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EXPEDITED PUBLICATIONS

Efficacy and Safety of a Novel Oral Inducer of Apolipoprotein A-I Synthesis in Statin-Treated Patients With Stable Coronary Artery Disease

A Randomized Controlled Trial

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Objectives The purpose of this study was to investigate the safety, tolerability, and efficacy of RVX-208, the first oral agent designed to enhance apolipoprotein (apo) A-I synthesis.

Background No agent that selectively induces synthesis of apoA-I has reached an advanced stage of clinical development.

Methods A total of 299 statin-treated patients with coronary artery disease were treated with placebo or with RVX-208 at a dose of 50, 100, or 150 mg twice daily for 12 weeks. Changes in lipid-related biomarkers, in addition to safety and tolerability, of RVX-208 were investigated.

Results For each dose of RVX-208, individual pairwise comparisons of apoA-I changes with placebo, the primary end point, did not achieve statistical significance. However, treatment with RVX-208 was associated with a dose-dependent increase in apoA-I levels by up to 5.6% ($p = 0.035$ for trend). Administration of RVX-208 resulted in significant increases in levels of high-density lipoprotein cholesterol (HDL-C) ranging from 3.2% to 8.3% ($p = 0.02$), and large HDL particles increased by 11.1% to 21.1% ($p = 0.003$). ApoA-I levels increased rapidly from 8 to 12 weeks, suggesting that peak pharmacological effect has not been achieved by the end of the 12-week study. Transient and reversible elevations in liver transaminases >3 times the upper limit of normal were observed in 18 patients treated with RVX-208, with no associated increase in bilirubin levels.

Conclusions Administration of RVX-208 for 12 weeks was associated with increases in apoA-I, HDL-C, and concentration of large HDL particles, consistent with facilitation of cholesterol mobilization. Maximal increases in apoA-I may require longer exposure. An increase in liver enzymes was observed with active treatment. (Clinical Trial for Dose Finding and Safety of RVX000222 in Subjects With Stable Coronary Artery Disease; NCT01058018) (J Am Coll Cardiol 2011;57: 1111–9) © 2011 by the American College of Cardiology Foundation

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Abbreviations and Acronyms

2D = 2-dimensional
apo = apolipoprotein
bid = twice daily
HDL-C = high-density lipoprotein cholesterol
IQR = interquartile range
ITT = intent to treat
LDL-C = low-density lipoprotein cholesterol
RCT = reverse cholesterol transport

Despite the widespread implementation of low-density lipoprotein cholesterol (LDL-C) lowering strategies, there remains significant residual risk of adverse cardiovascular events in developed countries (1). Accordingly, there exists an ongoing need to develop additional therapeutic approaches to achieve more effective reductions in cardiovascular risk.

For several decades, approaches that increase the levels of high-density lipoprotein cholesterol (HDL-C) have represented an attractive target for the development of novel cardioprotective therapies. This strategy approach is based upon observations from population (2) and animal (3,4) studies suggesting that levels of HDL-C are inversely correlated with the risk of cardiovascular morbidity and mortality. The finding that infusions of lipid-poor HDL subfractions promote rapid regression of coronary atherosclerosis further supports the concept that enhancing the biological activity of HDL is beneficial in humans (5–7).

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Although interest has focused primarily on therapies designed to elevate serum levels of HDL-C, an alternative approach involves up-regulation of endogenous synthesis of apolipoprotein (apo) A-I, the major protein carried on HDL particles. The theoretical advantages of enhancing apoA-I synthesis stem from the ability of this protein to generate new HDL particles, which act as the vehicle for promotion of reverse cholesterol transport (RCT). HDL particles also exert favorable effects on inflammatory, oxidative, apoptotic, endothelial, and thrombotic pathways, which may contribute to a beneficial effect on atherosclerotic disease (8).

RVX-208 is the first oral agent to reach clinical development that selectively induces hepatic synthesis of apoA-I. Mechanistic studies demonstrated that RVX-208 enhances apoA-I transcription in hepatic cell lines and does not appear to modulate peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), or cholesteryl ester transfer protein (CETP) mediated pathways (Johansson J, unpublished data, September 2010). In preclinical studies, administration of RVX-208 to nonhuman primates resulted in a sustained increase in circulating levels of preβ1-HDL, the lipid-deplete form of HDL, and α1-HDL, the fully mature particle. These changes produced an increase in systemic capacity to promote cholesterol efflux in ex vivo assays (9). Similar findings have been reported with short-term treatment in healthy volunteers (9). The ASSERT (ApoA-I Synthesis Stimulation Evaluation in Patients Requiring Treatment for Coronary Artery Disease) study assessed the safety, tolerability, and efficacy of RVX-208 at varying doses in patients with stable coronary artery disease, who were on statin therapy. This report represents the first published description of the potential lipid-related benefits, tolerability, and safety of a novel oral agent that stimulates endogenous production of apoA-I in patients with established coronary artery disease.

Methods

Study design. The trial was a multicenter, randomized, double-blind, placebo-controlled study performed at 35 sites in the U.S. A central institutional review board (Sterling, Atlanta, Georgia) approved the protocol, and all patients provided written informed consent. The study was appropriately registered on clinicaltrials.gov following commencement of patient enrollment.

