View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Journal of the American College of Cardiology Vol. 56, No. 14, 2010
© 2010 by the American College of Cardiology Foundation
Published by Elsevier Inc.
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.06.015

Effects of Varespladib Methyl on Biomarkers and Major Cardiovascular Events in Acute Coronary Syndrome Patients

Robert S. Rosenson, MD,* Colin Hislop, MD,† Michael Elliott, MA,† Yuri Stasiv, PhD,† Michael Goulder, BSc,‡ David Waters, MD§

New York, New York; Hayward and San Francisco, California; Nottingham, United Kingdom

Objectives The purpose of this study was to investigate the effects of varespladib on cardiovascular biomarkers in acute coronary syndrome patients.

Background Secretory phospholipase A_2 ($sPLA_2$) represents a family of proatherogenic enzymes that hydrolyze lipoprotein phospholipids, increasing their affinity for intimal proteoglycans; contribute to cholesterol loading of macrophages by nonscavenger receptor mediated pathways; and activate inflammatory pathways. In prospective studies, high $sPLA_2$-IIA levels predicted major adverse cardiovascular events in acute coronary syndrome (ACS) and stable coronary heart disease patients.

Methods This randomized, double-blind, prospective controlled clinical trial (phase 2B) was designed to investigate the effects of $sPLA_2$ inhibition with varespladib 500 mg daily versus placebo as adjunctive therapy to atorvastatin 80 mg daily on biomarkers (low-density lipoprotein cholesterol [LDL-C], high-sensitivity C-reactive protein [hsCRP], and $sPLA_2$-IIA levels), major adverse cardiovascular events (unstable angina, myocardial infarction, death), and safety. In all, 625 ACS subjects were randomized within 96 h of the index event and treated for a minimum of 6 months.

Results After 8 weeks (primary efficacy end point), varespladib/atorvastatin reduced mean LDL-C levels from baseline by 49.6% compared with 43.4% with placebo/atorvastatin ($p = 0.002$). Respective 8-week median reductions in $sPLA_2$-IIA levels were 82.4% and 15.6% ($p < 0.0001$), and hsCRP levels were lowered by 75.0% and 71.0% ($p = 0.097$). At 24 weeks, respective reductions with varespladib and placebo were as follows: LDL-C 43.5% versus 37.6% ($p < 0.05$), hsCRP 79.8% versus 77.0% ($p = 0.02$), and $sPLA_2$-IIA 78.5% versus 6.4% ($p < 0.0001$). Major adverse cardiovascular events were not different from placebo 6 months post-randomization (7.3% varespladib vs. 7.7% placebo). No treatment differences in elevated liver function studies on >1 occasion were observed.

Conclusions Varespladib therapy effectively reduced LDL-C and inflammatory biomarkers in ACS patients treated with conventional therapy including atorvastatin 80 mg daily. There were no treatment differences in clinical cardiovascular events. (FRANCIS [Fewer Recurrent Acute Coronary Events With Near-Term Cardiovascular Inflammation Suppression]-ACS Trial: A Study of the Safety and Efficacy of A 002 in Subjects With Acute Coronary Syndromes; NCT00743925). (J Am Coll Cardiol 2010;56:1079–88) © 2010 by the American College of Cardiology Foundation

High-dose statin therapy is evidence-based practice for secondary prevention of cardiovascular events in acute coronary syndrome (ACS) patients (1), and guideline-directed treatment to achieve "target levels" for low-density lipoprotein cholesterol (LDL-C) (2). However, the risk of cardiovascular events remains high among ACS patients treated with high-dose statin therapy.

Residual cardiovascular risk in statin-treated ACS patients has been associated with an LDL-C concentration that exceeds target levels, and a persistent systemic inflammatory state (3). C-reactive protein (CRP) is a biomarker of systemic inflammation that has been shown to predict cardiovascular events in patients presenting with ACS and treated with statins regardless of the achieved LDL-C level.

From the *Mount Sinai School of Medicine, New York, New York; †Anthera Pharmaceuticals, Hayward, California; ‡Worldwide Clinical Trials, Nottingham, United Kingdom; and the §University of California San Francisco, San Francisco, California. Dr. Rosenson has received honoraria from and served as a consultant to Anthera Pharmaceuticals; and has stock ownership in and served as a consultant to LipoScience. Dr. Hislop, Mr. Elliott, and Dr. Stasiv have ownership interest in and are employees/former employees of Anthera Pharmaceuticals. Mr. Goulder has reported that he has no relationships to disclose. Dr. Waters has served as a consultant for and has stock options in Anthera Pharmaceuticals.

Manuscript received May 2, 2010; revised manuscript received May 19, 2010, accepted June 1, 2010.

Abbreviations and Acronyms

ACS = acute coronary syndrome
CHD = coronary heart disease
CRP = C-reactive protein
hsCRP = high-sensitivity C-reactive protein
IL = interleukin
LDL = low-density lipoprotein
LDL-C = low-density lipoprotein cholesterol
Lp-PLA_2 = lipoprotein-associated phospholipase A_2
MACE = major adverse cardiac events
MI = myocardial infarction
$SPLA_2$ = secretory phospholipase A_2

However, a causal role of CRP in atherothrombosis has not been established (4-6). Other inflammatory proteins may be more specific targets for reducing cardiovascular risk, particularly in ACS patients (7).

Secretory phospholipase A_2 ($sPLA_2$) represents a family of isoenzymes that have direct and indirect involvement in atherosclerosis (8). Human atherosclerotic lesions have been shown to express 7 of 10 known $sPLA_2$ isoforms (9). In culprit coronary lesions of humans presenting with acute myocardial infarction (MI), atherosclerotic lesions contain abundant quantities of $sPLA_2$-IIA that are detected both intracellularly in macrophages of the intima and in vascular smooth muscle cells as well as in extracellular deposits (10). Expression of groups IIA and V have been shown to contribute to atherosclerosis in transgenic murine models (11,12).

In population studies, high $sPLA_2$-IIA levels have been shown to predict coronary heart disease (CHD) events in patients with stable CHD (13,14) and unstable angina (15), all-cause mortality in acute MI patients (16), and incident cardiovascular events in apparently healthy persons (17,18).

Varespladib methyl (A-002, Anthera Pharmaceutical, Hayward, California), a novel selective $sPLA_2$ inhibitor, has been shown to lower levels of $sPLA_2$-IIA by >90%, LDL-C by 12% to 18%, and high-sensitivity CRP (hsCRP) by 20% to 40% in stable CHD patients (19,20). This randomized, double-blind, prospective controlled clinical trial (phase 2B) was designed to investigate the effects of $sPLA_2$ inhibition with varespladib 500 mg daily versus placebo as adjunctive therapy to atorvastatin 80 mg daily on biomarkers (LDL-C, hsCRP, and $sPLA_2$-IIA levels), major adverse cardiac events (MACE [unstable angina, nonfatal MI, nonfatal stroke, urgent revascularization >60 days post-index event, and death]), and safety.

