Antisense inhibition of apolipoprotein (a) to lower plasma lipoprotein (a) levels in humans

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Abstract Epidemiological, genetic association, and Mendelian randomization studies have provided strong evidence that lipoprotein (a) [Lp(a)] is an independent causal risk factor for CVD, including myocardial infarction, stroke, peripheral arterial disease, and calcific aortic valve stenosis. Lp(a) levels >50 mg/dl are highly prevalent (20% of the general population) and are overrepresented in patients with CVD and aortic stenosis. These data support the notion that Lp(a) should be a target of therapy for CVD event reduction and to reduce progression of aortic stenosis. However, effective therapies to specifically reduce plasma Lp(a) levels are lacking. Recent animal and human studies have shown that Lp(a) can be specifically targeted with second generation antisense oligonucleotides (ASOs) that inhibit apo(a) mRNA translation. In apo(a) transgenic mice, an apo(a) ASO reduced plasma apo(a)/Lp(a) levels and their associated oxidized phospholipid (OxPL) levels by 86 and 93%, respectively. In cynomolgus monkeys, a second generation apo(a) ASO, ISIS-APO(a)Rx, significantly reduced hepatic apo(a) mRNA expression and plasma Lp(a) levels by >80%. Finally, in a phase I study in normal volunteers, ISIS-APO(a)Rx ASO reduced Lp(a) levels and their associated OxPL levels up to 89 and 93%, respectively. In cynomolgus monkeys, a second generation apo(a) ASO, ISIS-APO(a)Rx, significantly reduced hepatic apo(a) mRNA expression and plasma Lp(a) levels by >80%.

Supplementary key words atherosclerosis • aortic stenosis • myocardial infarction

Lipoprotein (a) [Lp(a)] is a highly polymorphic lipoprotein found in human plasma in levels ranging from <1 mg/dl to >250 mg/dl. Lp(a) consists of an LDL-like particle and apo(a), which are covalently bound via a disulfide bond between Cys4326 of apoB-100 and Cys4057 of apo(a) located in kringle IV (KIV) type 9 (KIV9). The apo(a) comprises 10 KIV subunits, of which KIV2 is present in variable identically sized repeats, kringle V (KV), and an inactive protease domain. The apo(a) protein shows a high degree of homology (75–100%) to plasminogen at both the nucleotide and the amino acid level (1). However, the apo(a) gene transcript is much larger due to the repetitive KIV2 domain (3 to >40 repeats) in the LPA gene. The majority of apo(a) mRNA is expressed in the liver, with minor amounts present in the testes, brain, adrenals, lung, and pituitary. Lp(a) levels are primarily genetically determined by the LPA alleles present within an individual (2, 3).

Epidemiological, genetic association, and Mendelian randomization studies have provided strong evidence that Lp(a) is an independent causal risk factor for CVD, including myocardial infarction, stroke, and peripheral arterial disease, as well as calcific aortic valve stenosis (4–9).

Due to the expanding evidence indicating that Lp(a) contributes to CVD, a search for potent and specific inhibitors of apo(a) was initiated. Among the various therapeutic platforms amenable to selective inhibition of Lp(a), antisense oligonucleotide (ASO) drugs have emerged as a

Abbreviations: ASO, antisense oligonucleotide; h-apoB, human APOB; HDL-C, HDL cholesterol; KIV, kringle IV; KV, kringle V; LDL-C, LDL cholesterol; Lp(a), lipoprotein (a); MOE, 2′-(2-methoxyethyl); m/s/year, meters per second per year; OxPL, oxidized phospholipid; OxPL-apoB, oxidized phospholipid on apoB-containing lipoprotein; P5S, phosphorothioate.

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promising approach in lowering Lp(a) levels in the clinical setting. ASOs represent the third major therapeutic discovery platform, distinct from small molecule and monoclonal antibody approaches, and have shown great promise in specific targeting of disease-associated genes in dyslipidemia, oncology, neurological dysfunction, and metabolic disorders. Due to their mode of action, by binding complementary mRNA targets via Watson-Crick base pairing, isoform-specific targeting is possible. In the case of Lp(a), this very important feature allows direct targeting of the apo(a) transcript without altering plasminogen transcript levels.

