Circulation
Cardiovascular Imaging
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Circ Cardiovasc Imaging 2010;3;298-307; originally published online Feb 27, 2010;
DOI: 10.1161/CIRCIMAGING.110.937169
Circulation: Cardiovascular Imaging is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 1941-9651. Online ISSN: 1942-0080

Treatment With 5-Lipoxygenase Inhibitor VIA-2291 (Atreleuton) in Patients With Recent Acute Coronary Syndrome

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Background—Production of leukotrienes by 5-lipoxygenase (5-LO) has been linked to unstable atherosclerotic plaques and cardiovascular events. VIA-2291 is a potent 5-LO inhibitor.

Methods and Results—In a double-blinded study, 191 patients were randomly assigned 3 weeks after an acute coronary syndrome to receive 25, 50, or 100 mg VIA-2291 or placebo daily for 12 weeks. The primary study end point, whole blood stimulated leukotriene LTB4 at trough drug level, was reduced in all VIA-2291 groups ($P<0.0001$) in a dose-dependent fashion, with approximately 80% inhibition in >90% of patients in the 100-mg group. A significant reduction of urine leukotriene LTE4 was obtained in all dose groups. No serious adverse events were considered related to study drug. A subset of 93 patients who had undergone a 64-slice coronary CT examination at baseline continued on study medication for a total of 24 weeks and underwent a repeat scan. Five of these patients withdrew or were noncompliant and 28 had nonevaluable scans. Among the 60 remaining patients, new coronary plaques were observed in 5 of 18 (27.8%) placebo-treated patients and in 2 of 42 (4.8%) VIA-2291–treated patients ($P=0.01$). A reduction in noncalcified plaque volume at 24 weeks versus placebo was observed in VIA-2291–treated groups in the 34 of these 60 patients in whom this end point was analyzable ($P<0.01$).

Conclusions—VIA-2291 reduces leukotriene production at 12 weeks after an acute coronary syndrome. Preliminary data from the CT substudy suggest that such a reduction in leukotriene production may influence atherosclerosis; however, this requires confirmation in a larger study.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00358826.

(*Circ Cardiovasc Imaging*. 2010;3:298-307.)

Key Words: coronary disease ■ inflammation ■ imaging ■ atherosclerosis ■ leukotrienes

Atherosclerosis is an inflammatory disorder of the arterial wall.[1–4] Most myocardial infarctions (MI) are caused by a rupture of the fibrous cap of an unstable atherosclerotic plaque, leading to release of thrombogenic material and blockage of the arterial lumen by a superimposed thrombus.[1,5,6] Despite standard-of-care treatment, patients with recent acute coronary syndromes (ACS) remain at high risk of recurrent vascular events,[7] and this risk is especially pronounced in patients with elevated high-sensitivity C-reactive protein (hs-CRP).[8] The underlying inflammatory disease process active in atherosclerosis and ACS is not directly addressed by currently available interventions.

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5-Lipoxygenase (5-LO) is the key regulatory enzyme in the biosynthesis of leukotrienes (LTs), including the 2 major active classes LTB4 and cysteinyl leukotrienes LTC4, D4, and E4. Leukotrienes are a class of eicosanoids that are known to exert various proinflammatory effects. Studies conducted in both animal models and patients have converged on the pivotal role of leukotrienes in unstable inflamed plaques and the identification of 5-LO as a potential target to reduce atherosclerotic plaque inflammation.[9–13] VIA-2291 is a potent 5-LO inhibitor that was previously studied in 1100 patients in clinical trials for asthma, another inflammatory disease.[14] The purpose of the current study was to test the safety and efficacy of 5-LO inhibition by VIA-2291 in a dose-ranging, double-blinded study in ACS patients and secondarily provide some insights into its effects on hs-CRP and coronary atherosclerosis.

Methods

Study Design

This was a prospective, randomized, double-blinded, parallel-group, placebo-controlled, multicenter, dose-ranging study. The study protocol was approved by the institutional review board at each center, and

Received August 17, 2009; accepted February 11, 2010.

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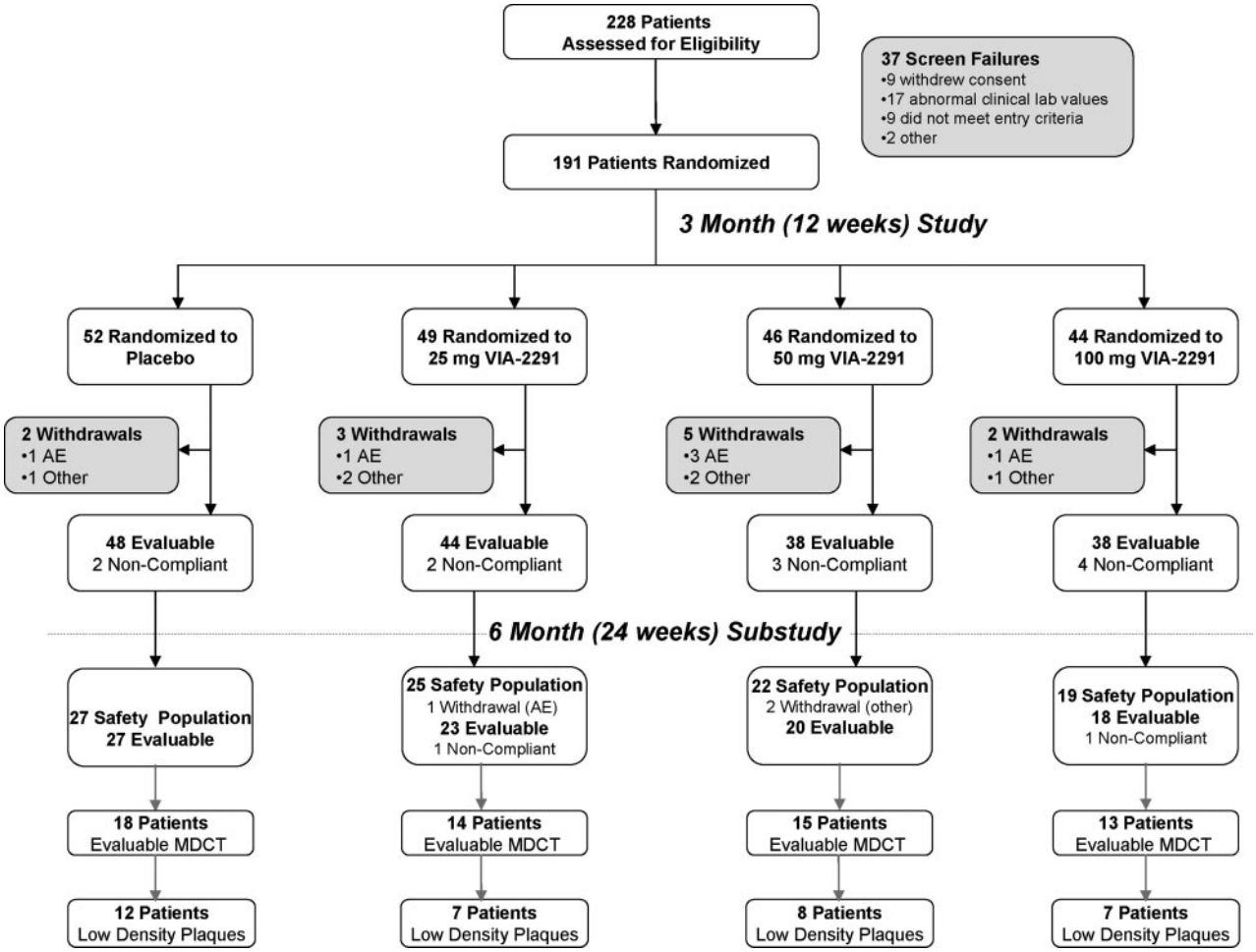

