Review

Gene silencing approaches for the management of dyslipidaemia

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The key role of dyslipidaemias in determining cardiovascular risk has been well established, and statins often provide effective therapeutic management. However, many patients do not achieve recommended lipid levels despite maximal therapy, and some cannot tolerate high-dose statin therapy. Recently, genetic insights into mechanisms underlying regulation of lipoprotein metabolism have expanded the potential targets of drug therapy and led to the development of novel agents, including development of gene silencing approaches. These therapeutic options include the modulation of synthesis in the liver, maturation in the circulation, and catabolism of lipoproteins. In this review, we discuss the pharmacological consequences of silencing apolipoprotein B, apolipoprotein (a), microRNA 33, proprotein convertase subtilisin/kexin type 9, and apolipoprotein C-III. New potential targets such as other microRNAs, diacylglycerol acyl transferase-1, and angiopoietin-like protein 3 are also presented. The pharmacological consequences of gene silencing and the advancement of these therapeutic approaches in clinical development will be examined.

Lipid lowering and cardiovascular risk

The key role of dyslipidaemias, and particularly of hypercholesterolaemia, in determining cardiovascular disease (CVD) has been proved beyond reasonable doubt. The discovery of drugs such as statins has provided a very effective approach to lower low-density lipoprotein cholesterol (LDL-C) and reduce cardiovascular risk, as documented by clinical trials and clinical practice [1]. However, research clearly suggests that other lipoprotein classes beyond LDL play important roles in determining cardiovascular risk and that further cardiovascular benefit could be derived from a greater reduction in LDL-C, especially in high-risk and very high-risk patients [1]. Recently, biological and genetic research on lipids and lipoprotein metabolism has led to the identification of several genes and proteins that may be pharmacologically targeted to improve the lipoprotein profile and possibly cardiovascular outcomes in patients with dyslipidaemia. This review will discuss emerging therapeutic approaches based on the silencing of gene transcripts involved in lipoprotein synthesis and catabolism.

Pharmacokinetics and pharmacodynamics of small nucleic acids used for gene silencing

Gene silencing strategies for the management of dyslipidaemia utilising the injection of small nucleic acid molecules are currently under preclinical and clinical development. The process is based on the intracellular hybridisation of a nucleic acid sequence to cognate messenger RNA (mRNA). The length of this molecule, approximately 20 nucleotides, favours the selective binding to one specific mRNA target, theoretically precluding off-target effects. The kinetics of these nucleic acid sequences following parental administration is characterised by a large and rapid distribution to the liver, thus making this approach rather attractive for inhibiting mRNA post-transcriptional processing at this site [2,3].

RNA interference (RNAi) is an evolutionarily highly conserved biological process used by plants, mammals, and even some fungi to post-transcriptionally inhibit gene expression [4]. In higher organisms, RNAi is triggered by long double-stranded RNA (dsRNA), usually derived from viral genomes or from transcription of nuclear DNA; in both cases, dsRNA are degraded intracellularly by the RNAse type III-like enzyme Dicer into endogenous microRNA or exogenous short interfering RNA (siRNA) that are 21–23 nucleotides in length. These small nucleic acids in the cytoplasm enter the RNA-induced silencing complex (RISC), where the argonaute 2 (Ago2) protein unwinds the siRNA and incorporates the guide strand. siRNAs selectively bind the target mRNA, with perfect or near-perfect base pairing, thus leading to its degradation catalysed by the PIWI domain of Ago2. By contrast, miRNA bind to not fully complementary sequences contained within the UTR of the target mRNA and induce translational repression [5].

To date, two approaches for gene silencing have been extensively investigated and are currently used in clinical studies: the injection of antisense oligonucleotides (ASOs) or siRNA that are complementary to the mRNA of interest (Figure 1).

ASOs

ASOs were the first to be developed. They are single-stranded oligodeoxynucleotides that inhibit gene expression by