Patients of at least 18 years of age were required to have documented coronary artery disease on the basis of coronary angiography or previous coronary revascularization or myocardial infarction, with no clinical event within the 90 days prior to randomization. All patients were treated with a statin, with no change in dose for at least 30 days prior to randomization. Investigators were instructed not to adjust statin dosages during the trial. Patients were excluded for severe heart failure, uncontrolled hypertension, evidence of hepatic or renal impairment, or the presence of a triglyceride level >400 mg/dl. Treatment with any fibric acid derivative or niacin at a dose >250 mg daily was not permitted within the 90 days prior to randomization and for the duration of the study. All patients entered an active-treatment phase for 12 weeks, during which they were randomized in a 1:1:1:1 fashion to treatment with RVX-208 at a dose of 50, 100, or 150 mg or with matching placebo twice daily (bid).

Clinic visits and laboratory tests. Patients were examined during scheduled clinic visits every 2 weeks during the treatment phase and a follow-up visit 4 weeks following cessation of the study drug (Fig. 1). A central laboratory performed all biochemical determinations (Icon, Farmingdale, New York). Levels of apoA-I were determined by turbidometric immunoassay (Wako Chemicals, Richmond, Virginia) and HDL subclasses characterized by 2-dimensional (2D) nondenaturing gel electrophoresis, immunoblotting, and image analysis (Boston Heart Labs, Boston, Massachusetts) as previously described (10). Image quantification was determined by the percentage distribution of each HDL subclass within the total HDL pool. Absolute concentrations were calculated by multiplying the plasma apoA-I concentration (mg/dl) by the percentile value of each HDL subclass. Measurements of lipoprotein particle number and size were performed by nuclear magnetic resonance (LipoScience, Raleigh, North Carolina) as previously described (11). Particle concentrations of lipoprotein subclasses of different sizes were calculated from the measured amplitudes of their spectroscopically distinct lipid methyl signals. Lipoprotein levels and safety laboratory measurements

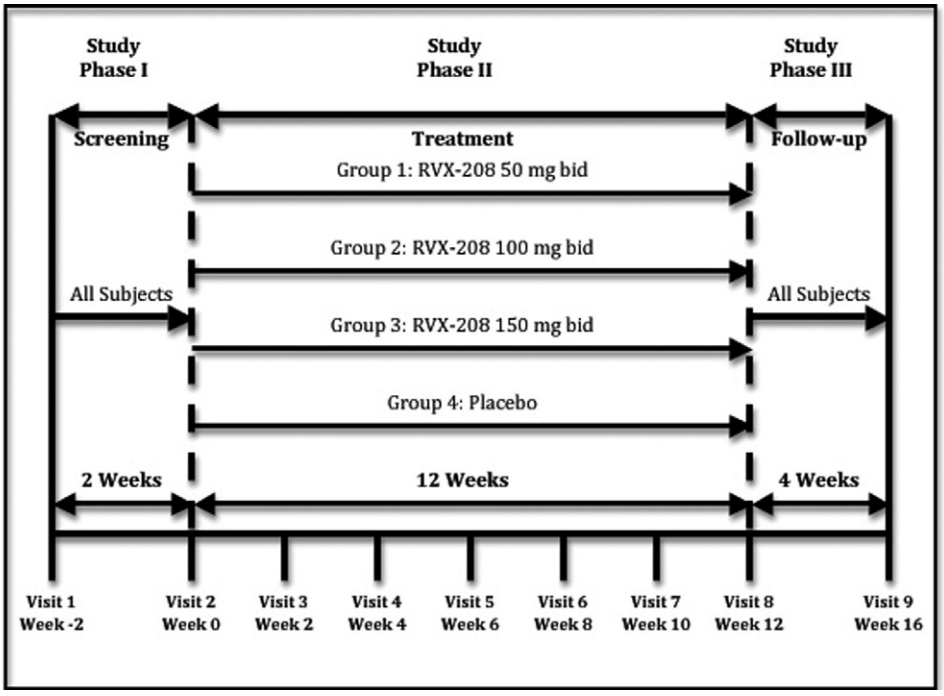

Figure 1 Flow of Patients Over the 18-Week Course of the ASSERT Study

The study consisted of: 1) a 2-week screening phase; 2) a 12-week treatment phase; and 3) a 4-week post-treatment phase.
ASSERT = ApoA-I Synthesis Stimulation Evaluation in Patients Requiring Treatment for Coronary Artery Disease; bid = twice daily.

were obtained and the occurrence of any adverse reactions recorded at each study visit.

Statistical tests. The primary end point was the percentage change in plasma apoA-I levels from baseline to 12 weeks post-randomization for each treatment arm compared with placebo. A sample size of 64 patients per group was selected to provide 80% power to detect an 8% increase in apoA-I, assuming an SD of 15% for the comparison of each dose of RVX-208 to placebo with a 5% type I error rate (1-sided test). With an anticipated dropout rate of approximately 10%, enrollment of 280 patients was calculated to provide an adequate number of evaluable patients.