Methods

Patient population. All patients provided written informed consent. Local and national ethics committees approved the study protocol in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Study protocol. The FRANCIS (Fewer Recurrent Acute coronary events with Near-term Cardiovascular Inflammatory Suppression) study is a randomized, double-blind, prospective controlled phase 2B clinical trial designed to investigate the effects of $sPLA_2$ inhibition with varespladib 500 mg daily versus placebo as adjunctive therapy to atorvastatin 80 mg daily on biomarkers (LDL-C, hsCRP, and $sPLA_2$-IIA levels), MACE (unstable angina, nonfatal MI, nonfatal stroke, urgent revascularization >60 days post-index event, and death), and safety after a minimum of 6 months of treatment. After screening, subjects were withdrawn from their prescribed statin and randomly assigned within 96 h of their index event by an interactive voice response system to receive treatment with atorvastatin 80 mg daily and either placebo or varespladib 500 mg once daily. Safety and efficacy data were collected at weeks 0 (baseline), 2, 4, 8, 16, and 24, and monthly thereafter until the last subject enrolled had completed 6 months of treatment. Clinical event data were collected throughout the study.

End points. The primary end point was comparison of active and placebo treatments with respect to percentage change from baseline to week 8 in LDL-C. Secondary biomarker end points included comparison of active and placebo treatments with respect to change from baseline in concentrations of $sPLA_2$-IIA and hsCRP. Assessment of treatment on MACE after 6 months was also a secondary end point.

Treatment-emergent adverse events and serious adverse events along with electrocardiographic and hemodynamic data, clinical chemistry, and hematology evaluations were used to assess safety.

Biochemical analysis. Plasma lipids and chemistry panels and hsCRP were measured in a central laboratory by standard procedures (Quest Diagnostics, Van Nuys, California). The LDL-C concentrations were calculated. Secretory PLA_2-IIA concentration was analyzed by a quantitative 2-site enzyme immunoassay (Cayman Chemical, Ann Arbor, Michigan).

Statistical analysis. Primary efficacy analysis was the comparison of percentage changes from baseline to week 8 on LDL-C between the varespladib group and placebo group. Assuming a dropout rate of 10%, we estimated that a sample size of 133 subjects per group was needed to provide 90% power to detect a difference in LDL-C of 8% with a 2-sided level of significance of 0.05. The primary efficacy analysis was performed using analysis of covariance, with baseline value as a covariate and factors for treatment group and country. Because of nonnormality, changes in hsCRP, interleukin (IL)-6, and $sPLA_2$ concentration were analyzed on natural log-transformed data using the same analysis of covariance model as described in preceding text. The dependent variable was the difference of the logarithm of the follow-up value and the logarithm of the baseline value. The proportion of subjects reporting the following were compared with the chi-square test: LDL-C values of <1.8 mmol/l [70 mg/dl]; CRP values of <3 mg/l; LDL-C values of <1.8 mmol/l [70 mg/dl], and CRP values of <1 mg/l.

All secondary end points were considered as descriptive evidence of efficacy or safety and were analyzed without any procedures to account for multiple comparisons.

The number of subjects with MACE was analyzed using a logistic regression model with factors for treatment and country. Summary descriptive statistics were derived for individual time points and for change and percent change from baseline. The SAS software version 9.1.3 (SAS Institute, Cary, North Carolina) was used to perform the data analysis.

Adverse events were coded using the MedDra dictionary, version 12.1, and categorized by primary system organ class and preferred term, by intensity, and by causal relationship to study agent. We assessed all adverse events that occurred from the start of administration of the first dose of study drug until 28 days after administration of the last dose of study drug.

Results

Demographic characteristics. In total, 673 subjects were screened, resulting in 625 subjects being randomly allocated to the varespladib plus atorvastatin, 80 mg daily, and standard of care arm (n = 313) or to placebo plus atorvastatin, 80 mg, and standard of care arm (n = 312) (Table 1, Fig. 1). One subject in the placebo group never received treatment. By chance, more subjects in the varespladib-treatment groups had revascularization procedures before the index event (p = 0.0055), but not as treatment for the qualifying index event. The treatment groups were balanced in terms of subjects withdrawing, 84 (27%) in the varespladib treatment group and 92 (30%) in the placebo group. The most common reasons for withdrawal were screening laboratory LDL-C and triglyceride values outside protocol limits or nondiagnostic criteria for MACE, 32 (10%) reporting in the varespladib group and 51 (16%) in the placebo group.

The treatment groups did not differ with respect to demographic and baseline characteristics (Table 1). Overall, the index event was unstable angina in 147 (23.6%), non–ST-segment elevation MI in 218 (34.9%), and ST-segment elevation MI in 259 (41.5%). The mean time from hospitalization to randomization was 57.5 h.

The use of standard cardiovascular medications was similar between treatment groups. All subjects had ongoing concomitant medication at baseline, with 96% reporting the use of aspirin, 84% beta-blocking agents, 84% agents acting on the renin-angiotensin system, and only 10% statins. The LDL-C levels were similar between the treatment groups at baseline. The mean baseline values for LDL-C were 3.40 mmol/l [131 mg/dl] and 3.35 mmol/l [130 mg/dl] for the varespladib group and placebo group, respectively.

Efficacy. Treatment with varespladib reduced LDL-C levels by 49.6% compared with 43.4% for placebo from baseline to week 8 (primary efficacy end point, p = 0.0021) (Table 2). The interaction term for previous revascularization by treatment interaction was not significant (p = 0.16). Statistical differences in favor of varespladib were preserved in other sensitivity analyses on the primary end point.

Table 1 Demographics and Baseline Characteristics: All Subjects

Variable	Varespladib 500 mg + Atorvastatin 80 mg (n = 313)	Placebo + Atorvastatin 80 mg (n = 311)
Age, yrs	58.5 ± 10.3	59.6 ± 10.5
Male, %	73.5	75.9
Female, %	26.5	24.1
Caucasian	313 (100)	310 (99.7)
BMI, kg/m^2	29.2 ± 4.4	29.1 ± 4.1
Prior CAD	214 (68.4)	222 (71.4)
Revascularization		
Before index event	97 (31.0)	66 (21.2)
For treatment of index event	16 (5.1)	18 (5.8)
Cerebrovascular disease	18 (5.8)	6 (1.9)
Peripheral vascular disease	19 (6.1%)	23 (7.4)
Risk factors		
Diabetes mellitus	84 (26.8)	87 (28.0)
Hypertension	271 (86.6)	274 (88.1)
Hyperlipidemia	120 (38.3)	109 (35.0)
Current cigarette smoking	73 (23.3)	68 (21.9)
Qualifying event		
STEMI	132 (42.2)	127 (40.8)
Mean cTnI, ng/ml	15.5 ± 21.4	50.5 ± 310.2
Median CRP, mg/l	12.0 (0–222)	9.9 (0–377)
NSTEMI	114 (36.4)	104 (33.4)
Mean cTnI, ng/ml	4.1 ± 8.2	3.7 ± 10.5
Median CRP, mg/l	5.6 (1–204)	6.0 (0–300)
Unstable angina	67 (21.4)	80 (25.7)
Mean cTnI, ng/ml	0.7 ± 22	0.4 ± 1.3
Median CRP, mg/l	3.8 (0–30)	5.0 (0–83)
Mean time from index event in-hospital to randomization, h	56.6 ± 24.5	58.4 ± 24.5
Aspirin	290 (92.7)	283 (91.0)
Beta-blockers	263 (84.0)	263 (84.6)
Drugs acting on RA system	269 (85.9)	254 (81.7)
Lipid-modifying agents	36 (11.5)	24 (7.7)
Thrombolytic therapy	34 (10.9)	34 (10.9)
Antiplatelet therapy	131 (41.9)	124 (39.9)
Anticoagulant therapy	36 (11.5)	50 (16.1)
LDL-C, mmol/l	3.39 ± 1.02	3.35 ± 1.06
hsCRP, mg/l	8.2 (3.5–33.6)	8.9 (3.3–32.5)
sPLA$_2$-IIA, pmol/l	22.4 (14.1–34.5)	24.8 (14.6–36.9)
IL-6, pg/ml	5.22 (2.79–12.10)	5.16 (2.69–10.94)