Figure 1. Patient disposition in the study. AE indicates adverse event.

patients provided written informed consent. The study was conducted at 12 centers in the United States and Canada between July 2006 and August 2008. Men and women between 30 to 80 years of age with an MI or unstable angina 21 (±3) days before study randomization were eligible to participate if they were clinically stable at the time of randomization. Major exclusion criteria included creatinine >1.5×upper limit of normal (ULN); cirrhosis, recent hepatitis, ALT >1.5×ULN or ALT > ULN, and at least 1 other abnormal liver function test or positive screening test for hepatitis B or C; uncontrolled diabetes defined as hemoglobin A1C >11% at screening; New York Heart Association class III or IV congestive heart failure; previous coronary artery bypass graft surgery or planned percutaneous coronary intervention or coronary artery bypass graft within the next 6 months; recurrence of MI or unstable angina less than 18 days before randomization; atrial fibrillation or other arrhythmia that might interfere with a multidetector CT (MDCT) substudy; or acetaminophen use in any form in the 7 days before enrollment.

One hundred ninety-one patients were randomly assigned to once-daily dosing of placebo or VIA-2291 (atreleuton) 25 mg, 50 mg, or 100 mg for 12 weeks; a substudy extended dosing to 24 weeks in patients who consented to undergo multidetector CT examinations at baseline and end of follow-up.

LTB4, LTE4, and hs-CRP Measurements

Measurements of whole blood LTB4, urine LTE4, and hs-CRP were conducted at a central laboratory (Mayo Laboratories, Rochester, Minn). For LTB4, heparinized whole blood was immediately incubated with a calcium ionophore (Calcimycin) for 60 minutes at 37°C, then chilled before centrifugation for 10 minutes at 4000*g* to isolate plasma. Samples were frozen at −20°C and shipped to the central laboratory for LTB4 measurement by enzyme-linked immunosorbent assay (Assay Design, Ann Arbor, Mich). Urine samples were shipped frozen for analysis of LTE4 using liquid chromatography with tandem mass spectrometry. Urine LTE4 concentrations were reported adjusted for urine creatinine. Hs-CRP concentrations were determined with an immunoturbidimetric method (Diasorin, Saluggia, Italy).

Study Outcomes

The predefined primary outcome was the effect of VIA-2291 versus placebo on change from baseline in ex vivo LTB4 synthesis in whole blood after 12 weeks of treatment. Secondary end points were changes in LTE4 in urine and hs-CRP. Safety and tolerability of VIA-2291 were evaluated by adverse events collected throughout the study.

Multidetector Coronary CT Substudy

CT examination was performed at baseline and after 24 weeks of treatment with a 64-slice scanner (GE LightSpeed VCT; GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). Patients with a resting heart rate of >65 bpm received 50 mg of metoprolol orally 1 hour before CT examination. Patients with a calcium score >800 Agatston Units were excluded. Sublingual nitroglycerin (0.4 mg) was administered immediately before the contrast-enhanced CT acquisition. Contrast-enhanced CT examination was performed using a triphasic bolus of 60 mL of nonionic, iso-osmolar iodine contrast (iodixanol 320), followed by 50 mL of mixed contrast (50%) and saline (50%), and finally followed by 25 mL of saline injected into an antecubital vein at 5 mL/s. The volume data set of 64 parallel slices using an algorithm of 180° multislice cardiac interpolation was obtained at