Demographic and laboratory characteristics are summarized for all randomized patients taking at least 1 dose of study drug using an intent-to-treat (ITT) approach. The analysis of safety was performed in all ITT patients through 4 weeks post-treatment. Categorical variables are described using frequencies, whereas continuous variables are reported as mean and SD, median and interquartile range (IQR), or geometric mean, as appropriate. The efficacy analyses were performed on patients in the ITT population with both a baseline and at least 1 post-baseline value through the 12-week treatment period. A last observation carried forward methodology was used when the 12-week value was not available. A nonparametric rank analysis of covariance (ANCOVA) was employed to compare percentage change from baseline between each active treatment group and placebo after controlling for the ranked baseline value. p Values were calculated for overall trend and for comparisons with placebo, with the pairwise p values adjusted according to the Dunnett method. If the overall p value was <0.05, pairwise comparisons were considered significant if <0.025. For the HDL subclass data, an ANCOVA analysis was performed following a log-transformation of the ratio of follow-up to baseline as the dependent variable and the log of baseline as a covariate. Ad hoc Spearman correlation coefficients were generated to evaluate relationships between changes in select lipid parameters. Analyses were performed using SAS statistical software (version 8.2, SAS Institute Inc., Cary, North Carolina). An independent statistician (K.W.) received the entire raw dataset, reviewed the analytic plan for appropriateness and accuracy, and confirmed all of the analyses.

Results

Participants. Between December 16, 2009, and February 2, 2010, 360 patients were enrolled in the study, of which 299 subjects were ultimately randomized to a treatment group. The disposition of these patients is shown in Figure 2. The baseline characteristics of the patients are shown in Table 1. Characteristics were similar for all treatment groups and are therefore presented in a summary fashion. The mean age was 66 years, 75% were male, and 93% were Caucasian.

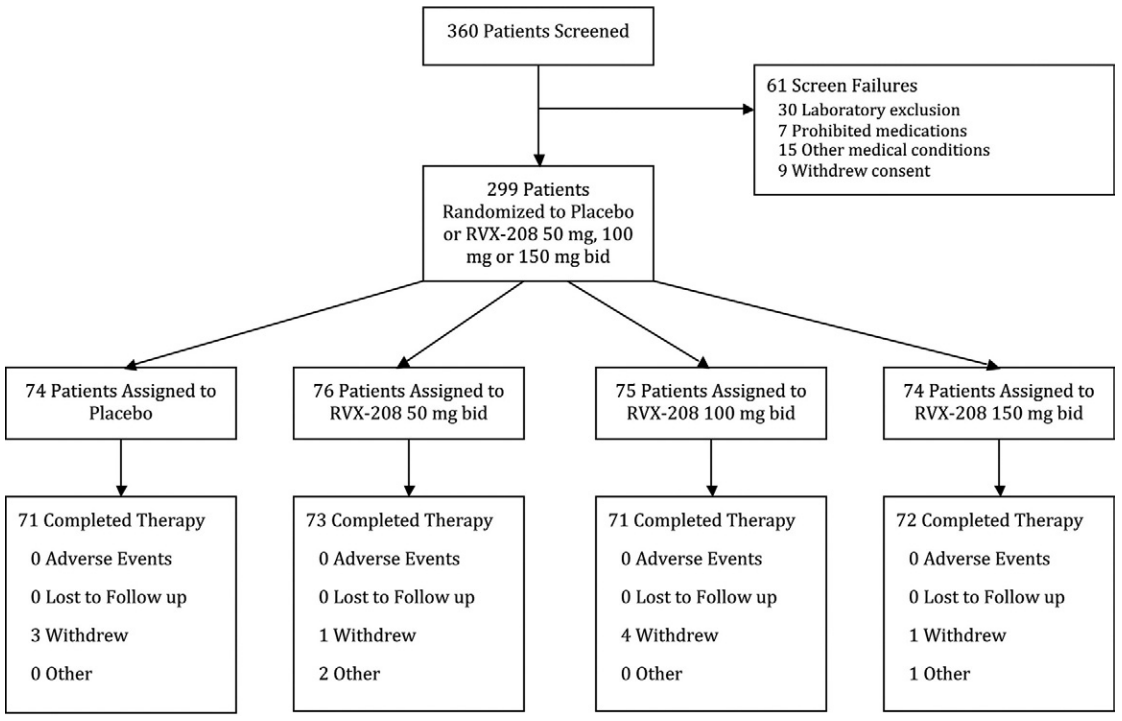

Figure 2 **Disposition of Patients Over the 18-Week Course of the ASSERT Study**

Major reasons for screen failure and discontinuation from the study are summarized. Abbreviations as in Figure 1.

Consistent with a population of patients with established coronary artery disease, there was a high prevalence of obesity, with a mean body mass index >30 kg/m^2, and with major cardiovascular risk factors. In association with universal use of statins, levels of LDL-C and triglycerides were consistent with contemporary treatment goals at 81 mg/dl and 115 mg/dl, respectively. In addition, average levels of HDL-C and apoA-I were within normal limits, averaging 46 and 141 mg/dl, respectively. Approximately 42% of patients had low levels of HDL-C (<40 mg/dl in males, <50 mg/dl in females).