Data are expressed as mean ± SD, n (%), or median (interquartile range).

ApoB = apolipoprotein B; BMI = body mass index; CAD = coronary artery disease; cTn1 = cardiac troponin 1; hsCRP = high-sensitivity C-reactive protein; IL = interleukin; LDL-C = low-density lipoprotein cholesterol; NSTEMI = non–ST-segment elevation myocardial infarction; PLA$_2$-IIA = group IIA secretory phospholipase A$_2$; RA = renin-angiotensin; STEMI = ST-segment elevation myocardial infarction.

Respective treatment-related reductions (least-squares mean) at week 8 observed using a repeated-measures mixed-model analysis were 49.2% versus 43.3% (p = 0.0014) and 49.2% versus 43.7% (p = 0.004) in a per-protocol analysis. Favorable treatment-related effects of varespladib on LDL-C levels were maintained through 24 weeks of the study: week 16, 43.8% compared with 36.6% (p = 0.007); and week 24, 43.5% compared with 37.6% (p = 0.03), as illustrated in Figure 2.

JACC Vol. 56, No. 14, 2010
September 28, 2010:1079–88

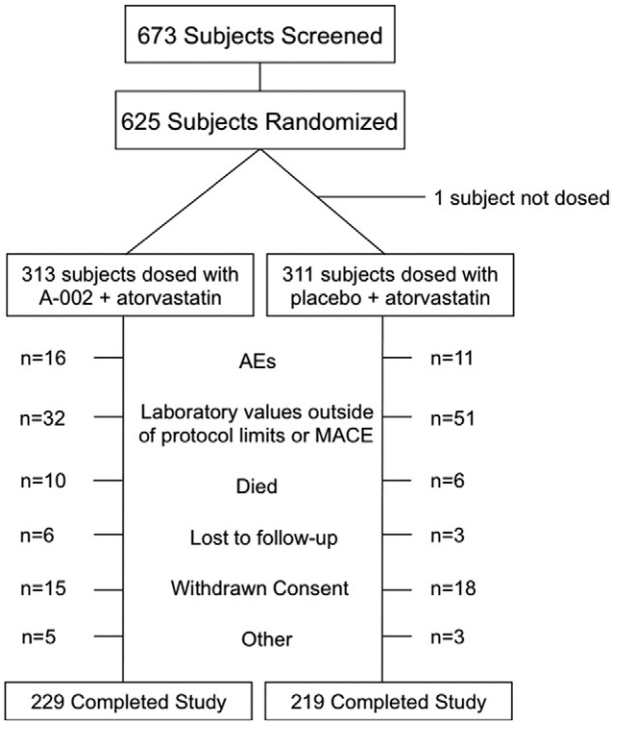

Figure 1 CONSORT Diagram

AE = adverse effect; CONSORT = CONsolidated Standards of Reporting Trials; MACE = major adverse cardiac events.

In addition to the mean reductions in LDL-C, the proportion of subjects treated with varespladib with LDL-C <1.8 mmol/l [70 mg/dl] at week 8 was 70.3% compared with 59.9% of those treated with placebo (p = 0.011) (Fig. 3). At 16 weeks, the respective proportions in each treatment group were 64.3% and 48.3% (p = 0.0005), and at 24 weeks, 61.5% and 50.0% (p = 0.02).

Varespladib treatment-related median reductions in hsCRP were seen at all time points and were statistically significantly different from placebo at week 16, 81.6% and 71.8% (p = 0.002), absolute difference 1 mg/l (p = 0.002), and at week 24, 79.8% and 77.0% (p = 0.02), respectively. A greater proportion of varespladib-treated subjects reported hsCRP values of <3 mg/l at all time points compared with placebo, and these changes were statistically significant at week 16, 75.1% compared with 64.0% (p = 0.008), and at week 24, 78.2% compared with 68.8% (p = 0.04). In addition, more subjects treated with varespladib (26.9%) had combined LDL-C levels <1.8 mmol/l [70 mg/dl] and hsCRP <1 mg/l at 24 weeks than subjects treated with placebo (15.9%, p = 0.012) (Fig. 4).

Varespladib treatment reduced $sPLA_2$-IIA levels at all time points through 16 weeks and at the end of the study. No samples were taken between 16 weeks and the end of the study. Respective median treatment-related decreases in $sPLA_2$-IIA levels were 82.4% in varespladib-treated subjects and 15.6% in placebo-treated subjects (p < 0.0001) at 8 weeks; 62.0% versus 12.1% (p < 0.0001) at 16 weeks; and 78.5% versus 6.4% (p < 0.0001) at the final study visit (Fig. 3).

Total MACE was not different between treatments at the end of the study (7.3% varespladib vs. 7.7% placebo, p = 0.93) (Table 3). There were no significant differences in cardiovascular risk for any component of the composite of all-cause death, nonfatal MI, documented unstable angina requiring urgent hospitalization, revascularization occurring >60 days after the index event, or fatal or nonfatal stroke. The difference in mortality between treatments was not statistically significant: p = 0.36 (odds ratio: 1.61, 95% confidence interval: 0.58 to 4.51; Fisher's exact test p =

Table 2 Plasma Levels of Lipids and Inflammatory Markers at Baseline and End of Treatment