ASOs TARGETING apo(a)

Second generation ASOs are single-stranded chimeric molecules generally 20 nucleotides in length, containing 2′-O-(2-methoxyethyl) (MOE) modifications at the 5′ and 3′ termini (positions 1-5 and 16-20) and DNA-like nucleotides in the central region (position 6-15) with a phosphorothioate (P=S) backbone throughout to enhance nuclease resistance (17–19). These molecules are up to 15-fold more potent than first-generation P=S only-modified ASO drugs, due to their enhanced mRNA affinity via the MOE moiety (19–21), their gapmer design supporting an RNase H1 enzymatic termination mechanism, and their improved pharmacokinetic properties that permit weekly, monthly, or potentially quarterly dosing (Fig. 1). These drugs also have an improved therapeutic index due to reduced pro-inflammatory properties (22–26).

Because ASO drugs are metabolized by cellular nucleases and not the cytochrome P450 system, they can be safely co-administered with traditional therapeutic agents with differing modes of action (29). Additionally, as they are hydrophilic, they may be administered in saline without special formulation via subcutaneous, intravenous, topical, aerosol, enema, intravitreal, intraventricular, intrathecal, and oral routes (27). The pharmacokinetic properties of ASOs have been extensively quantified in multiple species and in man (22). Following systemic administration, the liver, kidney, bone marrow, adipose tissue, spleen, and lymph nodes accumulate the highest drug concentrations, while distribution is poor to the intestine, skeletal muscle, heart, lung, reproductive organs, pancreas, and brain. MOE-modified ASOs are resistant to exonuclease degradation, resulting in prolonged tissue half-lives, ranging from 10 to 30 days. In general, ASOs are cleared from tissue by endonucleolytic degradation, producing lower molecular weight metabolites (8–12 nucleotides) that are eliminated by urinary excretion.

REVIEW OF ANTISENSE STUDIES REPORTING Lp(a) LEVELS

In the first described antisense study performed in vitro, a human apo(a) ribozyme oligonucleotide containing P=S DNA and RNA was designed to specifically target KIV of the apo(a) mRNA without altering plasminogen transcript levels (28). Because human cell lines do not adequately express the apo(a) gene, an apo(a) expression vector containing the 5′-untranslated region, the signal sequence, the first five KIV-like repeats, and 291 bp of the kringle repeat of apo(a) driven by the cytomegalovirus promoter was cotransfected into HepG2 cells with a hemagglutinating virus of Japan (HJV)-liposome-apo(a) DNA complex and either the apo(a) ribozyme or control oligonucleotide. After 72 h, the apo(a) ribozyme was shown to reduce HepG2 protein secretion by approximately 60%, while no significant change in plasminogen protein was observed. While these initial results were encouraging, the requirement of liposomal formulation of the apo(a) targeting ribozyme prior to delivery would have made in vivo pharmacology studies more challenging.

Mipomersen, a second generation ASO directed to apoB-100 and approved for clinical use in the United States for lowering LDL cholesterol (LDL-C) in patients with homozygous familial hypercholesterolemia (29), has been shown to lower Lp(a) levels in Lp(a)-transgenic mice (30) and in humans (31–34). In humans, four phase 3 trials were performed in 382 patients on maximally tolerated lipid-lowering therapy and randomized 2:1 to weekly subcutaneous mipomersen (200 mg) (n = 256) or placebo (n = 126) for 26 weeks. Mipomersen reduced plasma Lp(a) levels by 21–39%; whereas, no significant change was noted in the placebo groups (35). Interestingly, in the mipomersen group, only modest correlations were present between percent changes in Lp(a) and apoB (r = 0.43, P < 0.001) and Lp(a) and LDL-C (r = 0.36, P < 0.001), suggesting mechanisms of Lp(a) lowering related to liver synthesis of apoB that are not apparent by evaluating plasma apoB levels.

A study in Lp(a)-transgenic mice by Merki et al. (30) suggested that one potential mechanism of Lp(a) reduction by mipomersen may be through limiting hepatic production of newly formed apoB concomitantly when apo(a) is available to create an Lp(a) particle. Transgenic mice overexpressing both human APOB (h-apoB)-100 plus human LPA to generate genuine Lp(a) particles [human apo(a) does not form a covalent bond with mouse apoB-100] were treated with mipomersen. Mipomersen reduced hepatic apoB production and plasma levels of h-apoB-100 to very low levels (<20 mg/dl) and reduced LDL-C and Lp(a) levels by ~75%. However, the mice continued to produce similar amounts of apo(a) unbound to h-apoB as before treatment with mipomersen, suggesting that apoB-100 synthesis is a limiting factor in Lp(a) particle generation in this LPA transgenic model (Fig. 2A).