Effects on conventional lipid parameters. Table 2 summarizes the percentage change from baseline in measures of conventional lipid parameters. Compared with placebo, the effect of each dose of RVX-208 on apoA-I, the primary end point of the study, did not achieve statistical significance. However, a dose-dependent increase in levels of apoA-I, by up to 5.6% ($p = 0.035$ for overall trend) was observed across all treatment groups. Significant increases were observed in levels of HDL-C by 3.2% to 8.3% ($p = 0.02$ for overall trend) with increasing doses of RVX-208. The majority of the increase in HDL-C levels appeared to occur between weeks 8 and 12 of the treatment period, with no evidence of a plateau in this effect (Fig. 3). No changes in levels of atherogenic lipid parameters were observed at any dose of RVX-208.

Effects on lipoprotein particle parameters. Table 3 summarizes the percentage change from baseline in lipoprotein particle measures, determined by nuclear magnetic resonance spectroscopy analysis. Administration of RVX-208 was associated with a significant increase in mean size of circulating HDL particles by 1.1% to 1.2% ($p < 0.001$ for overall trend). This appeared to be predominantly associated with significant increases in the concentration of large HDL particles by 11.1% to 21.1% ($p = 0.003$ for trend). More pronounced effects were observed at the 2 highest doses of RVX-208. Although a trend towards dose-dependent reduction in levels of small HDL particles was observed, this just failed to reach conventional levels of statistical significance ($p = 0.07$ for trend). No change in the total number of circulating HDL particles was observed in association with administration of RVX-208. Similarly, RVX-208 treatment was not associated with significant changes in the number or size of LDL particles in the systemic circulation.

HDL subclass distribution was also characterized by 2D gel electrophoresis. Compared with placebo, RVX-208 administration was not associated with any significant change in the concentration of lipid-deplete forms of HDL with preβ1 mobility. In contrast, an increase in concentration of larger α1-HDL particles was observed in association with treatment with RVX-208 at the 100 mg bid (+8.0% [IQR: −0.7 to 17.5], $p = 0.046$) and 150 mg bid (+8.8% [IQR: −0.2 to 18.6], $p = 0.03$) dosages. This is consistent with the increase

Table 1 Baseline Demographics and Biochemical Parameters

Parameter	Cohort (n = 299)	Placebo (n = 74)	RVX 50 mg bid (n = 76)	RVX 100 mg bid (n = 75)	RVX 150 mg bid (n = 74)
Age, yrs	65.8 ± 9.7	66.8 ± 9.7	65.9 ± 9.9	65.8 ± 10.9	67.3 ± 8.2
Male	225 (75.3)	54 (73.0)	58 (76.3)	59 (78.7)	54 (73.0)
Caucasian	279 (93.3)	70 (94.6)	69 (90.8)	71 (94.7)	69 (93.2)
Body mass index, kg/m^2	30.7 ± 5.9	31.3 ± 6.0	30.2 ± 6.7	30.1 ± 5.0	31.1 ± 5.9
Hypertension	262 (87.6)	64 (86.5)	65 (85.5)	65 (86.7)	68 (91.9)
Diabetes	88 (29.4)	20 (27.0)	22 (28.9)	18 (24.0)	28 (37.8)
Smoker	51 (17.1)	10 (13.5)	12 (15.8)	17 (22.7)	12 (16.2)
Statin use					
Atorvastatin	75 (25.1)	18 (24.3)	17 (22.4)	25 (33.3)	15 (20.3)
Rosuvastatin	68 (22.7)	16 (21.6)	22 (28.9)	11 (14.7)	19 (25.7)
Simvastatin	123 (41.1)	28 (37.8)	28 (36.8)	33 (44.0)	34 (45.9)
Other	33 (11.1)	12 (16.2)	9 (11.8)	6 (8.0)	6 (8.1)
Total cholesterol, mg/dl	150 (131–171)	150 (129–177)	146 (133–167)	149 (133–171)	152 (131–169)
LDL-C, mg/dl	76 (66–93)	75 (63–97)	77 (65–91)	74 (67–88)	81 (67–94)
HDL-C, mg/dl	44 (37–53)	47 (39–53)	43 (36–51)	46 (37–51)	44 (36–54)
Triglycerides, mg/dl	115 (86–157)	121 (87–163)	125 (84–157)	114 (88–194)	109 (89–139)
Non–HDL-C, mg/dl	101 (87–124)	99 (84–127)	102 (90–118)	97 (90–124)	104 (86–124)
Apolipoprotein B, mg/dl	76 (65–90)	76 (64–92)	74 (65–86)	75 (65–84)	80 (65–93)
Apolipoprotein A-I, mg/dl	141 (123–161)	145 (124–166)	137 (117–158)	143 (126–158)	137 (122–164)
hsCRP, mg/l	1.8 (0.8–4.2)	1.7 (0.8–4.1)	1.8 (1.0–4.1)	2.3 (0.8–4.6)	1.2 (0.6–4.1)

Values are reported as mean ± SD, n (%), or median (interquartile range).
bid = twice daily; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; LDL-C = low-density lipoprotein cholesterol.

in large HDL particles observed in the nuclear magnetic resonance analysis.