Variable and Study Phase	Varespladib 500 mg	Placebo
$sPLA_2$-IIA concentration, pmol/l	n = 189	n = 186
Baseline	23.0 (0.41 to 407.4)	24.8 (1.3 to 522.8)
End point	4.83 (0.41 to 213.7)	21.4 (3.0 to 208.3)
Percent change	−78.5 (−99.7 to 1,898.7)*	−6.4 (−97.3 to 986.3)
Total cholesterol, mmol/l	n = 196	n = 194
Baseline	5.43 ± 1.21	5.36 ± 1.13
Week 24	3.54 ± 0.96	3.79 ± 0.96
Percent change	−32.9± 19.1†	−27.9 ± 18.4
LDL-C, mmol/l	n = 179	n = 184
Baseline	3.23 ± 0.93	3.20 ± 0.88
Week 24	1.72 ± 0.72	1.91 ± 0.68
Percent change	−43.5 ± 26.8‡	−37.6 ± 25.6
Non–HDL-C, mmol/l	n = 196	n = 194
Baseline	4.29 ± 1.17	4.20 ± 1.13
Week 24	2.42 ± 0.89	2.63± 0.91
Percent change	−41.2 ± 23.0‡	−35.6 ± 21.5
VLDL-C, mmol/l	n = 183	n = 189
Baseline	0.95 ± 0.37	0.92 ± 0.33
Week 24	0.68 ± 0.30	0.65± 0.29
Percent change	−19.3 ± 62.6	−25.3 ± 31.5
HDL-C, mmol/l	n = 196	n = 194
Baseline	1.08 ± 0.26	1.13 ± 0.31
Week 24	1.10 ± 0.26	1.14 ± 0.28
Percent change	5.1 ± 42.9	3.6 ± 23.1
Triglycerides	n = 196	n = 194
Baseline	2.28 ± 1.13	2.09 ± 0.88
Week 24	1.57 ± 0.81	1.50 ± 1.07
Percent change	−21.3 ± 59.0	−23.6 ± 45.7
hsCRP, mg/l	n = 188	n = 189
Baseline	7.9 (0.2 to 202)	8.3 (0.3 to 258)
Week 24	1.3 (0.2 to 17.8)	1.6 (0.2 to 59.5)
Percent change	−79.8 (−99.8 to 871.4)‡	−77.0 (−99.8 to 786.0)
Interleukin-6	n = 267	n = 246
Baseline	5.2 (0.5 to 126.6)	5.1 (0.5 to 272.1)
End point	3.1 (0.5 to 47.9)	3.0 (0.5 to 45.8)
Percent change	−37.4 (−98.4 to 920.3)	−32.0 (−97.7 to 1,503.3)

*Data are expressed as mean ± SD or median (range). To convert cholesterol values in mmol/l to mg/dl, multiply by 38.7; and to convert triglyceride values from mmol/l to mg/dl, multiply by 88.6. *p < 0.001 versus placebo. †p < 0.01 versus placebo. ‡p < 0.05 versus placebo.

HDL-C = high-density lipoprotein cholesterol; VLDL = very low-density lipoprotein-C; other abbreviations as in table 1.

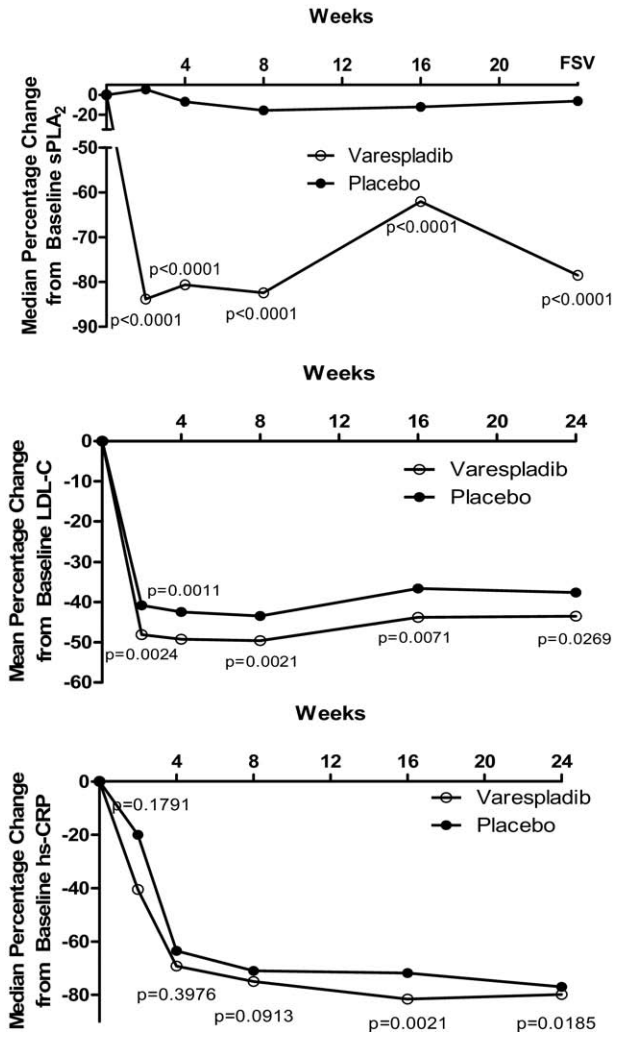

Figure 2 **Effects of Varespladib on Cardiovascular Biomarkers**

Data are expressed as least square mean ± SE of low-density lipoprotein cholesterol (LDL-C) and median (interquartile range) of high-sensitivity C-reactive protein (hsCRP) and $sPLA_2$-IIA concentration in placebo-treated patients **(solid circles)** and varespladib-treated patients **(open circles)**. Compared with placebo, varespladib reduced LDL-C, hsCRP, and $sPLA_2$-IIA levels at each time point except at week 8 for hsCRP. FSV = final study visit.

0.45). Furthermore, 2 of the deaths in the varespladib group occurred within 24 h of presentation of the index event after the subject had received only a single dose of study drug. A further 4 subjects died in the placebo group within the 6-month period after study completion compared with 1 in the varespladib group.

Safety. The median duration of exposure to both study medication and atorvastatin was 224 days for varespladib and 223 days for placebo.

Adverse events were reported in 70% of varespladib-treated subjects compared with 76% of placebo-treated subjects ($p = 0.12$) (Table 4). Fewer subjects treated with varespladib reported treatment emergent adverse events related to the renal tract compared with placebo: 10 (3.2%) and 19 (6.1%), respectively. Nine percent of subjects in each group reported events related to the study medication.

The only biochemical changes of note were transient increases in alanine aminotransferase and aspartate aminotransferase. A total of 58 subjects reported liver function tests ≥3 times the upper limit of normal at some time point during the course of the study: 31 (9.9%) subjects treated with varespladib and 27 (8.7%) treated with placebo (atorvastatin 80 mg). All 58 subjects were asymptomatic, and the number withdrawn due to elevated serum transaminases was similar between the varespladib group (n = 6) and placebo group (n = 4). The proportions of subjects with aminotransferase or aspartate aminotransferase increases >3 times the upper limit of normal on >1 occasion was not different between treatments: varespladib 3 (1.0%) and placebo 2 (0.7%).

No varespladib treatment-related effects were observed on blood pressure or the QT interval.