In a follow-up investigation, Merki et al. (12) evaluated ISIS 144367, a second generation apo(a)-specific ASO, in the following mouse models: 1) LPA transgenic mice expressing a truncated human LPA cDNA with eight KIV repeats [8K-L(a) mice] that has very high Lp(a) levels (11, 15, 36). This construct contains wild-type human apo(a) cDNA encoding kringle, KIV′, KIV′2, a fusion of KIV′3 and KIV′, KIV′5–KIV′10, KV, and the protease-like domain. The promoter for this construct consists of the apoE hepatic control region LE6 and apoE intron 1. 2) Lp(a)-transgenic...
mice expressing both human genomic APOB and the human LPA [8K-Lp(a) mice]. LPA transgenic mice expressing the natural human apo(a) gene containing 12 KIV repeats in a complete genomic construct [12K-apo(a) mice] that contains the natural apo(a) promoter and regulatory sequences. All transgenic mice administered ISIS 144367 for 4 weeks showed significant reductions in hepatic apo(a) mRNA expression. ISIS 144367 significantly reduced Lp(a) by 24.8% in 8K-Lp(a) mice and reduced apo(a) levels by 19.2% in 8K-apo(a) mice, and 86% in 12K-apo(a) mice (Fig. 2B). The most potent effect was documented in 12K-apo(a) mice expressing apo(a) with multiple KIV 2 repeats containing the natural LPA promoter and regulatory sequences. Importantly, in the 12K-apo(a) mice, ISIS 144367 also significantly reduced plasma-OxPLs on apoB-containing lipoproteins (OxPL-apoB) by 86%. A large number of clinical studies have demonstrated that OxPL-apoB, mainly reflecting the biological activity of small apo(a) isoforms with high Lp(a) levels, are strong predictors of CVD.

Fig. 1. Mechanism by which ISIS-APO(a) Rx suppresses apo(a) protein synthesis. A ubiquitous intracellular RNase, RNase H1, recognizes the RNA:DNA duplex formed when ISIS-APO(a) Rx binds to the complementary apo(a) mRNA sequence and cleaves the target, thereby reducing apo(a) protein and preventing generation of Lp(a) particles. PL, phospholipid; FC, free cholesterol; CE, cholesteryl ester. This figure was adapted from (62) and (19) with permission.
As the apo(a) transcript is not expressed in rodents, in vivo preclinical efficacy assessments were limited to studies in transgenic mice expressing a 12 kringle KIV apo(a) isoform which expressed the entire human LPA genomic sequence, without h-apoB (43). Administration of ISIS-APO(a) Rx to 12K-apo(a) mice produced dose-dependent reductions in apo(a) liver mRNA and apo(a) in plasma after 2 weeks of ASO administration at 1.5, 5, 15, and 50 mg/kg/week (Fig. 3). The 50% effective dose values for ISIS-APO(a) Rx apo(a) mRNA and plasma apo(a) reductions were 9.7 and 12.4 mg/kg/week, respectively, in this transgenic model.

PHARMACOLOGY OF ISIS-APO(a) Rx IN LEAN CYNOMOLGUS MONKEYS

While the ISIS-APO(a) Rx binding site in the rhesus monkey contains a single mismatch relative to the nonhuman primate sequence, the potential pharmacodynamic effects of this compound were evaluated in chow-fed cynomolgus monkeys when administered up to 40 mg/kg/week for 12 weeks. Cynomolgus monkeys, in a similar fashion to humans, have a wide range of plasma Lp(a) levels due to variability in the KIV 2 repeats similar to humans, but lack KV of human apo(a) (44). As described previously in both humans and nonhuman primates, our data show that cynomolgus monkeys had highly variable hepatic mRNA expression levels (44, 45). Results from this
Another 13-week study evaluated the effects of ISIS-APO(a) Rx inhibition as a function of dose in chow-fed cynomolgus monkeys. At the 4, 8, 12, and 40 mg/kg/week doses, hepatic apo(a) mRNA was reduced to 84 ± 12%, 70 ± 18%, 79 ± 17%, and 97 ± 3%, respectively, of mean saline control expression levels by day 93 of ISIS-APO(a) Rx study. Furthermore, due to some conservation of apo(a) and plasminogen nucleotide sequences (there are three base mismatches within the near homologous binding site), plasminogen mRNA levels were also measured. There was no significant change in hepatic plasminogen mRNA detected, relative to the PBS cohort, after 12 weeks of ISIS-APO(a) Rx administration (Table 2).