Table 1. Baseline Characteristics of Patients According to Treatment Group

Characteristics	Placebo (n=52)	VIA-2291 25 mg (n=49)	VIA-2291 50 mg (n=46)	VIA-2291 100 mg (n=44)
Age, y, mean (SD)	56.6 (9.8)	56.4 (9.5)	57.3 (10.0)	57.6 (9.4)
Male sex, n (%)	41 (78.8)	41 (83.7)	41 (89.1)	38 (86.4)
Caucasian, n (%)	48 (92.3)	44 (89.8)	45 (97.8)	42 (95.5)
Body mass index, mean (SD)	29.1 (5.4)	29.9 (4.7)	30.9 (4.3)	29.4 (4.6)
Risk factors, n (%)				
Diabetes	9 (17.3)	7 (14.3)	11 (23.9)	6 (13.6)
Hypertension	30 (57.7)	20 (40.8)	27 (58.7)	27 (61.4)
Prior MI	6 (11.5)	7 (14.3)	11 (23.9)	9 (20.5)
Smoking				
Current, n (%)	19 (36.5)	13 (26.5)	12 (26.1)	13 (29.5)
Ex-smoker, n (%)	15 (28.8)	18 (36.7)	13 (28.3)	18 (40.9)
Percutaneous coronary intervention, n (%)				
Before index event	10 (19.2)	6 (12.2)	12 (26.1)	11 (25.0)
For treatment of index event	47 (90.4)	44 (89.8)	39 (84.8)	42 (95.5)
Index event, n (%)*				
STEMI	25 (48.1)	20 (40.8)	19 (41.3)	22 (50.0)
Non-STEMI	17 (32.7)	14 (28.6)	15 (32.6)	12 (27.3)
Unstable angina	10 (19.2)	15 (30.6)	12 (26.1)	9 (20.5)
Blood pressure, mm Hg				
Systolic, mean (SD)	116.1 (21.7)	114.8 (15.4)	119.8 (16.9)	118.4 (17.8)
Diastolic, mean (SD)	69.8 (14.0)	69.4 (8.1)	70.2 (9.5)	70.7 (10.8)
Lipid values, mmol/L				
Total cholesterol, mean (SD)	3.50 (0.85)	3.39 (0.84)	3.29 (0.74)	3.44 (0.86)
LDL-C, mean (SD)	2.02 (0.73)	1.87 (0.58)	1.88 (0.56)	1.97 (0.66)
HDL-C, mean (SD)	1.01 (0.26)	0.92 (0.28)	0.95 (0.23)	0.95 (0.19)
Triglycerides, mean (SD)	1.55 (0.77)	1.97 (1.37)	1.77 (0.84)	1.80 (0.83)
Other cardiovascular history				
CHF	4 (7.7)	3 (6.1)	1 (2.2)	2 (4.5)
CABG, prior	0 (0.0)	0 (0.0)	1 (2.2)	1 (2.3)
Angina, prior	12 (23.1)	12 (24.5)	13 (28.3)	11 (25.0)

MI indicates myocardial infarction; STEMI, ST segment elevation MI; CHF, chronic heart failure; and CABG, coronary artery bypass grafting.

The table reflects an intent-to-treat population. For lipid values, n is the same in all groups except for the 100-mg group, in which n=43.

*Information on type of index event is missing for 1 patient in the 100-mg group.

0.625-mm intervals. The rotation time was 350 ms; tube voltage was 120 kV at a current of 550 to 800 mA (depending on patient weight) from the carina to the diaphragm, without ECG dose modulation. All images were acquired on a single breath-hold of approximately 6 seconds using monosegmented or multisegmented algorithms. Images were reconstructed to a slice thickness of 0.625 mm with an interslice gap of 0.625 mm using retrospective ECG gating from 0% to 90% of the R-R interval at 10% increments. Automatic reconstructions of the 75% phase were also obtained. The CT substudy evaluable population was defined on the basis of compliance with study medication (80% to 120% of scheduled doses) and having interpretable images at 24 weeks.

Scans were evaluated at the Montreal Heart Institute CT core laboratory. Target plaque lesions were selected for assessment when there were no artifacts or technical acquisition problems interfering with the analysis on baseline and follow-up studies. Image analysis was performed on the Advantage Windows Workstation 4.4 to measure changes in (1) noncalcified plaque volume (mm^3); (2) mean plaque density (Hounsfield units, HU); and (3) percentage stenosis at a target lesion from baseline to follow-up.

Target plaque lesions were defined prospectively as noncalcified plaque with measurable low-density components of ≤60 HU situated in the proximal or middle portion of either the left main, left anterior ascending, left circumflex, or right coronary artery causing at least 20% luminal stenosis. To determine the volume of noncalcified plaque of a target lesion, the space between the luminal and adventitial surfaces at the lesion site was determined for each 1-mm axial section and all of the volume elements were then summed to achieve volume quantification. The percentage reduction in lumen diameter or percentage stenosis was determined manually for each target plaque lesion. Finally, the number of new plaque lesions was assessed in patients with 2 evaluable scans including all 4 vessels in a semiquantitative analysis.

A total of 12 patients had their MDCT examinations evaluated twice by the same reviewer and also by a second reviewer for evaluation of intraobserver and interobserver variability of measurements. The intra-

Table 2. Concomitant Medications of Study Patients According to Treatment Group

	Placebo (n=52)	VIA-2291 25 mg (n=49)	VIA-2291 50 mg (n=46)	VIA-2291 100 mg (n=44)	Overall (n=191)
Any concomitant medications, n (%)	51 (98.1)	48 (98.0)	46 (100.0)	44 (100.0)	189 (99.0)
Platelet inhibitors, n (%)	51 (98.1)	48 (98.0)	46 (100.0)	43 (97.7)	188 (98.4)
Acetylsalicylic acid, n (%)	50 (96.2)	47 (95.9)	46 (100.0)	42 (95.5)	185 (96.9)
Clopidogrel, n (%)	47 (90.4)	43 (87.7)	44 (95.7)	42 (95.5)	176 (92.1)
HMG CoA reductase inhibitors, n (%)	48 (92.3)	46 (93.9)	46 (100.0)	44 (100.0)	184 (96.3)
Atorvastatin 80 mg, n (%)	10 (19.2)	10 (20.4)	9 (19.6)	5 (11.4)	34 (17.8)
No statin at time of ACS, n (%)	35 (67.3)	33 (67.3)	29 (63.0)	27 (61.4)	124 (64.9)
β-blockers, n (%)	48 (92.3)	36 (73.5)	39 (84.8)	40 (90.9)	163 (85.3)
ACE inhibitors, n (%)	35 (67.3)	32 (65.3)	27 (58.7)	29 (65.9)	123 (64.4)
Angiotensin II antagonists, n (%)	7 (13.5)	8 (16.3)	9 (19.6)	7 (15.9)	31 (16.2)

class coefficients for intraobserver variability of lumen and vessel volume measurements were 0.98 and 0.95, respectively. The corresponding values for interobserver variability were 0.94 and 0.97.

Statistical Methods

The assessment of 42 patients per group allows detection of a 35% difference (or greater) versus placebo in the 12-week change from baseline in serum LTB4, assuming an adjusted α level of 0.017 (2-sided) for the 3 VIA-2291 dose arms and a power of 90%. Because this was a proof-of-principle study, an evaluable patient data set constituted the primary analysis data set. An intent-to-treat analysis data set was also analyzed. The primary outcome measure was the change in ex vivo LTB4 synthesis in whole blood from baseline after 12 weeks of dosing; an analysis of covariance (ANCOVA) using the baseline level of whole blood LTB4 synthesis as a covariate and Dunnett method at an overall α level of 0.05 (2-sided) was used to assess significance. The secondary biomarker outcomes (change from baseline at 12 weeks) were analyzed using ANCOVA as described for the primary outcome. It was prespecified that either logarithmic transformations or nonparametric analyses would be used as appropriate. An exploratory repeated-measures analysis was performed for hs-CRP that included time, group, baseline value, and time×group interaction term as factors to further assess the changes at 12 and 24 weeks.