Discussion

This study of ACS patients treated with 80-mg atorvastatin plus standard of care extends the understanding of the treatment-related effects of varespladib on lipid and inflammatory biomarkers beyond that reported in stable CHD patients (19,20). Varespladib was more effective than placebo in lowering biomarkers that identify patients at high risk for recurrent cardiovascular events. The treatment differences in LDL-C and hsCRP persisted over the duration of the 6-month trial. The study was not powered to detect a difference in clinical events, and the number of events was small in both groups and not significantly different at the end of the study.

Varespladib methyl (1-H-indole-3-glyoxamide; A-002, Anthera Pharmaceuticals, Hayward, California; or previously LY333013, Eli Lilly & Co., Indianapolis, Indiana; or S3013, Shionogi & Company, Osaka, Japan) is the oral prodrug of varespladib sodium (sodium 2-[1-benzyl-2-ethyl-3-oxamoylindol-4-yl]) oxyacetate; A-001, Anthera Pharmaceuticals, or previously LY315920, Eli Lilly & Co.; or S5920, Shionogi & Company), which is a small molecule inhibitor of $sPLA_2$ with specificity toward group IIA $sPLA_2$ (IC_{50}: 9 to 14 nM), group V $sPLA_2$ (IC_{50}: 77 nM), and group X $sPLA_2$ (IC_{50}: 15 nM) (21). In 1 study, varespladib has been shown to reduce the extent of aortic atherosclerosis in $ApoE^{-/-}$ mice fed a high fat diet for 2 weeks, and treated with varespladib methyl or placebo for 16 weeks (21). In a study of accelerated atherosclerosis induced by a combination of high fat diet and continuous infusion of angiotensin II, 4 weeks of treatment with varespladib methyl reduced the extent of aortic plaque coverage and aneurysm formation (21). In $ApoE^{-/-}$ mice fed a Western diet for 3 months, combined low doses of varespladib methyl (1.5 mg/kg daily) and pravastatin caused a greater decrease in atherosclerosis than either drug when used alone, suggesting a synergistic

JACC Vol. 56, No. 14, 2010
September 28, 2010:1079–88

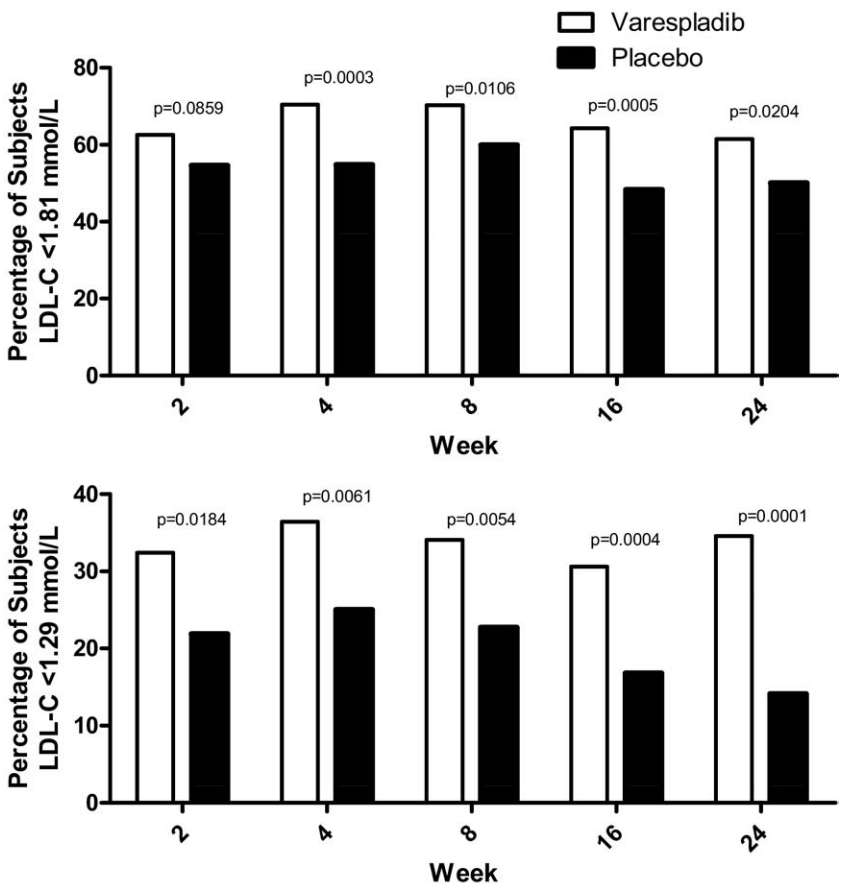

Figure 3 **LDL-C Goal Attainment With Varespladib and Placebo**

Percentage of patients who achieved goal low-density lipoprotein cholesterol (LDL-C) levels <1.81 mmol/l (70 mg/dl) in varespladib-treated patients **(open bars)** and placebo-treated patients **(solid bars)** at each time point in the study.

effect (22). Pravastatin combined with low-dose varespladib methyl reduced plaque area by 50%. These experimental studies provided a rationale for investigation into the role of varespladib methyl in the treatment of atherosclerosis, and the potential synergistic effect of varespladib methyl and statin therapy.

Previously, we reported that varespladib 500 mg daily reduced LDL-C from 12% to 18% in stable CHD patients, whereas hsCRP was nonsignificantly reduced by 20% to 40% (19,20). In the prior studies of stable CHD patients, 8 weeks of treatment with varespladib reduced $sPLA_2$-IIA concentrations by >90% and reduced LDL-C from 12% to 18%.

Several members of the $sPLA_2$ family of enzymes catalyze hydrolysis of surface phospholipids on lipoproteins, and result in structural alteration in these particles (8). The ensuing conformational change in apolipoprotein B (23) reduces clearance of circulating very low-density lipoprotein remnants and low-density lipoprotein (LDL) particle by the apolipoprotein B/E (LDL) receptor, resulting in increased residence time in the circulation that increases their susceptibility to oxidative modification (24). In the vessel wall, the conformational changes in apolipoprotein B increase their binding to intimal proteoglycans (23,25), promote retention of these atherogenic lipoproteins in the vessel wall (26–28), and increase cholesterol accumulation in macrophages (29,30). It has been shown that inhibition of secretory phospholipase A_2 with varespladib increases LDL particle size and lowers LDL-C levels (19,20). The LDL-C lowering effect is augmented with statin therapy (19), presumably because of enhanced LDL receptor-mediated clearance of the larger LDL particles.

Proinflammatory effects of $sPLA_2$ derive from multiple pathways that include production of lysophosphatidylcholine and nonesterified fatty acids (31), and direct effects on activation of the mitogen-activated protein kinase pathway, extracellular signal-regulated protein kinase-1/2 activation, and increased release of arachidonic acid (32-36). Arachidonic acid is an important inflammatory lipid mediator that increases expression of adhesion molecules on endothelial cells, including intercelluar adhesion molecule-1 and vascular cell adhesion molecule-1, that subsequently causes increased adhesion of monocytes to endothelial cells (37).