In another 13-week study, the effects of ISIS-APO(a) Rx inhibition as a function of dose were evaluated in chow-fed cynomolgus monkeys. At the 4, 8, 12, and 40 mg/kg/week doses, hepatic apo(a) mRNA was reduced to 84 ± 12%, 70 ± 18%, 79 ± 17%, and 97 ± 3%, respectively, of mean saline control expression levels by day 93 of ISIS-APO(a) Rx study. Furthermore, due to some conservation of apo(a) and plasminogen nucleotide sequences (there are three base mismatches within the near homologous binding site), plasminogen mRNA levels were also measured. There was no significant change in hepatic plasminogen mRNA detected, relative to the PBS cohort, after 12 weeks of ISIS-APO(a) Rx administration (Table 2).

Table 1. ISIS-APO(a) Rx complementary binding sites within the human apo(a) transcript

| ISIS-APO(a) Rx Binding Site | Position on NM_005577.2 apo(a) mRNA transcript | Binding Site on First Exon | Binding Site on Second Exon |
|-----------------------------|-------------------------------------------------|---------------------------|---------------------------|
| kringle IV2 repeat 2         | Exon 4-5                                         | 505-524 bp                | CTGTGTC                  |
| kringle IV2 repeat 3         | Exon 6-7                                         | 847-866 bp                | CTGTGTC                  |
| kringle IV2 repeat 4         | Exon 8-9                                         | 1189-1208 bp              | CTGTGTC                  |
| kringle IV2 repeat 5         | Exon 10-11                                       | 1531-1550 bp              | CTGTGTC                  |
| kringle IV2 repeat 6         | Exon 12-13                                       | 1873-1892 bp              | CTGTGTC                  |
| kringle IV2 repeat 7         | Exon 14-15                                       | 2215-2234 bp              | CTGTGTC                  |
| kringle IV2 repeat 8         | Exon 16-17                                       | 2557-2576 bp              | CTGTGTC                  |
| kringle IV2 repeat 9         | Exon 18-19                                       | 2899-2918 bp              | CTGTGTC                  |
| kringle IV2 repeat 11        | Exon 22-23                                       | 3583-3602 bp              | CTGTGTC                  |
| kringle IV2 repeat 12        | Exon 24-25                                       | 3901-3920 bp              | CTGTGTC                  |
| Exon 24-25                   | kringle IV2 repeat 14                            | 4584-4604 bp              | CTGTGTC                  |
| kringle IV2 repeat 15        | Exon 28-29                                       | 4927-4946 bp              | CTGTGTC                  |
| kringle IV2 repeat 12        | Exon 30-31                                       | 4927-4946 bp              | CTGTGTC                  |

ISIS-APO(a) Rx complementary binding sites within the human apo(a) transcript (GenBank accession NM_005577.2) at position 3901-3920 bp. ISIS-APO(a) Rx was designed to perfectly match only the exon 24-25 splice site (indicated with bold type) but may also bind at 11 other apo(a) exon splice sites containing one to four mismatched nucleotides (indicated by underlined letters).

Fig. 3. Effect of APO(a) Rx on murine liver apo(a) mRNA expression and plasma apo(a) levels. The apo(a) liver mRNA (A) and apo(a) plasma protein levels (B) were measured after 2 weeks of ISIS-APO(a) Rx administration of 1.5, 5, 15, and 50 mg/kg/week or saline in LPA transgenic mice (n = 4 per group). Dosing was performed once weekly by intraperitoneal injection for 2 weeks. Mean ± SD is plotted for each analyte as a percentage of the saline control.
Interestingly, when plasma lipids were measured after 13 weeks, there were no significant changes observed in total cholesterol, HDL cholesterol (HDL-C), LDL-C, or apoB levels, even at the highest administered dose (Table 2).

In order to evaluate the heterogeneity of apo(a) allelic expression patterns within the PBS and ISIS-APO(a) Rx cynomolgus monkey treatment groups, Western blotting was performed to directly compare day 1 (predose) and day 93 samples in both saline and 12 mg/kg/week ISIS-APO(a) Rx cohorts (Fig. 5A). The apo(a) band intensities observed were consistent with derived plasma apo(a) levels (Fig. 5B).