The changes from baseline on coronary CT scans after 24 weeks were analyzed through an ANCOVA model with baseline level of the CT outcome as covariate. The Dunnett test of each of the 3 doses of VIA-2291 compared with placebo was done at the 0.05 level of

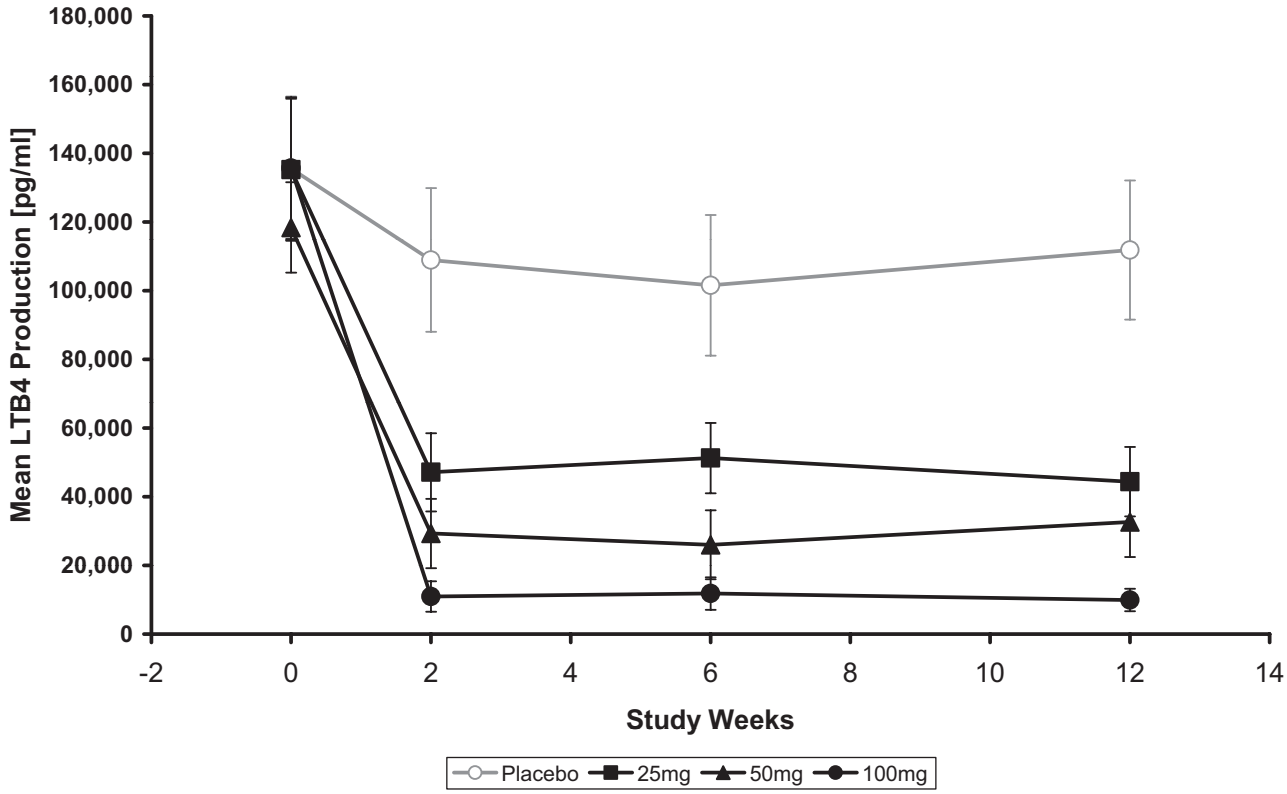

Figure 2. Whole blood stimulated LTB4 production by indicated dose over time. Stimulation as described in the Methods section occurred on a trough blood sample at indicated times after randomization. Error bars indicate 95% confidence intervals; $P<0.0001$ at all doses.