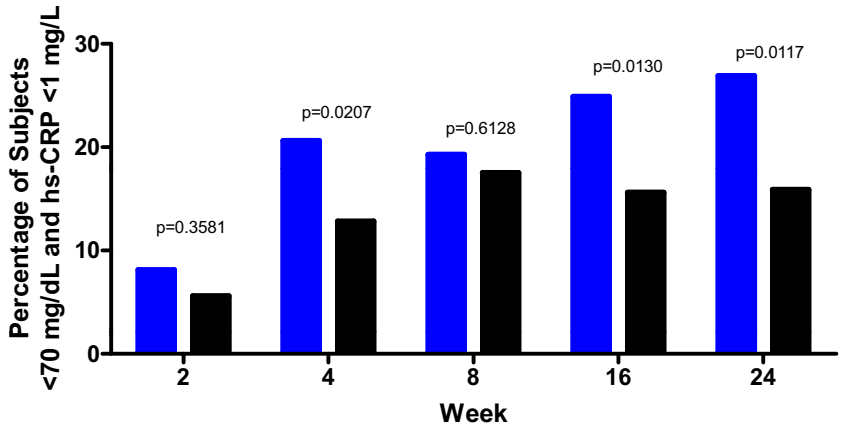

Figure 4 **Percentage of Treated Patients Who Achieved LDL-C Levels and hsCRP Levels**

Percentage of patients who achieved low-density lipoprotein cholesterol (LDL-C) levels <1.81 mmol/l (70 mg/dl) and high-sensitivity C-reactive protein (hsCRP) levels <1 mg/l in varespladib-treated patients **(blue bars)** and placebo-treated patients **(black bars)** at each time point in the study.

Although the precise mechanism for the benefit of $sPLA_2$ inhibition with varespladib treatment cannot be established solely on the basis of the findings from this trial, similar on-trial levels of LDL-C and hsCRP have been accompanied by lower rates of MACE (3,37). In the PROVE IT–TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction 22) trial, ACS patients were randomly assigned to atorvastatin 80 mg/day or pravastatin 40 mg/day (3). Among treated patients with LDL-C levels of 1.8 mmol/l (70 mg/dl) or higher and hsCRP levels of 2 mg/l or higher, the risk of recurrent MI of CHD death was 4.6 per 100 person-years, and 2.4 per 100 person-years for patients with LDL-C levels <1.8 mmol/l (70 mg/dl) and hsCRP levels <2 mg/l. In phase Z of the A-to-Z (Aggrastat-to-Zocor) trial, more intensive statin therapy was not accompanied by a reduction in the incidence of the primary end point (cardiovascular death, MI, readmission for ACS, or stroke) (38). However, patients in the intensive therapy arm who achieved both aggressive lowering of LDL-C (<1.8 mmol/l [70 mg/dl]) and hsCRP reductions (<2 mg/l) had lower cardiovascular event rates.

Table 3 **Breakdown of Major Adverse Cardiac Events at Week 16 and at End of Study**

	Varespladib	Placebo
Week 16		
Overall, ITT analysis	n = 313	n = 311
Total MACE	14	19
ITT event rate	4.2%	6.1%
UA requiring hospitalization	5 (1.6%)	9 (2.9%)
Myocardial infarction	2 (0.6%)	4 (1.3%)
Stroke	1 (0.3%)	1 (0.3%)
Death	6 (1.9%)	5 (1.6%)
Revascularization >60 days	0 (0%)	0 (0%)
End of study		
Overall, ITT analysis	n = 313	n = 311
Total MACE	23	24
ITT event rate	7.3%	7.7%
UA requiring hospitalization	8 (2.6%)	10 (3.2%)
Myocardial infarction	3 (1.0%)	6 (1.9%)
Stroke	1 (0.3%)	1 (0.3%)
Death	10 (3.2%)	6 (1.9%)
Revascularization >60 days	1 (0.3%)	1 (0.3%)

ITT = intention to treat; MACE = major adverse cardiac events; UA = unstable angina.

Although CRP has been the most extensively studied inflammatory biomarker associated with residual risk in statin-treated patients, $sPLA_2$ is more proximate inflammatory protein in the inflammatory cascade and a more specific target of therapy (7). Median secretory PLA_2 and hsCRP levels were elevated at baseline in both treatment groups, but the levels of these inflammatory proteins were even higher among those subjects who had a secondary event during the course of the study: $sPLA_2$ 23.9 pmol/l versus 26.9 pmol/l, and hsCRP 8.6 mg/l versus 16.5 mg/l, respectively.

Of the various $sPLA_2$ isoenzymes, $sPLA_2$-IIA is an acute phase reactant expressed in arterial smooth muscle cells and hepatocytes that activates transcription factors that induce synthesis of proinflammatory cytokines such as tumor necrosis factor-α, IL-1β, and IL-6 (8). These proinflammatory cytokines induce hepatic and myocardial CRP. We observed an increase in $sPLA_2$-IIA levels in ACS patients that preceded the rise in hsCRP levels. These temporal differences in inflammatory markers are inconsistent with the earlier activation of $sPLA_2$ from leukocytes after acute tissue injury than is observed from hepatic synthesis of CRP evident 48 to 72 h after an acute event (39). In this trial, varespladib reduced acute phase mediated increases in $sPLA_2$-IIA concentrations. As on-trial $sPLA_2$-IIA levels were highest among subjects with recurrent major cardio-

JACC Vol. 56, No. 14, 2010
September 28, 2010:1079–88

Table 4 MedDRA Summary of Treatment Emergent Adverse Events Occurring in >3% in Either Treatment Group by Primary System Organ Class and Preferred Term: All Subjects Population

MedDRA Primary System Organ Class and Preferred Term	Varespladib 500 mg + Atorvastatin 80 mg (n = 313)		Placebo + Atorvastatin 80 mg (n = 311)	
	No. of Reports	No. of Subjects Reporting, n (%)	No. of Reports	No. of Subjects Reporting, n (%)
Any	760	220 (70.3)	749	236 (75.9)
Cardiac disorders				
Angina pectoris	13	10 (3.2)	20	15 (4.8)
Cardiac failure chronic	16	16 (5.1)	7	7 (2.3)
General disorders and administration site conditions				
Asthenia	5	5 (1.6)	12	11 (3.5)
Infections and infestations				
Respiratory tract infection	11	10 (3.2)	9	8 (2.6)
Respiratory tract infection viral	11	11 (3.5)	9	8 (2.6)
Investigations				
Alanine aminotransferase increased	33	31 (9.9)	17	16 (5.1)
Aspartate aminotransferase increased	19	18 (5.8)	7	7 (2.3)
Blood alkaline phosphatase increased	9	8 (2.6)	10	10 (3.2)
Blood creatine phosphokinase increased	10	9 (2.9)	11	11 (3.5)
Blood glucose increased	12	11 (3.5)	14	11 (3.5)
C-reactive protein increased	55	45 (14.4)	62	58 (18.6)
Gamma-glutamyltransferase increased	36	34 (10.9)	35	35 (11.3)
Metabolism and nutrition disorders				
Hyperglycemia	12	9 (2.9)	12	11 (3.5)
Nervous system disorders				
Headache	15	13 (4.2)	16	14 (4.5)
Vascular disorders				
Hypertension	12	11 (3.5)	9	8 (2.6)

vascular events, these data are consistent with the prognostic significance of elevated $sPLA_2$-IIA levels shown in ACS patients (15,16).