Furthermore, as described previously, monkeys in this study were both heterozygous and homozygous for different apo(a) isoform sizes. Importantly, in all four monkeys treated with ISIS-APO(a) Rx, plasma apo(a) levels were reduced to nearly undetectable levels at the 12 mg/kg/week dose by Western blot, irrespective of apo(a) protein isoform size; while in the saline cohort, no changes in expression levels were observed in any of the plasma samples. These results demonstrate that ISIS-APO(a) Rx treatment is highly effective in lowering plasma apo(a) levels in nonhuman primates regardless of individual variation in isoforms or circulating concentrations.

ISIS-APO(a)Rx PHASE 1 TRIAL

A double-blinded placebo-controlled dose-escalation phase 1 trial in healthy volunteers with Lp(a) concentration of ≥25 nmol/l (≥10 mg/dl) was initiated to assess the efficacy, safety, and pharmacokinetics of ISIS-APO(a)Rx (19). A total of 16 subjects were enrolled into the APO(a)Rx single-dose cohorts and 31 subjects into the multiple-dose cohorts. Participants were randomly assigned to receive ISIS-APO(a)Rx by subcutaneous injection (50, 100, 200, or 400 mg) or placebo (3:1) in the single-dose part of the study or to receive six subcutaneous injections of ISIS-APO(a)Rx (100, 200, or 300 mg, for a total dose exposure of 600, 1,200, or 1,800 mg) or placebo (4:1) during a 4 week period in the multi-dose part of the study.

In the multi-dose cohort, ISIS-APO(a)Rx (100–300 mg) resulted in dose-dependent mean percentage decreases in plasma Lp(a) concentration of 39.6% from baseline in the 100 mg group (P = 0.005), 59.0% in the 200 mg group (P = 0.001), and 77.8% in the 300 mg group (P = 0.001). The largest decrease in an individual patient was 88.8% at day 36 after multiple doses of 300 mg ISIS-APO(a)Rx. Maximum plasma concentrations of ISIS-APO(a)Rx were dose-dependent over the studied dose range, and maximum

| Treatment     | Dose        | Duration | Apo(a) mRNA * | Plasminogen mRNA * | TC (%) | HDL-C (%) | LDL-C (%) | apoB (%) |
|---------------|-------------|----------|---------------|-------------------|--------|-----------|-----------|----------|
| Saline        | 0 mg/kg/week| 13 weeks | 100 ± 26      | 100 ± 3           | 89 ± 7 | 73 ± 8    | 103 ± 8   | 123 ± 12 |
| APO(a)Rx      | 40 mg/kg/week| 13 weeks | 10 ± 3        | 107 ± 3           | 96 ± 6 | 83 ± 6    | 109 ± 9   | 122 ± 18 |

ISIS-APO(a)Rx was administered to lean cynomolgus monkeys at 40 mg/kg/week over 13 weeks (n = 4 per group). A loading regimen of three doses in first week followed by once per week subcutaneous administration was utilized.

*Data are expressed as the mean percentage of values observed in saline (±SEM) treated monkeys for apo(a) and plasminogen mRNA levels.

**Plasma total cholesterol (TC), HDL-C, LDL-C, and apoB protein are expressed as a percentage of baseline levels.

*Indicates statistically significant from saline using Mann Whitney two-tailed test (P < 0.05).
plasma concentration was followed by an initial relatively rapid distribution phase. Post-distribution plasma concentrations in the 300 mg multi-dose cohort reached steady state just before day 36, which coincided with the nadir of Lp(a) and OxPL-apoB and OxPL-apo(a) response (Fig. 6). Similar reductions were observed in the amount of OxPLs associated with apoB-100 (up to 90.2%) and apo(a) (up to 93.1%), but no significant changes were noted in other lipoproteins (Fig. 7). No significant changes were noted in OxPLs on plasminogen or plasminogen levels.

In the combined multi-dose cohorts, an inverse correlation was noted between the size of the predominantly expressed apo(a) isoform and baseline plasma Lp(a) and OxPL-apo(a) concentrations. However, there was no significant correlation between the major apo(a) isoform and the mean percent change from baseline to day 36 in Lp(a)

Fig. 5. Effect of ISIS-APO(a)Rx on cynomolgus monkey apo(a) protein expression and plasma Lp(a) levels. A: Western analysis of male and female cynomolgus monkey plasma apo(a) protein levels at day 1 predose and day 93 postdose in saline- and 12 mg/kg/week ISIS-APO(a)Rx-administered groups. Plasma protein was diluted 1:100 and separated by PAGE on a 4–12% Tris glycine gel. The apo(a) was detected using the LPA4 anti-apo(a) antibody. B: Plasma samples for the individual monkey samples were analyzed on a Beckman clinical analyzer using Randox™ isoform-independent measurements shown in (A). The apo(a) levels are expressed in milligrams per deciliter.