Significant correlations in the combined RVX-208 dosage groups were observed between changes in apoA-I and changes in HDL-C ($r = 0.70$, $p < 0.001$), preβ1-HDL ($r = 0.30$, $p < 0.001$), and α1-HDL ($r = 0.57$, $p < 0.001$). Similar relationships were noted between changes in levels of HDL-C and HDL subclasses. No significant relationship was observed between changes in preβ1 and α1 forms of HDL ($r = -0.07$, $p = 0.31$), which may reflect the ability of a given HDL particle to continue to accumulate cholesterol with ongoing lipid mobilization.

Safety assessment. Laboratory measures of drug safety are summarized in Table 4 and Figure 4. A dose-dependent increase in liver transaminase levels, peaking at week 8, was observed. Elevations of liver transaminases >3 times the upper limit of normal were observed in patients treated with RVX-208 (n = 18, p = 0.009 for trend), with a greater incidence in patients receiving either 100 or 150 mg twice daily, with elevations greater than eight times the upper limit of normal in 8 of these patients. The majority of these substantial elevations were observed between 4 and 8 weeks during the treatment period, demonstrating a pattern that included a rapid increase in levels, and return to baseline levels within 2 weeks of drug discontinuation. One episode occurred during the post-treatment phase, which was similarly observed to rapidly resolve. Furthermore, an additional episode with rapid resolution was observed in a patient who was seropositive for hepatitis C. A greater proportion of patients treated with high-dose statin therapy demonstrated substantial transaminase elevations when treated with RVX-208 (13.3% vs. 6.7%, p = 0.14), although this finding

Table 2 Percentage Changes in Conventional Lipid and Inflammatory Parameters

Parameter	Placebo (n = 74)	RVX-208 50 mg bid (n = 76)	RVX-208 100 mg bid (n = 75)	RVX-208 150 mg bid (n = 74)	Overall p Value
Apolipoprotein A-I, mg/dl	0.9 (−5.7 to 7.6)	0.1 (−4.0 to 7.7)	3.8 (−3.7 to 9.4)	5.6 (−1.1 to 14.3)	0.035
HDL-C, mg/dl	0 (−6.1 to 8.8)	3.2 (−1.9 to 10.1)	6.3 (−3.4 to 14.9)	8.3 (−1.9 to 17.9)*	0.02
LDL-C, mg/dl	4.2 (−8.5 to 15.0)	0.7 (−8.6 to 8.9)	1.6 (−9.4 to 15.4)	1.0 (−14.2 to 10.1)	0.79
Triglycerides, mg/dl†	1.6 (−15.8 to 25.3)	2.2 (−14.0 to 25.5)	4.5 (−15.5 to 26.8)	6.5 (−12.7 to 26.7)	0.85
Total cholesterol, mg/dl	1.6 (−6.1 to 10.7)	0.7 (−4.9 to 9.0)	3.5 (−4.1 to 14.4)	4.3 (−4.7 to 12.5)	0.60
Non-HDL-C, mg/dl	1.8 (−6.5 to 12.7)	0 (−6.2 to 9.9)	3.6 (−7.0 to 12.8)	2.2 (−11.2 to 15.1)	0.99
Apolipoprotein B, mg/dl	−3.8 (−12.7 to 7.2)	−6.6 (−17.4 to 1.3)	−6.7 (−16.4 to 4.9)	−2.0 (−16.3 to 4.8)	0.45
hsCRP, mg/l†	2.5 (−33.3 to 45.2)	−13.0 (−40.8 to 27.9)	−17.5 (−48.1 to 24.2)	−22.0 (−39.5 to 28.6)	0.33

Values are reported as median (interquartile range) or percentage change in conventional lipid and inflammatory markers in patients treated with placebo, or RVX-208 at a dose of 50, 100, or 150 mg twice daily, except as noted. p values were generated from a model adjusting for baseline values. *p ≤ 0.01 compared with placebo. †Values are reported as geometric mean percentage change.
Abbreviations as in Table 1.

