrease the chances of inducing unspecific side effects.

A disadvantage of ASOs is their rapid degradation by nucleases. To circumvent this, various chemical modifications have been designed to stabilise ASOs and promote their cellular uptake.[13] Because ASOs selectively inhibit the production of selected proteins, specific targets can be identified based on knowledge of the lipid metabolism. Below is a review of targets investigated thus far and ASOs developed against these targets.

POTENTIAL TARGETS AND AVAILABLE ASO'S

ApoB-100

ApoB-100 is an essential protein in all pro-atherogenic lipoproteins (e.g. VLDL, LDL and Lp(a)).[14] Patients

expressing increased apoB-100 are characterised by increased CVD risk. In contrast, genetically decreased apoB-100 offers protection against CVD.[15]

Mipomersen: Mipomersen (ISIS 301012) is one of the first ASOs specifically targeted against apoB-100.[16] It is administered subcutaneously followed by almost complete absorption. Due to minimal degradation by endonucleases it exhibits a relatively long half-life of 23-46 days. Following absorption, highest concentrations are measured in liver and kidneys. LDL-C reductions of 44% were achieved with 200 mg administered once a week subcutaneously, whereas on top of statins mipomersen decreased LDL-C by 36%.[17,18] In phase III studies identical dosages during 26 weeks abated both LDL-C and apoB by 36% in patients with severe hypercholesterolaemia or increased CVD risk.[19] Most of the side effects reported are related to injection site reactions. These are experienced by up to 90% of patients, comprising erythema and itching. Of the patients 50% also report flu-like symptoms.[20] Another significant side effect of mipomersen relates to its mechanism of action, namely the decreased excretion of VLDL. In 10% of patients a steatotic response of the liver was observed, which was shown to be reversible after discontinuation of treatment.[21] Increased liver transaminases were associated with hepatic steatosis.[18] Noteworthy, there were no signs of impaired liver synthesis function (PTT, bilirubin).[22]

ISIS 147764: A second ASO against apoB-100 is ISIS 147764. Thus far its effects have only been investigated in mice. In LDL-receptor knockout mice 12 weeks of treatment resulted in LDL-C reduction of 60-90% with a concomitantly decreased formation of atherosclerotic lesions.[23]

PCSK9

PCSK9 promotes the degradation of LDL receptors.[24] Gain-of-function mutations in the gene encoding this protein result in low abundance of hepatic LDL receptors and consequently increased levels of LDL-C in serum and eventually premature CVD. Conversely, loss-of-function mutations are accompanied by decreased levels of LDL-C and decreased risk of CVD.[25] These observations led to development of antibodies targeted against PCSK9, which have shown very positive effects on LDL-C in humans.[26] ASOs against PCSK9 are expected to offer potent LDL-C lowering effects, comparable with those reported following the treatment with PCSK9 antibodies. In a mouse model these ASOs produced a strong reduction in PCSK9 mRNA (92%) with a concomitant doubling of LDL receptors. This resulted in an LDL-C decrease of 38%.[27]

Apo(a)

Lipoprotein (a) (Lp(a)) is an important risk factor for myocardial infarction and other forms of coronary diseases.[28] Lp(a) is formed by the fusion of apolipoprotein (a) and apoB-100 containing lipoproteins (e.g. LDL). To date, there are no treatments to lower Lp(a) besides niacin.[29] Therefore it remains unclear whether Lp(a) reduction will lead to decreased CVD risk. Apolipoprotein (a) is assimilated in the liver and an ASO was developed against the responsible gene.[16] In mice this ASO diminished Lp(a) by 27% and total cholesterol by 22%. Interestingly, mipomersen, the apoB antisense, also lowers Lp(a).[30]

Apolipoprotein C-3

The apolipoprotein C-3 (apoC-3) is present on triglyceride-rich lipoproteins, such as VLDL. ApoC-3 impairs clearance of these particles by inhibition of the enzyme lipoprotein lipase (LPL) which has a triglyceridase effect. Patients with a genetic defect in the gene encoding apoC-3 are characterised by decreased levels of VLDL and triglycerides.[31] Furthermore these patients exhibit low coronary calcium scores, a surrogate marker for atherosclerosis. ASOs directed against apoC-3 theoretically possess therapeutic effects in patients with increased levels of triglycerides. In mice studies treatment with such ASO resulted in 90% lower apoC-3 mRNA and subsequently 80% and 95% decreased levels of triglycerides in serum and liver respectively.[11] Currently the ASO 'ISIS APOCIII Rx', targeted to apoC-3, is being examined in a phase II study.

Diglyceride acyltransferase (DGAT2)

DGAT is the enzyme catalysing the last step in the synthesis of triglycerides. It is expressed in the liver, small intestine and adipose tissue. Two subtypes of DGAT (DGAT1 and DGAT2) have been identified. DGAT2 has been shown to play a role in cholesterol metabolism as shown in several mice studies.[32] Due to its central role in triglyceride metabolism, inhibition of DGAT2 is suggested to result in decreased levels of triglycerides. To verify this hypothesis the effect of the ASO ISIS-217376 targeted against DGAT2 was investigated in mice.[32] It abates mRNA of DGAT2 in liver and adipose tissue by 75% while leaving expression of DGAT1 unaltered. Hepatic synthesis of triglycerides decreased significantly and this led to diminished secretion of triglycerides. A positive effect on lipid profile and hepatic steatosis was also observed. Antisense therapy against DGAT2 appears to have a selective effect on cholesterol metabolism which makes DGAT2 an interesting target in patients with lipid disorders.

miRNA 33a/b

ATP binding cassette A1 (ABCA1) is a transmembrane protein which exerts essential functions in cholesterol transport from peripheral cells to HDL particles.

Deficiency of this protein results in Tangier disease which is characterised by, among other things, extremely low levels of HDL-C and increased CVD risk.[33,34] Production of ABCA1 is inhibited by microRNAs 33a and 33b (miRNA 33a/b). Of note, the latter does not only inhibit ABCA1 but also other genes involved in lipid metabolism.[35] Rayner and colleagues showed an increased expression of ABCA1 in monkeys after treatment with an ASO directed against miRNA33a/b.[35] This translated into diminished plasma levels of VLDL and triglycerides (50%) and increased levels of HDL-C (50%). MiRNA 33a/b is therefore a promising target in the treatment to promote cholesterol efflux.

CONCLUSION

ASOs provide a potentially novel therapeutic paradigm allowing highly specific inhibition of selected proteins. They are considered a promising moiety to treat a wide variety of lipid disorders due to the fact that ASOs accumulate in the liver, which is also the major orchestrator of lipid metabolism. Since ASOs retain their efficacy on lipid levels, also on top of statin therapy, they hold a promise as add-ons in patients not reaching their target LDL-C despite maximum lipid-lowering therapy or those experiencing serious side effects on currently available lipid-lowering drugs. Mipomersen, the most advanced ASO decreasing apoB production in the liver, is developed for use in patients with homozygous and/or severe heterozygous FH. In view of the less favourable side effect profile, comprising injection site reactions and liver steatosis, further efforts should be aimed at reducing these side effects. In view of the high similarity of the production process of the various ASOs, it is to be expected that the development of novel ASOs for clinical use can be performed in an expedited manner.

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