Fig. 6. Plasma ISIS-APO(a)Rx trough concentrations measured 7 days after the last dose in the 300 mg dose cohort in relation to change in concentration of plasma Lp(a), OxPL-apoB, and OxPL-apo(a). The shaded area represents the dosing window and the arrows indicate dosing at days 1, 3, 5, 8, 15, and 22. This figure was adapted from (19) with permission.
or OxPL-apoB concentrations, consistent with the independence of lowering of Lp(a) and OxPL-apoB on isoform size (Fig. 8). In the combined single-dose and multi-dose cohorts, at all time points, a strong correlation was noted between Lp(a) concentrations and OxPL-apoB ($r = 0.86, P < 0.0001$) and Lp(a) and OxPL-apo(a) ($r = 0.91, P < 0.0001$).

In summary, ISIS-APO(a)$_{Rx}$ resulted in potent dose-dependent selective reductions of plasma Lp(a) and represents a potential therapeutic drug to reduce the risk of CVD and calcific aortic valve stenosis in patients with elevated Lp(a) concentration.

**Fig. 7.** Mean percent change in Lp(a), OxPL-apoB, OxPL-apo(a), total cholesterol (TC), LDL-C, apoB, HDL-C, and TG from baseline to day 36 among the different doses in the multi-dose groups. *$P = 0.020$; **$P < 0.008$; ***$P < 0.001$ compared with placebo. This figure was adapted from (19) with permission.

**Fig. 8.** Mean percent change in Lp(a), OxPL-apoB, and OxPL-apo(a) at day 36 in the 300 mg multiple-dose group, demonstrating that the extent of lowering was independent of baseline Lp(a) levels. The x axis shows individual patients (denoted A–G) and their baseline Lp(a) levels. This figure was adapted from (19) with permission.

**ALTERNATIVE THERAPIES TO LOWER Lp(a)**

Recent data has demonstrated that Lp(a) can be significantly lowered by 20–40% with ASOs to apoB (35), monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (46–48), and cholesterol ester transfer protein inhibitors (49, 50). However, in patients at or above the 80th percentile, corresponding to ~50 mg/dl plasma Lp(a) concentrations, much greater reduction than is currently achieved with these indirect therapeutic agents would be required to significantly reduce CVD risk, which is thought to occur at levels which exceed 25–30
the need for aortic valve replacement. Elevated Lp(a) levels and the LPA SNP, s10455872, which is associated with elevated Lp(a) levels, have recently been identified in epidemiologic and genome-wide association studies as predictors of aortic valve stenosis, aortic valve replacement, and aortic valve calcification (6, 8). Our group has recently evaluated the role of Lp(a) and OxPL-apoB, which reflects the biological activity of Lp(a), in predicting the rate of progression of preexisting aortic stenosis in the ASTRONOMER (Aortic Stenosis Progression Observations: Measuring Effects of Rosuvastatin) trial. Elevated levels of both Lp(a) and OxPL-apoB predicted aortic stenosis progression [measured by the annualized increase in peak aortic jet velocity in meters per second per year (m/s/year) by Doppler echocardiography], as well as the need for aortic valve replacement and cardiac death during 3.5 ± 1.2 years of follow-up (9). The rate of progression was faster in patients in the top tertiles of Lp(a) (peak aortic jet velocity 0.26 ± 0.26 m/s/year vs. 0.17 ± 0.21 m/s/year; \( P < 0.005 \)) and OxPL-apoB (0.26 ± 0.26 m/s/year vs. +0.17 ± 0.21 m/s/year; \( P < 0.01 \)) (Fig. 9). These findings support the hypothesis that Lp(a) mediates aortic stenosis progression through its associated OxPLs and provide a rationale for randomized trials of Lp(a)- and OxPL-apoB-lowering therapies in aortic stenosis (Fig. 10). A clinical trial can be performed to assess whether lowering Lp(a) may reduce progression of aortic stenosis and the need for aortic valve replacement.