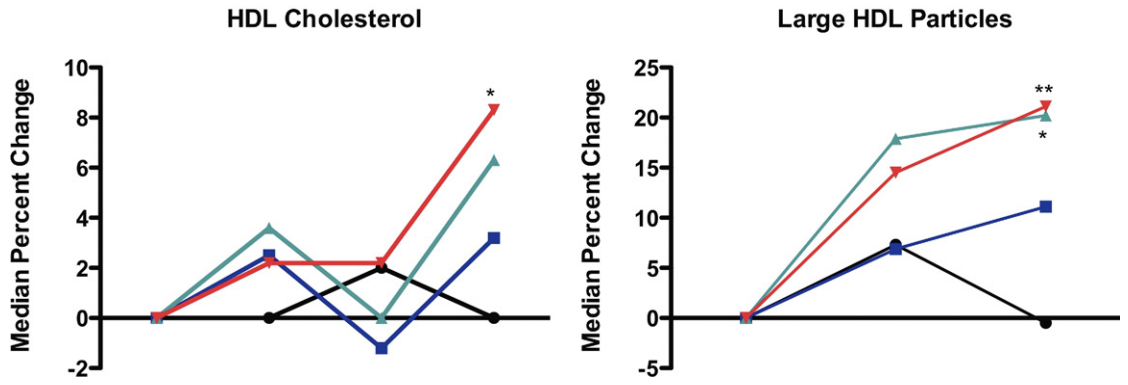

Figure 3 Time Course of Changes

Time course of median percentage change in high-density lipoprotein (HDL) cholesterol and number of large HDL particles in patients treated with placebo **(black circles)** or RVX-208 at a dose of 50 mg **(blue squares)**, 100 mg **(green triangles)**, or 150 mg **(inverted red triangles)** twice daily. *p < 0.01 and **p < 0.001 for comparison with placebo. p values for changes in parameters were generated from a model adjusting for baseline values.

was not statistically significant. No episode of transaminase elevation was accompanied by an increase in levels of bilirubin. Similarly, increases in creatinine levels by more than 1.5-fold greater than baseline levels were observed in 6 patients treated with 100 or 150 mg of RVX-208 twice daily, although this did not achieve statistical significance compared with placebo (p = 0.07). Addition of RVX-208 to background statin therapy was not associated with increases in levels of creatinine phosphokinase. One patient treated with RVX-208 50 mg bid died, and 2 patients treated with RVX-208 100 mg bid experienced a myocardial infarction during the treatment period. None of these

Table 3 Baseline and Percentage Change in Nuclear Magnetic Resonance–Derived Lipoprotein Particle Parameters

Parameter	Placebo (n = 74)	RVX-208 50 mg bid (n = 76)	RVX-208 100 mg bid (n = 75)	RVX-208 150 mg bid (n = 74)	Overall p Value
Total HDL particles, particles/l					
Baseline	32.3 (28.1 to 36.3)	31.2 (26.9 to 35.9)	32.7 (28.7 to 35.6)	29.9 (27.2 to 34.7)	0.16
Percentage change	1.2 (−8.4 to 8.6)	4.0 (−4.7 to 13.5)	2.8 (−5.1 to 11.0)	5.1 (−4.8 to 16.5)	0.80
HDL particle size, nm					
Baseline	8.6 (8.4 to 9.0)	8.7 (8.4 to 8.9)	8.7 (8.4 to 8.9)	8.7 (8.5 to 9.0)	0.50
Percentage change	0 (−2.2 to 1.2)	1.1 (−1.1 to 2.3)	1.2 (0 to 2.4)*	1.1 (−1.1 to 2.4)*	<0.001
Large HDL particles, particles/l					
Baseline	5.0 (3.5 to 6.9)	4.6 (3.2 to 6.6)	4.8 (3.3 to 6.7)	5.3 (3.7 to 7.3)	0.74
Percentage change	−0.5 (−12.7 to 22.2)	11.1 (−7.1 to 31.4)	20.2 (−1.5 to 52.1)†	21.1 (−8.8 to 50.8)*	0.003
Small HDL particles, particles/l					
Baseline	23.8 (21.2 to 27.5)	24.0 (20.4 to 27.0)	24.8 (22.7 to 27.2)	22.8 (20.4 to 26.2)	0.19
Percentage change	2.6 (−8.9 to 14.7)	−0.4 (−13.9 to 9.0)	−2.6 (−14.0 to 6.3)	−4.0 (−17.3 to 7.9)	0.07
Total LDL particles, particles/l					
Baseline	1,077 (841 to 1,290)	1,020 (905 to 1,221)	1,022 (891 to 1,152)	1,079 (848 to 1,246)	0.82
Percentage change	3.8 (−4.6 to 17.4)	3.8 (−12.2 to 16.8)	0.8 (−12.7 to 16.0)	4.1 (−8.5 to 19.2)	0.25
LDL particle size, nm					
Baseline	20.6 (20.3 to 21.1)	20.5 (20.2 to 21.1)	20.6 (20.3 to 21.1)	20.6 (20.3 to 21.2)	0.84
Percentage change	−1.0 (−2.1 to 0.5)	0 (−1.9 to 1.0)	−0.5 (−1.9 to 1.5)	−0.5 (−2.5 to 1.5)	0.59
Large LDL particles, particles/l					
Baseline	285 (194 to 380)	238 (163 to 371)	269 (172 to 404)	282 (187 to 400)	0.84
Percentage change	−7.9 (−38.9 to 20.5)	−3.4 (−28.1 to 22.6)	0.4 (−35.4 to 26.3)	−1.6 (−28.2 to 35.7)	0.83
Small LDL particles, particles/l					
Baseline	770 (554 to 960)	703.5 (555 to 927)	707 (578 to 878)	727 (538 to 934)	0.89
Percentage change	8.4 (−11.2 to 30.5)	8.5 (−15.2 to 26.4)	3.5 (−16.5 to 29.8)	10.4 (−11.7 to 32.7)	0.34

Values are reported as the median (interquartile range) baseline and percentage change in nuclear magnetic resonance spectroscopy–derived lipoprotein parameters in patients treated with placebo, or RVX-208 at a dose of 50, 100, or 150 mg twice daily. p values for changes in parameters were generated from a model adjusting for baseline values. *p ≤ 0.001 compared with placebo. †p ≤ 0.01 compared with placebo.