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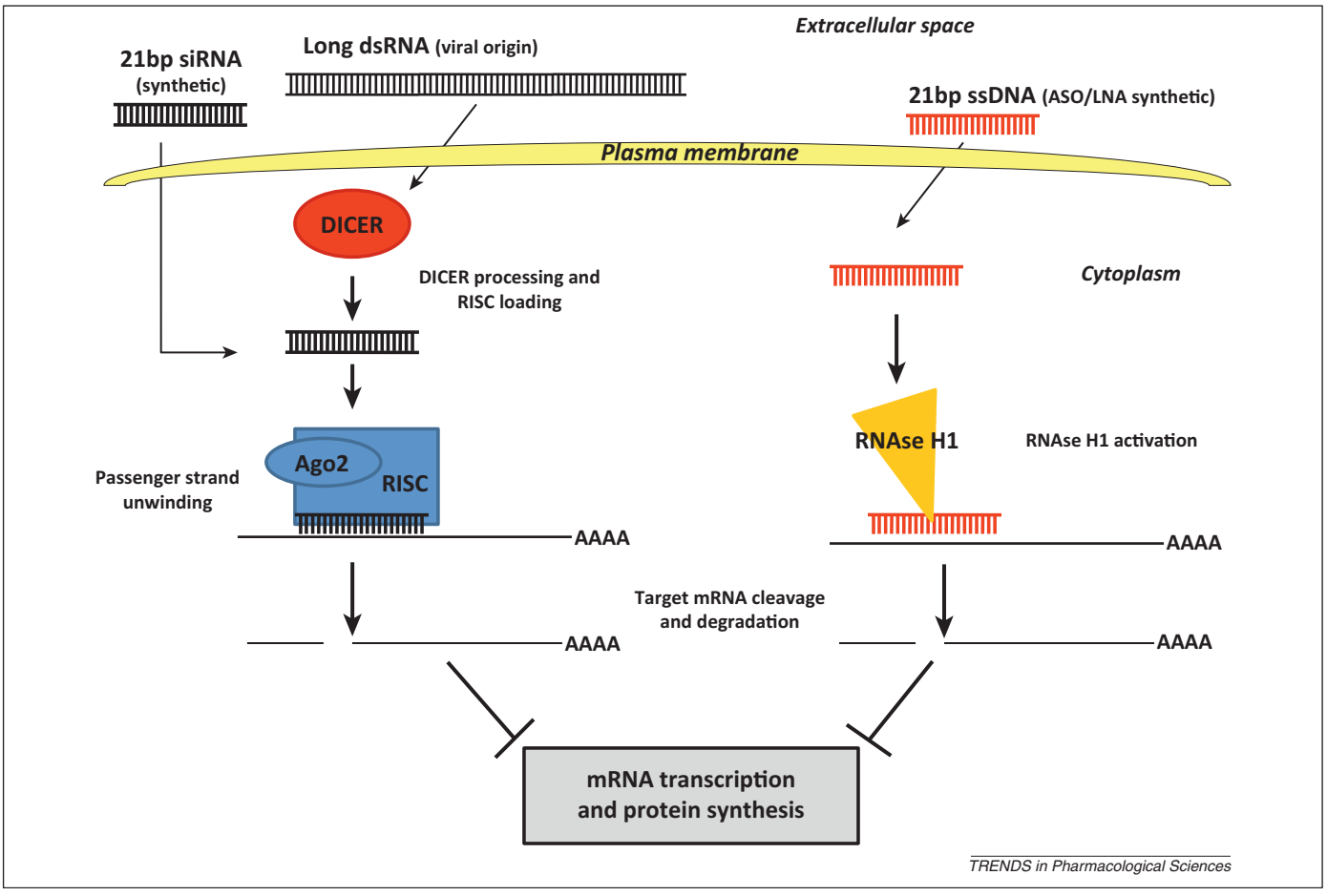

Figure 1. Mechanisms of action of short interfering RNA (siRNA) and antisense oligonucleotides (ASOs). siRNA can be introduced directly into the cell or may derive from long double-strand RNA of viral origin cleaved by the Dicer enzyme. These siRNAs are incorporated into the RNA-induced silencing complex (RISC) and unwound, then the passenger strand is removed and the guide strand binds specifically to the target mRNA and directs its cleavage mediated by the nuclease activity of argonaute 2 (Ago2). ASOs are synthetic molecules that enter into the cytoplasm and recognise their complementary sequence within the target mRNA thus triggering ribonuclease H1 activation and mRNA degradation.

different pathways: (i) activation of the RNA-specific nuclease RNase H1; (ii) inhibition of the translation by steric interference on the ribosome; and (iii) destabilisation of the pre-mRNA [6]. RNase H1 activation is the most potent mechanism for antisense activity. Activation is triggered by chemically modified ASOs that have a phosphorothioate (PS) backbone. However, PS-modified ASOs are sensitive to exonucleases and therefore highly unstable in many biological fluids such as plasma or serum. Replacement of the fructose 2′ hydrogen with an O-methyl (OMe) or an O-methoxyethyl (MOE) on the flanking 3′ and 5′ ends of the oligomer further increases the binding efficiency and stability [7,8]. Locked nucleic acid (LNA)-based ASOs, in which the ribose moiety of the nucleotide is modified with an extra bridge connecting the 2′ oxygen and 4′ carbon, also demonstrate increased stability in biological fluids, thus improving their activity (http://www.santaris.com/science/lna-drug-platform).

A severe limitation of ASOs therapy is their poor adsorption by the gastrointestinal tract and the requirement for an intravenous or subcutaneous injection. Once in systemic circulation, ASOs bind to plasma proteins and are cleared from the plasma with an initial rapid distribution (half-life of hours) to organs of the reticulo-endothelial system, primarily liver and kidneys but also spleen and lymph nodes. This first phase is followed by a prolonged (several days) elimination phase determined by endonucleases-dependent intracellular degradation and terminated mainly by urinary excretion [9].

siRNA

In brief, siRNAs are chemically synthesised dsRNAs with a passenger (sense) strand and a guide (antisense) strand complementary to the target mRNA sequence (Figure 1). Many of the concepts described for ASOs also apply to siRNAs, with a few notable differences. Specifically, naked siRNAs, despite being less susceptible to nuclease-mediated degradation, have a decreased plasma half-life compared with ASOs, as a consequence of the relatively high-negative charge given by the phosphate groups and the high-molecular weight. This makes it more difficult for siRNAs to cross cellular membranes, reducing their binding to plasma proteins and promoting a more rapid kidney excretion as compared with ASOs [10]. For this reason, systemic administration of siRNAs requires conjugation with lipophilic groups (cholesterol), cationic polymers or cell-penetrating peptides, and poly-ethylene glycol (PEG) to improve cell delivery and prevent excretion in the urine [11]. However, the best results have been obtained when siRNAs are complexed with nanoparticles or liposomal