Another phospholipase A_2 inhibitor under clinical investigation is lipoprotein-associated phospholipase A_2 (Lp-PLA_2) (7). The Lp-PLA_2 inhibitor represents a calcium-independent phospholipase that is predominantly synthesized by macrophages. In plasma, Lp-PLA_2 is bound to LDL and high-density lipoprotein, with a greater affinity for the polar surface of LDL particles, particularly electronegative small LDL particles that have been minimally oxidatively modified. The requirement for a minimally oxidatively modified LDL particle is one feature that distinguishes Lp-PLA_2 from $sPLA_2$ that acts on native LDL. In a multicenter trial of stable CHD or CHD risk equivalent patients, treatment with darapladib 160 mg daily reduced IL-6 levels by 12.3% (95% confidence interval: −22% to −1%, p = 0.028) compared with placebo, whereas there were no significant group differences in hsCRP (40). In this study, at week 2, varespladib also reduced median IL-6 levels by 16.6% compared with 5.2% for placebo (p = 0.19). Treatment with darapladib has been shown to reduce lipid necrotic core volume as assessed by intravascular ultrasonography (41). The data from the IBIS-2 (Integrated Biomarkers and Imaging Study-2) suggest that Lp-PLA_2 inhibition may reduce the vulnerability of rupture-prone plaques.

The FRANCIS study was primarily conducted as a biomarker trial in ACS patients, and the findings in trial are consistent with the results reported in stable CHD patients (19,20). In the PLASMA I (Phospholipase Levels and Serological Markers of Atherosclerosis) and PLASMA II trials, we also reported that varespladib was more effective than placebo treatment for lowering levels of LDL-C and $sPLA_2$-IIA levels. In contrast to the earlier trials in stable CHD patients, we report significant treatment differences in hsCRP that occurred on a background of high-dose atorvastatin treatment. The reduction in hsCRP in FRANCIS may result from the larger study population or higher baseline hsCRP levels (median 8.6 mg/l in the FRANCIS study versus 1.10 to 1.89 mg/l in the PLASMA I and II trials). A secondary outcome of FRANCIS was a pilot study of MACE. Although there were more deaths in varespladib-treated subjects at week 24 (10 vs. 6), 2 subjects assigned to varespladib died within 24 h after ST-segment elevation MI. Six months after the trial, 1 additional patient died in the varespladib group and 4 subjects died in the placebo group. Based on a reduction in MIs and unstable angina among varespladib-treated patients, we have designed a larger event-driven trial.

Study limitations. Limitations of the current trial include small numbers of ACS patients that limit the ability to detect differences in cardiovascular events. Nonetheless, the trend toward reduction in MIs and unstable angina was seen beyond maximal use of evidence-based therapies, inclusive of high-dose atorvastatin. Second, serum $sPLA_2$ activity was not measured, as we previously reported in the PLASMA I trial, because low $sPLA_2$-IIA concentration in

the samples from varespladib-treated subjects was below the limit required for the activity assay (19); and, when $sPLA_2$ inhibitors bind to the active site of $sPLA_2$, the enzyme-inhibitor complex binds tighter to membranes than does the enzyme without inhibitor (42). Thus, it may be necessary in the presence of inhibitor to measure total $sPLA_2$ activity on whole blood samples.

Conclusions

The FRANCIS study demonstrates that treatment with varespladib reduces concentration of LDL-C, hsCRP, and $sPLA_2$ in ACS patients treated with evidence-based therapies inclusive of high-dose atorvastatin. Elevated levels of these biomarkers have been shown to identify CHD patients who remain at high risk for recurrent cardiovascular events; however, in this trial, there were no treatment differences in clinical cardiovascular events. A large, prospective, double-blind, placebo-controlled trial in ACS patients will be required to explore the future role of varespladib treatment in cardiovascular event reduction.

Acknowledgments

The authors thank Heather Fraser for technical assistance, and Paul Truex and James Pennington for editorial comments.

Reprint requests and correspondence: Dr. Robert S. Rosenson, Mount Sinai Heart, Mount Sinai School of Medicine, One Gustave Levy Place, New York, New York 10029. E-mail: robert.rosenson@mssm.edu.