Abbreviations as in Table 1.

Table 4 Biochemical Safety Parameters

Parameter	Placebo (n = 74)	RVX-208 50 mg bid (n = 76)	RVX-208 100 mg bid (n = 75)	RVX-208 150 mg bid (n = 74)	Overall p Value
ALT or AST >3× ULN	0 (0)	3 (3.9)	8 (10.7)	7 (9.5)	0.009
ALT or AST >8× ULN	0 (0)	2 (2.6)	4 (5.3)	2 (2.7)	0.28
Total bilirubin >2× ULN	0 (0)	0 (0)	0 (0)	0 (0)	1.00
CK elevation >3× ULN	4 (5.4)	1 (1.3)	0 (0)	3 (4.1)	0.10
Creatinine elevation >1.5× baseline	0 (0)	0 (0)	3 (4.0)	3 (4.1)	0.07

Values are reported as the n (%) of subjects experiencing laboratory measures of adverse events.
ALT = alanine transaminase; AST = aspartate transaminase; bid = twice daily; CK = creatinine phosphokinase; ULN = upper limit of normal.

clinical episodes were considered by the investigator to be related to study medication. There was no difference between treatment groups with regard to the rate of investigator reported adverse events.

Discussion

Considerable attention has focused on the development of new therapeutic agents that substantially elevate levels of HDL-C. However, development of pharmacological therapies that raise HDL-C levels has been challenging, in part because the underlying biology is substantially more complex than other lipoprotein-directed therapies. HDL circulates in many forms, including both lipid-rich and lipid-poor subfractions (12). It appears that lipid-poor preβ1-HDL fractions acquire cholesterol from macrophages in atherosclerotic plaques. These particles increase in size as they accumulate cholesterol. Cholesterol is ultimately taken up by the liver from these larger, lipid-rich α1-HDL particles or transferred to apoB-containing particles, a process known as RCT (13). Preβ1-HDL represents the most efficient substrate for enhancing RCT, which is considered a pivotal mechanism underlying the potential benefits of HDL-C–raising therapies (14). Because apoA-I has the capacity to generate more lipid-poor preβ1-HDL particles, most authorities believe that enhancing production of apoA-I represents the most promising approach to HDL modulation.

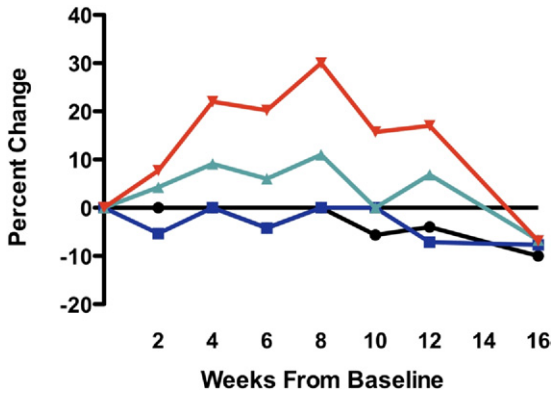

Figure 4 Time Course of Alanine Transaminase Changes

Time course of median percentage change in alanine transaminase in patients treated with placebo **(black circles)** or RVX-208 at a dose of 50 mg **(blue squares)**, 100 mg **(green triangles)**, or 150 mg **(inverted red triangles)** twice daily.

In this study, we characterized the early efficacy, tolerability, and safety of a novel inducer of endogenous apoA-I synthesis in patients with stable coronary artery disease who received concomitant statin treatment. Although the primary end point was not achieved, modest dose-dependent increases in circulating levels of apoA-I, HDL-C, and the concentration of large HDL particles were observed. The time course of the observed changes suggests that the maximal drug effects were not observed during this 12-week trial (Fig. 3). The increase in large HDL particles, exceeding 20% for the highest dosage, is consistent with the proposed mechanism of action of RVX-208. The increase in apoA-I should theoretically increase levels of lipid-poor preβ1-HDL particles, which subsequently mature into lipid-rich large HDL particles by acquiring cholesterol from macrophages in atherosclerotic plaques. Although these findings suggest that administration of RVX-208 is likely to facilitate cholesterol mobilization, this requires further investigation.

The 2D gel electrophoresis analysis revealed an increase in the circulating pool of larger, lipid-rich HDL particles with α1 mobility. Although significant correlations were observed between changes in levels of apoA-I and all additional HDL-related parameters, there was no apparent relationship between changes in the lipid-deplete and lipid-rich particle subclasses. Given that RVX-208 has no effect on the activity of CETP (9), this could be consistent with the ability of a given HDL particle to continue to promote lipid mobilization over time. The finding that these correlations are similar in both placebo and active treatment groups suggests that the potential benefit of RVX-208 may be exclusively related to its ability to act as a physiological switch of the HDL synthetic process, rather than having any additional activity at other stages that regulate HDL metabolism.