Finally, the ongoing development of tri-antennary N-acetyl galactosamine conjugates is expected to further enhance

mg/dl (8). Therefore, potent and specific inhibitors of Lp(a) represent an unmet medical need for high risk patients.

**FUTURE DIRECTIONS**

Future studies of ISIS-APO(a)Rx will include gaining more experience on the safety and efficacy in various populations where it may be used clinically. For example, potential indications may include patients with elevated Lp(a) levels and otherwise controlled risk factors, such as patients with refractory angina (51, 52), recurrent cardiovascular events [including patients undergoing apheresis for elevated Lp(a)] (53–56), young patients (i.e., <50–60 years old) with CVD (57), calcific aortic valve stenosis (9), patients with familial hypercholesterolemia of whom 40–50% have Lp(a) levels >50 mg/dl (35), stroke (particularly in the pediatric age group) (58), chronic renal disease, and secondary and primary prevention. With the current potent ASO-lowering Lp(a) levels of 80–90%, it may be possible to lower Lp(a) levels in most patients to what is considered least atherogenic, i.e., <25–30 mg/dl, and to test the hypothesis that lowering Lp(a) levels will lead to reduction in CVD events.

An attractive population in which to reduce Lp(a) levels is patients with preexisting aortic valve stenosis. The prevalence of aortic valve stenosis is increasing rapidly due to the aging of the population and there is a clinical need to reduce the progression of aortic stenosis and, ultimately, the need for aortic valve replacement. Elevated Lp(a) levels and the LPA SNP, s10455872, which is associated with elevated Lp(a) levels, have recently been identified in epidemiologic and genome-wide association studies as predictors of aortic valve stenosis, aortic valve replacement, and aortic valve calcification (6, 8). Our group has recently evaluated the role of Lp(a) and OxPL-apoB, which reflects the biological activity of Lp(a), in predicting the rate of progression of preexisting aortic stenosis in the ASTRONOMER (Aortic Stenosis Progression Observations: Measuring Effects of Rosuvastatin) trial. Elevated levels of both Lp(a) and OxPL-apoB predicted aortic stenosis progression [measured by the annualized increase in peak aortic jet velocity in meters per second per year (m/s/year) by Doppler echocardiography], as well as the need for aortic valve replacement and cardiac death during 3.5 ± 1.2 years of follow-up (9). The rate of progression was faster in patients in the top tertiles of Lp(a) (peak aortic jet velocity 0.26 ± 0.26 m/s/year vs. 0.17 ± 0.21 m/s/year; \( P < 0.005 \)) and OxPL-apoB (0.26 ± 0.26 m/s/year vs. +0.17 ± 0.21 m/s/year; \( P < 0.01 \)) (Fig. 9). These findings support the hypothesis that Lp(a) mediates aortic stenosis progression through its associated OxPLs and provide a rationale for randomized trials of Lp(a)- and OxPL-apoB-lowering therapies in aortic stenosis (Fig. 10). A clinical trial can be performed to assess whether lowering Lp(a) may reduce progression of aortic stenosis and the need for aortic valve replacement.

Finally, the ongoing development of tri-antennary N-acetyl galactosamine conjugates is expected to further enhance

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**Fig. 9.** Calcific aortic valve stenosis progression rate according to plasma levels of Lp(a) and OxPL-apoB. Annualized progression rate of peak velocity across the aortic valve (V peak) is compared by tertiles in the whole cohort for Lp(a) (A) and for OxPL-apoB (B) and after dichotomization by median age (C, D). \(* P < 0.05\) tertile 3 (>58.5 mg/dl) compared with tertiles 1 and 2 (<58.5 mg/dl) of the Lp(a) age ≤57 group; † \( P < 0.05\) tertile 3 (>5.5 nM) compared with tertiles 1 and 2 (<5.5 nM) of the OxPL-apoB age ≤57 group. Error bars = SEM. This figure was adapted from (9) with permission.
the potency of ASOs by as much as 10- to 20-fold for mRNA targets expressed in hepatic parenchymal cells (59). This is possible because the physiological ligands for N-acetyl galactosamine uptake, the asialoglycoprotein receptors-1 and -2, are abundantly and exclusively expressed on hepatocytes (60, 61). Such potency improvement may ultimately lead to extended dosing periods, such as monthly or quarterly, or even orally bioavailable antisense drugs, greatly enhancing the ease of administration and utility of this emerging therapeutic platform.

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