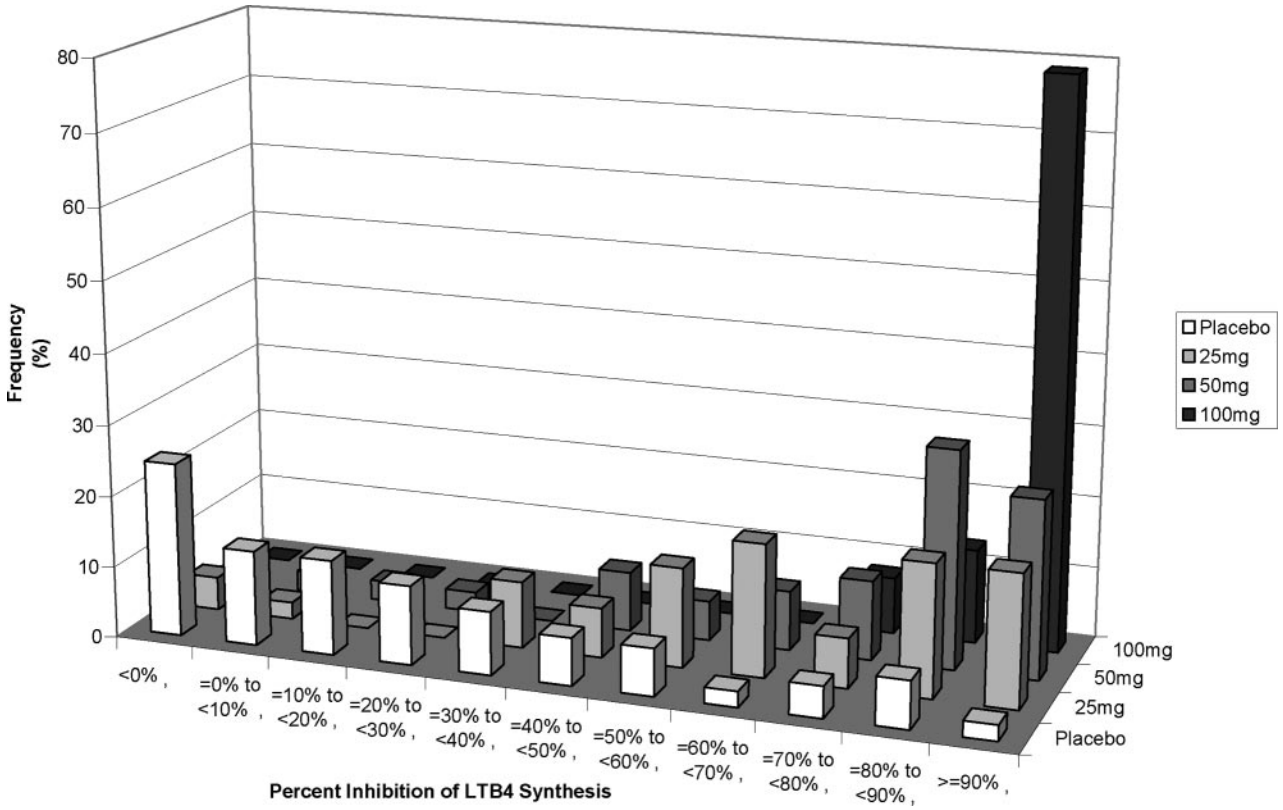

Figure 3. Percent LTB4 inhibition by dose at trough at 12 weeks. Percent inhibition was calculated based on baseline LTB4 value.

significance (2-sided). In addition, using the appropriate contrast under the ANCOVA model, the mean of the 3 active groups was compared with the mean of the placebo group. Frequencies and percentages of new lesions were presented within each treatment group and compared across groups using a χ^2 test. Sensitivity analyses that imputed missing data were undertaken on categorical end points, and groups (placebo versus VIA-2291) were compared based on imputed data by χ^2 tests.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Patient Disposition and Baseline Characteristics

One hundred ninety-one patients were enrolled into the trial (Figure 1), and 179 of them (93.7%) completed the main study protocol as planned. Of the 93 patients participating in the MDCT substudy, 90 (96.8%) completed the substudy as planned.

Baseline characteristics and concomitant medications were similar among treatment groups as shown in Tables 1 and 2. The clinical features of patients in the 12-week main study and the CT substudy were similar.

Inflammatory Biomarkers

Baseline values for ex vivo stimulated LTB4 were similar across all 4 patient groups (Figure 2). VIA-2291 inhibited LTB4, the primary study end point, in each of the VIA-2291 groups compared with placebo ($P<0.0001$). Significant reductions were observed at 2 weeks, the earliest time point assessed. The intent-to-treat population also showed a highly significant change from baseline at all doses ($P<0.0001$). A dose-related effect was observed, with >90% of the patients taking 100 mg VIA-2291 having at least 80% inhibition of LTB4 at trough drug concentrations (Figure 3).

Urine leukotriene levels were assessed as a measurement of systemic leukotriene production. Baseline values for urine LTE4 were similar across all patient groups. VIA-2291 decreased production of leukotrienes at all doses compared with placebo (25 mg, $P=0.0003$; 50 mg and 100 mg, $P<0.0001$), as depicted in Figure 4. Significant reductions of LTE4 were observed at 2 weeks, and a linear dose-response was seen ($P<0.0001$). LTB4 and LTE4 findings in the main 12-week study were replicated in the 24-week CT substudy (not shown).

Hs-CRP levels differed at baseline but decreased to 12 weeks in all groups (Table 3). The changes in hs-CRP in the VIA-2291 groups compared with placebo were not statistically significant. In a prespecified assessment, there was a 67% decrease in hs-CRP in the 100-mg group at 24 weeks, compared with placebo ($P=0.0002$, Table 3 and Figure 5). There was a significant reduction in hs-CRP within the VIA-2291 100-mg group between 12 and 24 weeks ($P<0.01$) and a significant increase in hs-CRP within the placebo group during the same period ($P<0.02$).

Exploratory Coronary CT Substudy

Of the 88 patients who qualified for the coronary CT substudy, 28 had poor or nondiagnostic examinations (motion or banding artifacts in 10, noise or misregistration in 9, poor contrast opacification in 3, and other artifacts such as stents or pacemaker wires in 6 patients), resulting in 60 compliant patients with acceptable CT scans both at baseline and 24