Although the observed changes in lipid parameters in statin-treated patients were modest, increases of a similar magnitude in HDL-related parameters for other therapies, such as statins and fibrates, have reduced adverse clinical outcomes and exerted a beneficial effect on the progression

of atherosclerosis (15–18). In fact, regression of coronary atherosclerosis was observed with infusions of lipid-deplete forms of HDL without any increase in steady-state levels of HDL-C (5–7). The most striking effect of RVX-208 was elevation of the concentration of larger HDL particles. Levels of this HDL subclass were a strong predictor of cardiovascular protection in population studies (10,19). Niacin, the approved therapeutic agent with the greatest HDL-C raising also appears to promote large HDL particles, a finding that strongly associates with slowing of coronary disease progression (20). The majority of changes were observed between 8 and 12 weeks following initiation of treatment, with no clear evidence of a plateau in efficacy. Further incremental benefit may be observed during longer duration of treatment. This would be consistent with the experience of many other agents that raise HDL-C, that often take many months to achieve maximal elevations (21–23).

In general, administration of RVX-208 was well tolerated. However, compared with placebo, a greater number of patients treated with RVX-208 demonstrated elevations in transaminase levels. These increases were transient and reversible, characterized by rapid increases in levels, typically between weeks 4 and 8, with rapid return to baseline levels upon discontinuation of the study drug. The mechanism underlying this pattern remains to be fully elucidated. However, it should be noted that increases in transaminases are common with virtually all lipid-modulating therapies, including statins, fibrates, and niacin (24), and more recently with HDL infusions (5,6). The lack of an associated rise in bilirubin levels suggests that there was no apparent hepatocellular injury. However, because of the short duration of the trial and limited sample size, we cannot rule out adverse hepatic effects during long-term administration. Ultimately, the overall balance between efficacy and safety must be determined in future clinical trials of longer duration.

Recent evidence has supported the concept that the functional quality of HDL may be a pivotal factor in determining its ability to protect against cardiovascular disease. Findings that patients with established coronary artery disease, despite having very high levels of HDL-C, often have pro-inflammatory forms of HDL suggest that the functional activity, rather than the quantity, may be a more important therapeutic target (25). This is further supported by observations that relatively modest changes in levels of HDL-C are associated with the cardiovascular benefit of many lipid-modifying therapies (15–18), whereas substantial HDL-C increases with the cholesteryl ester transfer protein inhibitor, torcetrapib, were associated with an adverse clinical outcome (21). For several decades, up-regulating endogenous expression of apoA-I has presented an attractive option for the development of novel antiatherosclerotic agents. Theoretically, this approach should result in the generation of nascent HDL particles with the capacity to perform a range of favorable biological functions. The objective of this approach is primarily to enhance functionality, as opposed to substantially elevate HDL-C. However, to date, identification of a compound that selectively increases apoA-I transcription has remained elusive. Accordingly, although the changes observed in this study are modest, it remains to be determined whether this will translate into any cardiovascular benefit.

One of the major challenges in the evaluation of therapies that target HDL and its biological functions is the lack of standardized and well-validated methods to characterize HDL functionality in humans. Traditional measures of apoA-I and HDL-C simply quantify the mass of protein and cholesterol, respectively, carried within the circulating HDL pool. Although systemic measures of these values have been demonstrated in population studies to predict cardiovascular risk (2,26), there is currently no evidence that they accurately reflect the functional quality of HDL particles. As a result, there remains an urgent need to develop additional assays that will provide complementary information with regard to HDL quality. Although it is possible to measure the ability of HDL function to promote cholesterol efflux (27) and modify cellular inflammatory, oxidative, and apoptotic pathways in ex vivo assays (25,28,29), it has not been established to what extent these reflect in vivo activity. Similarly, although measurement of fecal sterol excretion has been employed as an indirect measure of RCT (30), it is unknown to what degree this reflects a precise measurement of lipid removal from peripheral tissues. Furthermore, reports of a lack of benefit of elevated HDL-C levels in recent statin trials places additional uncertainty regarding the potential efficacy of HDL targeted therapies (26,31). This will be resolved only in the context of large-scale clinical trials.

Conclusions

Administration of an oral inducer of endogenous apoA-I synthesis resulted in changes in circulating HDL particles that may be consistent with enhanced cholesterol mobilization, although this requires further investigation. Although an increase in reversible elevations in liver enzymes was also observed, the balance with cardiovascular benefit remains to be determined in large, prospective clinical trials that will evaluate the impact of this novel agent. Ultimately, the ability to bring this strategy to clinical practice would represent a significant paradigm change, in which the focus is predominantly on specifically targeting the functional quality, rather than quantity of HDL.

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Key Words: apoA-I ■ coronary artery disease ■ high-density lipoprotein ■ prevention ■ risk factors.

APPENDIX

For a complete list of the trial investigators, see the online version of this article.