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Novel therapeutic agents for lowering low density lipoprotein cholesterol

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ABSTRACT

Elevated low density lipoprotein cholesterol (LDL-C) levels have been associated with an increased risk for cardiovascular disease (CVD). Despite a 25–30% reduction in CVD risk with LDL-C reducing strategies, there is still a significant residual risk. Moreover, achieving target LDL-C values in individuals at high CVD risk is sometimes limited because of tolerability and/or efficacy. Thus, novel therapeutic agents are currently being developed to lower LDL-C levels further. This review will highlight some of these therapeutic agents including anti-sense oligonucleotides focused on apolipoprotein B, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, microsomal triglyceride transfer protein inhibitors, and thyromimetics. For each therapeutic class, an overview of the mechanism of action, pharmacokinetic data, and efficacy/safety evidence will be provided.

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1. Introduction

Lowering low-density lipoprotein cholesterol (LDL-C) levels reduces the risk of cardiovascular disease (CVD) by approximately 25–30%. Currently, LDL-C reduction is primarily achieved through the use of statins although there are several other marketed medications that can lower LDL-C levels, albeit to a lesser degree. Despite these strategies for LDL-C reduction, there still remains a significant residual cardiovascular risk. Moreover, achieving target LDL-C values in individuals with high cardiovascular risk is sometimes limited because of tolerability and/or efficacy. Indeed, up to 40% of high-risk and 80% of very-high-risk individuals do not achieve their respective LDL-C goals (Yan et al., 2006). Thus, alternative physiologic strategies to further lower LDL-C levels effectively and safely are being actively sought. This paper will discuss several novel LDL-C lowering pharmacologic agents under consideration, including anti-sense oligonucleotides (ASOs) to apolipoprotein B (apo B), proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, microsomal triglyceride transfer protein (MTP) inhibitors, and thyromimetics. For each category, an overview of the mechanism of action, pharmacokinetic data, and efficacy/safety evidence will be provided.

2. Anti-sense oligonucleotides directed at apolipoprotein B

2.1. Mechanism of action

Antisense oligoneucleotides (ASOs) are short, deoxyribonucleotide strands (8 to 50 nucleotides in length) that bind using Watson–Crick

Abbreviations: Apo, apolipoprotein; ASO, antisense oligonucleotide; CHD, coronary heart disease; CVD, cardiovascular disease; CYP, cytochrome P450; ED_{50}, effective dose; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; IC_{50}, half maximal inhibitory concentration; IHTG, intrahepatic triglyceride; IV, intravenous; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; Lp(a), lipoprotein (a); mRNA, messenger ribonucleic acid; MTP, microsomal triglyceride transfer protein; PON, paraoxonase; RCT, randomized placebo-controlled trial; SC, subcutaneous; siRNA, small interfering ribonucleic acid; $t_{1/2}$, half-life; TG, triglyceride; TR, thyroid hormone receptor; VLDL, very low density lipoprotein.

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hybridization to a target messenger ribonucleic acid (mRNA) to cause inhibition of gene expression (Fig. 1). This inhibition of gene expression can occur through several mechanisms, including activation of enzymes (RNAse H or Argonaute 2) which in turn cause targeted degradation of the mRNA of interest; translational arrest through interference with ribosomal activity; or deterrence of maturation of the mRNA itself (Bennett & Swayze, 2010). The design of ASOs must ensure that these molecules have a high specificity for their target gene and are relatively resistant to degradation by endogenous nucleases. This is achieved through varying chemical modifications of the ASO, such as incorporation of a phosphodiester- or phosphorothioate-modified backbone, alteration of the ring structure, and creation of a central gap region of typically 10 modified deoxynucleotides flanked on the 5′ and 3′ ends (termed wings). The gap region activates RNAse H while the wings prevent degradation of the ASO (Ito, 2007).

ISIS 301012 or mipomersen is a second-generation 20 nucleotide ASO inhibiting *APOB* gene expression via RNAse H activation (Ito, 2007). *APOB* encodes both apo B-48, required for chylomicron assembly, and apo B-100, the major lipoprotein present in LDL, very-low density lipoprotein (VLDL), and intermediate-density lipoprotein (IDL) particles. Since there is a single copy of apo B-100 in each of these atherogenic particles, apo B-100 levels are indicative of the number of atherogenic particles. Apo B-100 serves as the ligand for the LDL receptor (LDLR) and thus plays an important role in the clearance of LDL particles from the bloodstream (Hussain et al., 1999). Importantly, mutations in *APOB* have been associated with a clinical phenotype of significantly elevated LDL-C levels, physical stigmata (xanthelasmas, xanthomas, corneal arcus), and premature cardiovascular disease (Marsh et al., 2002). Mipomersen reduces the levels of apo B-100 and not apo B-48. Thus, using ASOs like mipomersen to directly reduce the production of apo B-100 could have a significant impact on LDL-C levels and atherosclerotic outcomes.

2.2. Pharmacokinetic and pharmacodynamic data

Mipomersen is ≥85% protein-bound (Yu et al., 2007). The initial distribution half-life ($t_{1/2}$) is rapid at 1.26 ± 0.16 h (Yu et al., 2007). The mean time to maximum plasma concentration is 3.4 to 4.0 h, and a loading dose is most likely not needed (Akdim et al., 2011). Urinary excretion is a minor contributor to initial plasma clearance of the drug with <4% being excreted in urine. However, distribution to tissue is the primary mechanism of plasma clearance as the volume of distribution of mipomersen is quite large at 48.3 ± 14.7 L/kg with the tissues with highest concentration of mipomersen being liver and kidney in rodents and non-human primates (Yu et al., 2007). Mipomersen was not found to be present in the brain (Yu et al., 2007). The terminal elimination tissue $t_{1/2}$ ranges from 23 (±1) day for the 50 mg dose to 30 (±11) days in the 200 mg dose (Kastelein et al., 2006). Whole-body clearance involves slow metabolism by endo- and exonucleases, followed by urinary excretion, and to a lesser extent, fecal excretion (Yu et al., 2007).