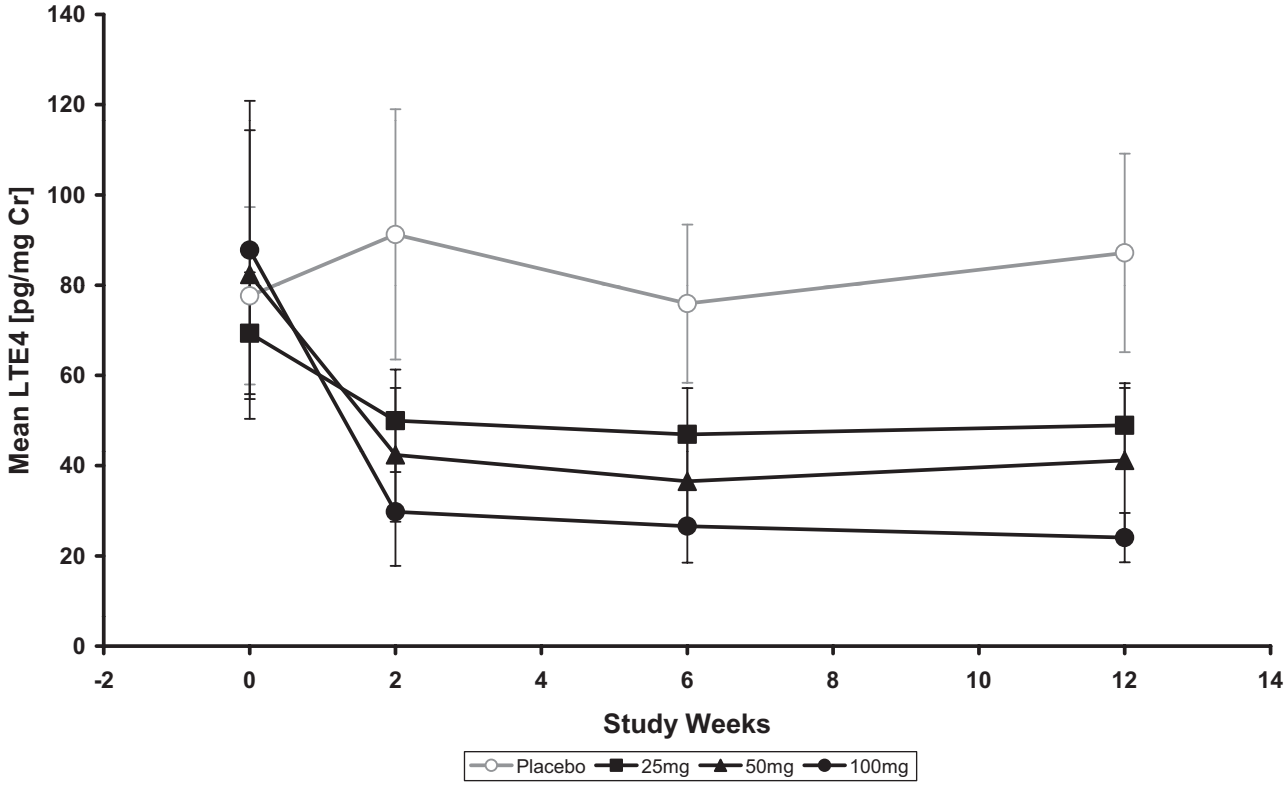

Figure 4. Urinary LTE4 production by dose over time. LTE4 as described in the Methods section was calculated at indicated times at trough drug level. Ninety-five percent confidence intervals are shown (25 mg, $P=0.0003$; 50 mg and 100 mg, $P<0.0001$ versus placebo).

weeks. Scans from 26 patients could not be analyzed quantitatively for plaque volume because only calcified or dense plaques were present, resulting in 34 patients who met eligibility criteria for the presence of ≥1 target (≤60 HU) atherosclerotic plaque or stenosis site. A summary of the quantitative imaging analysis is presented in Table 4. Although an average increase of 2.83 mm^3 in noncalcified plaque volume was observed in the placebo group, the average noncalcified plaque volume in all VIA-2291–treated patients decreased by 2.33 mm^3 or 4.9% ($P<0.01$, Figure 6A and Figure 7). The largest decrease in noncalcified plaque volume (5.6 mm^3 or 11.6%) was observed in patients treated with VIA-2291 50 mg ($P<0.005$ when compared with placebo). To extrapolate these results to the population of 60 patients who had serial imaging, we created an index in which patients with an increase in plaque volume (change >0 mm^3) or in the number of lesions were counted as "increasers" and patients with no increase in plaque volume (change ≤0 mm^3) and no change in lesion number were counted as "no change." When this index was used to compare placebo with VIA-2291 groups, more "no change" patients were in the active treatment groups ($P=0.003$).

There appeared to be a slight increase in mean plaque density in all study groups over 24 weeks. There was no significant change from baseline in mean plaque density or percent stenosis in any of the VIA-2291 groups when compared with placebo.

The overall number of lesions in all main coronary arteries was assessed at baseline and after 24 weeks of treatment in 60 patients (Table 5 and Figure 6B) in a post hoc analysis. In the placebo group, 5 of 18 patients (27.8%) showed an increased number of lesions, compared with only 2 of 42 patients (4.8%) in the VIA-2291 groups ($P=0.01$). A sensitivity analysis was done to extrapolate these results to the 88 patients who completed follow-up in the MDCT study by imputing data to the 28 patients without satisfactory serial images. We assumed that a similar proportion of patients developed new lesions in the placebo patients (n=9) and in the VIA-2291 patients (n=19) who did not have serial imaging as in the overall group of 60 patients with serial imaging (7 of 60, 11.7%). Under this assumption, the difference in the number of patients with new lesions between the placebo and VIA-2291 groups remained statistically significant ($P=0.033$) for the population of 88 patients.

Safety Data

VIA-2291 was generally well tolerated. The most common adverse events during treatment are listed in Table 6. Six subjects were discontinued for adverse events (1 placebo for lymphoma, 1 VIA-2291 25 mg for abnormal liver function tests, 3 in the 50-mg group including 1 for mild ALT elevation, and 1 in the 100-mg group for lightheadedness). During the extension study, 1 patient in the 25-mg group was discontinued for mild ALT elevation. No serious adverse events were considered by local investigators to be related to VIA-2291. All ALT elevations resolved after discontinuation of statins and/or VIA-2291 or while therapy was continued.

Table 3. High-Sensitivity CRP Results in the Main Study (12 Weeks) and MDCT Substudy (24 Weeks) According to Treatment Group

12-Week Main Population	Statistics	Placebo (n=48)	VIA-2291 25 mg (n=44)	VIA-2291 50 mg (n=38)	VIA-2291 100 mg (n=38)
Baseline	Median (25–75)	1.1 (0.4, 4.0)	1.5 (0.9, 2.5)	2.0 (0.5, 4.7)	0.7 (0.5, 2.6)
12 Weeks	Median (25–75)	0.7 (0.3, 2.0)	1.1 (0.6, 2.5)	1.3 (0.4, 2.7)	0.6 (0.3, 2.5)
Change from baseline*	Median (25–75)	−0.2 (−0.9, 0.1)	−0.2 (−1.1, 0.4)	−0.1 (−1.9, 0.1)	−0.3 (−0.8, 0.1)
	LSMEANS geometric mean (%)	−36.97%	−25.32%	−29.13%	−38.99%
	P value change from baseline within group	0.0002	0.0213	0.0119	0.0004
	P value (adj) vs placebo		0.6558	0.8627	0.9962
24-Week CT Substudy Population	**Statistics**	**Placebo (n=27)**	**VIA-2291 25 mg (n=23)**	**VIA-2291 50 mg (n=20)**	**VIA-2291 100 mg (n=18)**
Baseline	Median (25–75)	1.2 (0.4, 4.6)	1.9 (0.8, 3.3)	2.0 (0.4, 7.7)	0.9 (0.5, 4.1)
12 Weeks	Median (25–75)	0.7 (0.3, 2.3)	1.1 (0.6, 2.4)	1.7 (0.4, 4.3)	0.6 (0.4, 2.0)
12 Weeks change from baseline*	Median (25–75)	−0.1 (−0.7, 0.1)	−0.1 (−1.5, 0.8)	−0.1 (−5.0, 0.6)	−0.3 (−2.4, 0.1)
	LSMEANS geometric mean (%)	−35.82%	−24.82%	−22.71%	−39.05%
	P value change from baseline within group	0.0108	0.1185	0.1877	0.0178
	P value (adj) vs placebo		0.8688	0.8230	0.9953
24 Weeks	Median (25–75)	1.6 (0.5, 3.3)	1.2 (0.7, 2.1)	1.5 (0.6, 2.6)	0.3 (0.2, 0.9)
24 Weeks change from baseline*	Median (25–75)	0.0 (−0.5, 1.2)	−0.4 (−1.2, 0.3)	−0.2 (−3.7, 0.2)	−0.4 (−3.9, −0.2)
	LSMEANS geometric mean (%)	−4.19%	−32.91%	−27.05%	−67.16%
	P value change from baseline within group	0.7896	0.0271	0.1217	<0.0001
	P value (adj) vs placebo		0.3313	0.6060	0.0002

LSMEANS indicates least-squares means from ANCOVA model adjusted (adj) for baseline value.