REFERENCES

1. Anderson JL, Adams CD, Antman EM, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non–ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non–ST-Elevation Myocardial Infarction). J Am Coll Cardiol 2007;50:e1–157.
2. Grundy SM, Cleeman JI, Merz CN, et al. National Heart, Lung, and Blood Institute; American College of Cardiology Foundation; American Heart Association. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004;110:227–39.
3. Ridker PM, Cannon CP, Morrow D, et al., Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI 22) Investigators. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.
4. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. N Engl J Med 2008;359:1897–908.
5. Koike T, Kitajima S, Yu Y, et al. Human C-reactive protein does not promote atherosclerosis in transgenic rabbits. Circulation 2009;120:2036–9.
6. Danesh J, Pepys MB. C-reactive protein and coronary disease: is there a causal link? Circulation 2009;120:2088–94.
7. Rosenson RS. Future role for selective phospholipase A_2 inhibitors in the prevention of atherosclerotic cardiovascular disease. Cardiovasc Drugs Ther 2009;23:93–101.
8. Rosenson RS, Gelb MH. Secretory phospholipase A_2: a multifaceted family of proatherogenic enzymes. Curr Cardiol Rep 2009;11:445–51.
9. Kimura-Matsumoto M, Ishikawa Y, Komiyama K, et al. Expression of secretory phospholipase A_2s in human atherosclerosis development. Atherosclerosis 2008;196:81–91.
10. Nijmeijer R, Meuwissen M, Krijnen PAJ, et al. Secretory type II phospholipase A_2 in culprit coronary lesions is associated with myocardial infarction. Eur J Clin Invest 2008;38:205–10.
11. Webb NR, Bostrom MA, Szilvassy SJ, et al. Macrophage-expressed group IIA secretory phospholipase A_2 increases atherosclerotic lesion formation in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol 2003;23:263–8.
12. Bostrom MA, Boyanovsky BB, Jordan CT, et al. Group V secretory phospholipase A_2 promotes atherosclerosis: evidence from genetically altered mice. Arterioscler Thromb Vasc Biol 2007;27:600–6.
13. Kugiyama K, Ota Y, Takazoe K, et al. Circulating levels of secretory type II phospholipase A_2 predict coronary events in patients with coronary artery disease. Circulation 1999;100:1280–4.
14. Liu PY, Li YH, Tsai WC, et al. Prognostic value and the changes of plasma levels of secretory type II phospholipase A_2 in patients with coronary artery disease undergoing percutaneous coronary intervention. Eur Heart J 2003;24:1824–32.
15. Kugiyama K, Ota Y, Sugiyama S, et al. Prognostic value of plasma levels of secretory type II phospholipase A_2 in patients with unstable angina pectoris. Am J Cardiol 2000;86:718–22.
16. Porela P, Pulkki K, Voipio-Pulkki LM, Pettersson K, Leppänen V, Nevalainen TJ. Level of circulating phospholipase A_2 in prediction of the prognosis of patients with suspected myocardial infarction. Basic Res Cardiol 2000;95:413–7.
17. Mallat Z, Benessiano J, Simon T, et al. Circulating secretory phospholipase A_2 activity and risk of incident coronary events in healthy men and women: the EPIC-NORFOLK study. Arterioscler Thromb Vasc Biol 2007;27:1177–83.
18. Koenig W, Vossen CY, Mallat Z, Brenner H, Benessiano J, Rothenbacher D. Association between type II secretory phospholipase A_2 plasma concentrations and activity and cardiovascular events in patients with coronary heart disease. Eur Heart J 2009;30:2742–8.
19. Rosenson RS, Hislop C, McConnell D, et al. Effects of a selective inhibitor of secretory phospholipase A_2 on low density lipoproteins and inflammatory pathways. Lancet 2009;373:649–58.
20. Rosenson RS, Elliott M, Stasiv Y, Hislop C. Randomized trial of an inhibitor of secretory phospholipase A_2 on atherogenic lipoprotein subclasses in statin-treated patients with coronary heart disease. Eur Heart J 2010. In press.
21. Fraser H, Hislop C, Christie RM, et al. Varespladib (A-002), a secretory phospholipase A_2 inhibitor, reduces atherosclerosis and aneurysm formation in ApoE–/– mice. J Cardiovasc Pharmacol 2009;53:60–5.
22. Shaposhnik Z, Wang X, Trias J, Fraser H, Lusis AJ. The synergistic inhibition of atherogenesis in $ApoE^{-/-}$ mice between pravastatin and the $sPLA_2$ inhibitor varespladib (A-002). J Lipid Res 2008;50:623–9.
23. Kleinman Y, Krul ES, Burnes M, et al. Lipolysis of LDL with phospholipase A_2 alters the expression of selected ApoB-100 epitopes and the interaction of LDL with cells. J Lipid Res 1988;29:729–43.
24. Tietge UJF, Pratico D, Ding T, et al. Macrophage-specific expression of group IIA $sPLA_2$ results in accelerated atherogenesis by increasing oxidative stress. J Lipid Res 2005;46:1604–14.
25. Flood C, Gustafsson M, Pitas RE, et al. Molecular mechanism for changes in proteoglycan binding on compositional changes of the core and the surface of low-density lipoprotein-containing human apolipoprotein B100. Arterioscler Thromb Vasc Biol 2004;24:564–70.
26. Hurt-Camejo E, Andersen S, Standal R, et al. Localization of nonpancreatic secretory phospholipase A_2 in normal and atherosclerotic arteries: activity of the isolated enzyme on low-density lipoproteins. Arterioscler Thromb Vasc Biol 1997;17:300–9.
27. Sartipy P, Camejo G, Svensson L, et al. Phospholipase A_2 modification of low density lipoproteins forms small high density particles with increased affinity for proteoglycans and glycosaminoglycans. J Biol Chem 1999;274:25913–20.
28. Hakala JK, Oorni K, Pentikainen MO, et al. Lipolysis of LDL by human secretory phospholipase A_2 induces particle fusion and enhances the retention of LDL to human aortic proteoglycans. Arterioscler Thromb Vasc Biol 2001;21:1053–8.
29. Wooton-Kee CR, Boyanovsky BB, Nasser MS, et al. Group V $sPLA_2$ hydrolysis of low-density lipoprotein results in spontaneous particle

aggregation and promotes macrophage foam cell formation. Arterioscler Thromb Vasc Biol 2004;24:762–7.
30. Hanasaki K, Yamada K, Yamamoto S, et al. Potent modification of low density lipoprotein by group X secretory phospholipase A_2 is linked to macrophage foam cell formation. J Biol Chem 2002;277: 29116–24.
31. Leitinger N, Watson AD, Hama SY, et al. Role of group II secretory phospholipase A_2 in atherosclerosis: 2. Potential involvement of biologically active oxidized phospholipids. Arterioscler Thromb Vasc Biol 1999;19:1291–8.
32. Balboa MA, Balsinde J, Winstead MV, et al. Novel group V phospholipase A_2 involved in arachidonic acid mobilization in murine P388D1 macrophages. J Biol Chem 1996;271:32381–4.
33. Kim YJ, Kim KP, Han SK, et al. Group V. phospholipase $A_{,2}$ induces leukotriene biosynthesis in human neutrophils through the activation of group IVa phospholipase A_2. J Biol Chem 2002;277:36479–88.
34. Henderson WR Jr., Chi EY, Bollinger JG, et al. Importance of group X-secreted phospholipase A_2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. J Exp Med 2007;204: 865–77.
35. Bingham CO III, Murakami M, Fujishima H, et al. A heparin-sensitive phospholipase A_2 and prostaglandin endoperoxide synthase-2 are functionally linked in the delayed phase of prostaglandin d_2 generation in mouse bone marrow-derived mast cells. J Biol Chem 1996;271:25936–44.
36. Bezzine S, Koduri RS, Valentin E, et al. Exogenously added human group X secreted phospholipase A_2 but not the group Ib, IIA, and V enzymes efficiently release arachidonic acid from adherent mammalian cells. J Biol Chem 2000;275:3179–91.
37. Saegusa J, Akakura N, Wu C-Y, et al. Pro-inflammatory secretory phospholipase A_2 type IIA binds to integrins alpha V beta 3 and alpha 4 beta 1 and induces proliferation of monocytic cells in an integrin-dependent manner. J Biol Chem 2008;283:26107–15.
38. Morrow DA, de Lemos JA, Sabatine MS, et al. Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor trial. Circulation 2006;114: 281–8.
39. Jahangiri A, de Beer MC, Noffsinger V, et al. HDL remodeling during the acute phase response. Arterioscler Thromb Vasc Biol 2009;29: 261–7.
40. Mohler ER III, Ballantyne CM, Davidson MH, et al., for the Darapladib Investigators. The effect of darapladib on plasma lipoprotein-associated phospholipase A_2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. J Am Coll Cardiol 2008;51: 1632–41.
41. Serruys PW, García-García HM, Buszman P, et al., for the Integrated Biomarker and Imaging Study-2 Investigators. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. Circulation 2008;118: 1172–82.
42. Lambeau G, Gelb MH. Biochemistry and physiology of mammalian secreted phospholipases A_2. Annu Rev Biochem 2008;77:495–520.

Key Words: acute coronary syndromes ■ inflammation ■ low-density lipoprotein ■ secretory phospholipase A_2 ■ varespladib.