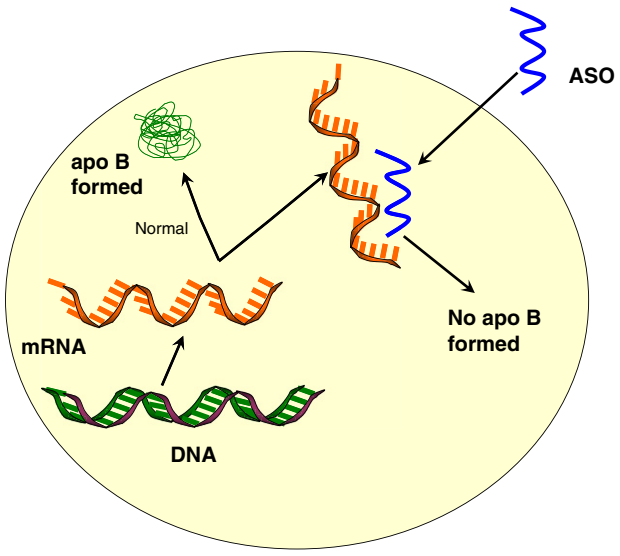

Fig. 1. Mechanism of action of anti-sense oligonucleotides (ASOs).Typically, once DNA is transcribed to messenger RNA (mRNA), translation of mRNA leads to formation of apolipoprotein B. ASOs bind to the mRNA and thereby inhibit apo B formation and this decreased apo B production, in turn, decreases formation of apo B containing lipoproteins, including LDL.

2.3. Efficacy

Preclinical data for a murine ASO against apo B named ISIS 147764 has demonstrated significant dose-dependent reductions in hepatic apo B mRNA and LDL-C of 60–90% in addition to decreases in aortic atherosclerosis of 46–89% (Mullick et al., 2011). Meanwhile, use of mipomersen in transgenic mice overexpressing human apo B-100 or those overexpressing human apo B-100 and human apo (a) (to generate lipoprotein (a) [Lp(a)] particles) resulted in ~75% reduction in LDL-C and ~60% reduction in Lp(a) without a significant change in apo(a) levels at 4 weeks (Merki et al., 2008). The reduction in apo B and Lp(a) levels was sustained post-discontinuation of mipomersen, taking ~10 weeks to return to baseline values (Merki et al., 2008). Since apo B is needed for the movement of VLDL out of the liver into peripheral tissues, inhibition of apo B by ASOs could theoretically be associated with accumulation of VLDL and thus, hepatic steatosis. However, murine models testing ISIS 147764 have not demonstrated significant hepatic steatosis (Crooke et al., 2005; Mullick et al., 2011).

Mipomersen effects a dose-dependent decrease in apo B, VLDL, and LDL-C levels (Kastelein et al., 2006). In those with mild LDL-C elevations receiving the 200 mg/week subcutaneous (SC) dose of mipomersen, the maximal reductions in apo B, VLDL, and LDL-C were 46%, 53%, and 45%, respectively (Akdim et al., 2011). Given the long $t_{1/2}$ of mipomersen, these reductions remained significantly below baseline for up to 90 days after the last dose of mipomersen (Kastelein et al., 2006; Akdim et al., 2011).

Phase II randomized placebo-controlled trials (RCTs) have been conducted in 74 individuals with hypercholesterolemia receiving stable statin therapy (Akdim et al., 2010a) and in 44 patients with heterozygous familial hypercholesterolemia (FH) (Table 1) (Akdim et al., 2010b). Significant dose-dependent decreases in apo B and LDL-C of approximately 20–50% were achieved with mipomersen at doses of 200 mg/week to 400 mg/week but more importantly, these effects demonstrated minimal attenuation at 90 days after discontinuation of the mipomersen (Akdim et al., 2010a). Moreover, 63% and 88% of those receiving the 200 mg/week and 300 mg/week mipomersen dose, respectively, were able to achieve LDL-C < 100 mg/dL. These results thereby demonstrate that mipomersen is effective in significantly lowering LDL-C levels, even when the LDL receptor is defective (Akdim et al., 2010b). This latter trial in patients with FH also demonstrated that although Lp(a) levels were not significantly decreased at 6 weeks, those receiving 300 mg/week had a 29% reduction in Lp(a) at 13 weeks follow-up (Akdim et al., 2010b).

Similar results were obtained in a Phase III RCT of mipomersen in those with homozygous FH (Table 1) (Raal et al., 2010). In this 26-week trial, 34 individuals received mipomersen 200 mg/week while 17 received placebo. Compared with the placebo arm, those receiving mipomersen had significant decreases in apo B, LDL-C, and Lp(a) of 24%, 21%, and 23%, respectively. Interestingly, this was the first trial to demonstrate a significant increase of 15% in HDL-C levels in those receiving mipomersen. No significant changes in highly sensitive C-reactive protein (hsCRP) were demonstrated.

A recent press release by Isis Pharmaceutical, Inc. identified positive results from 2 Phase III RCTs which have yet to be published. The first