*Change from baseline was calculated as postbaseline value minus baseline value for each patient who had both a baseline and a postbaseline value for the visit in question. Baseline is last nonmissing assessment before first dose of study drug; median (25 to 75) indicates median value 25th to 75th percentile. One patient in the 25-mg group did not take study drug within 6 days of the 24-week visit and was not included in the 24-week analysis.

P value (adj): Dunnett test of each of the 3 doses of VIA-2291 compared with placebo.

Discussion

In this study, the safety and efficacy of the potent specific 5-LO inhibitor VIA-2291 were evaluated in patients with a recent ACS event. VIA-2291 at all doses tested met the primary end point of significant inhibition of ex vivo stimulated whole blood LTB4 production as compared with placebo at trough drug levels. The inhibition was maximal at the earliest time assessed (2 weeks) and continued through 12 and 24 weeks. LTB4 inhibition was dose-related and exceeded 90% at 100 mg. Urinary leukotrienes (LTE4) were similarly significantly inhibited at all VIA-2291 doses tested. Although unchanged at 12 weeks in the main study, hs-CRP was reduced significantly, by 67%, in the VIA-2291 100-mg treatment group relative to placebo at 24 weeks among patients in the extension substudy. Our results suggest that more prolonged therapy (up to 24 weeks) with VIA-2291 may reduce ongoing inflammation in post-ACS patients.

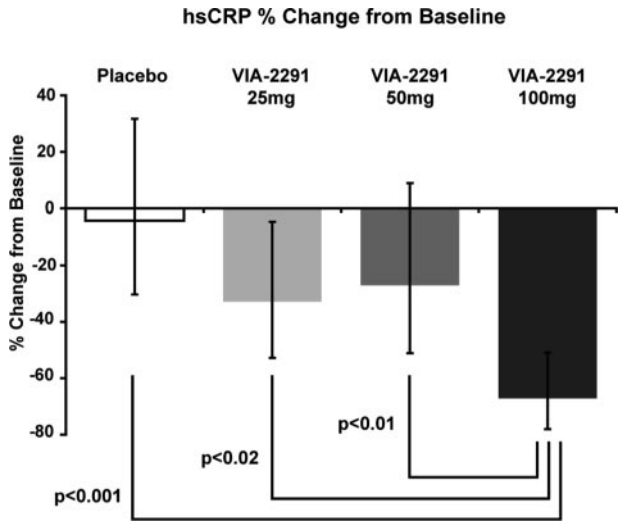

Figure 5. Hs-CRP percent change from baseline to 24 weeks in the CT substudy evaluable population based on log-transformed hs-CRP values. The significance indicated is based on the repeated analysis approach.

Role of Leukotrienes in Inflammation and Unstable Atherosclerotic Plaques

Evidence suggests that both LTB4 and cysteinyl leukotrienes (eg, LTE4) play a role in increasing inflammation in atherosclerotic plaque. High levels of LTB4 lead to migration of inflammatory cells into the plaque, and cysteinyl leukotrienes lead to increased vascular permeability through the vessel walls.[11] Studies in humans and rodents have demonstrated increased expression of 5-LO in atherosclerotic plaques, particularly in unstable lesions.[12,13,15] Moreover, human genetic studies have demonstrated a significant contribution of 5-LO polymorphisms in CAD.[10] Inhibition of the leukotriene pathway has been associated with a reduction in hs-CRP,[16]

Table 4. Coronary CT Image Analysis According to Treatment Group

	Placebo (n=12)	VIA-2291 25 mg (n=7)	VIA-2291 50 mg (n=8)	VIA-2291 100 mg (n=7)	All VIA-2291 Groups (n=22)
Average noncalcified plaque volume, mm^3					
Baseline	34.50±18.89	22.71±11.40	45.10±24.89	26.00±11.97	31.90±19.77
Follow-up	37.25±21.18	21.64±11.84	38.92±21.61	26.48±10.67	29.46±16.90
Percent change from baseline	6.03±17.91	−6.76±19.25	−11.61±10.55	4.79±18.00	−4.85±16.89
LSM* change from baseline (95% CI)	2.83 (−0.18 to 5.84)	−1.55 (−5.62 to 2.51)	−5.60 (−9.49 to −1.71)	0.15 (−3.84 to 4.14)	−2.33 (−4.56 to −0.10)
P value vs placebo†		0.22	<0.005	0.60	<0.01
Average mean plaque density					
Baseline	68.54±18.74	69.47±25.45	62.09±22.14	64.36±20.43	65.16±21.86
Follow-up	79.86±32.76	87.02±26.82	71.62±29.03	77.58±17.83	78.42±24.89
LSM* change from baseline (95% CI)	12.42 (−3.32 to 28.17)	19.11 (−1.51 to 39.74)	7.39 (−11.96 to 26.74)	12.22 (−8.38 to 32.82)	12.91 (1.27 to 24.55)
P value vs placebo†		0.92	0.96	1.00	0.96
Average percent stenosis (%)					
Baseline	32.92±12.08	27.57±11.25	40.94±29.08	30.43±7.41	33.34±19.23
Follow-up	34.17±8.97	28.79±6.90	50.56±29.92	33.44±11.02	38.19±21.03
LSM* change from baseline (95% CI)	1.19 (−4.95 to 7.32)	−0.11 (−8.24 to 8.03)	11.45 (3.73 to 19.17)	2.36 (−5.70 to 10.42)	4.57 (0.03 to 9.11)
P value vs placebo†		0.99	0.11	0.99	0.37

Values are mean±SD, unless indicated otherwise.
*LSM indicates least-squares means from ANCOVA model adjusted for baseline value.
†Dunnett test of each of the 3 doses of VIA-2291 compared with placebo.

and one of its products, LTB4, has been shown to be a regulator of interleukin-6, a known modulator of hs-CRP,[17] providing plausible mechanistic explanation for the favorable hs-CRP result that was observed at 24 weeks in the current study. This finding must be confirmed in future trials because it was not seen at 12 weeks in the main study.

Effects of VIA-2291 on Coronary Atherosclerotic Plaques

Coronary CT allows noninvasive visualization of the coronaries and thus assessment of coronary atherosclerotic lesions. A good correlation exists between 64-slice CT and invasive measurements with angiography and intravascular ultrasound for overall plaque volume and stenosis measurements.[18] CT may have lesser ability to assess meaningful variations in plaque density.[19]

To our knowledge, our study is one of the first to use serial contrast-enhanced CT measurements to directly assess drug effects on coronary plaques. A statistically significant decrease in noncalcified coronary plaque volume was observed in the combined VIA-2291–treated groups when compared with placebo. These noncalcified plaques have been shown to be the type most likely to rupture and cause an ACS both in retrospective[20] and prospective studies.[21] A reduction in noncalcified plaque volume might reasonably be expected to reduce subsequent coronary events; however, this supposition remains to be proven. Only 34 of the 60 patients (57%) in this study with evaluable MDCT images had noncalcified plaques seen by MDCT. Whether a drug that favorably affects noncalcified plaques is of any benefit to ACS patients without this type of plaque is not known.

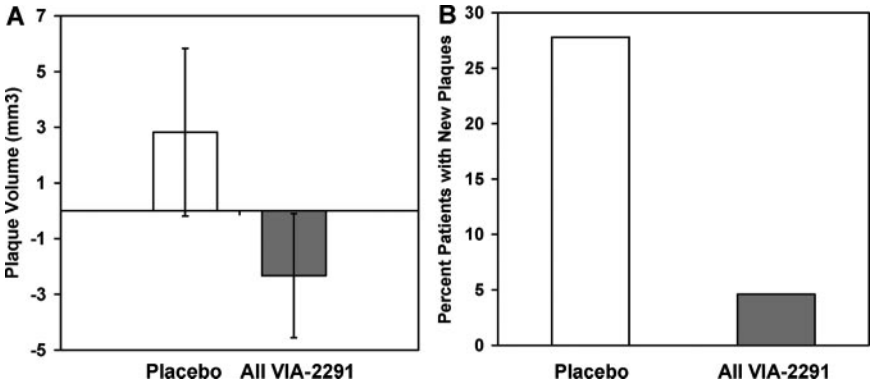

Figure 6. Change in noncalcified plaque volume (A, $P<0.01$) and percentage of patients with new plaque lesions (B, $P=0.01$) on serial coronary CT scans.

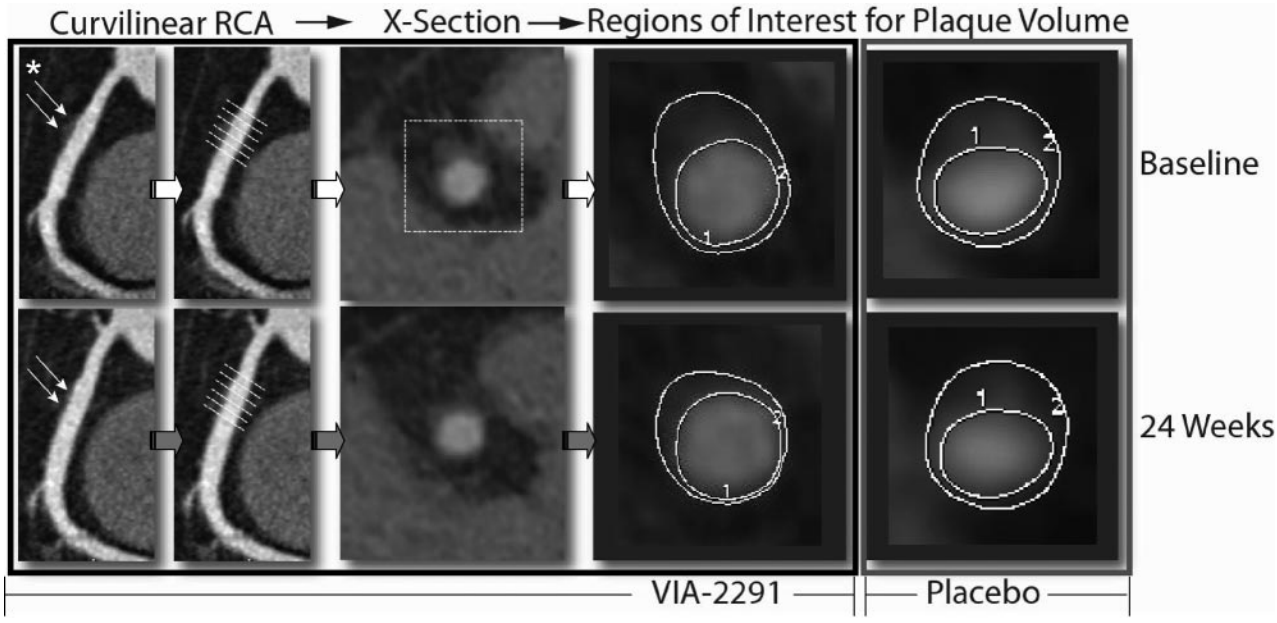

Figure 7. Representative low-density plaque serial measurements of noncalcified plaque volume demonstrating method of measuring plaque volume at baseline and 24-week scans.

Among the 60 patients with evaluable MDCT images, new lesions developed during the 24-week interval between MDCT studies in only 2 of 42 VIA-2291–treated patients compared with 5 of 18 placebo-treated patients (4.8% versus 27.8%, $P=0.01$). This finding supports the notion that any benefit of treatment may not be restricted to the subset of ACS patients with noncalcified plaques. Taken together, these preliminary data from the CT substudy suggest that such reduction in leukotriene production may influence atherosclerosis; however, this requires confirmation in a larger study.

Study Limitations

A significant limitation of the current study is that relatively small numbers of patients were studied to assess the effects on inflammatory biomarkers and coronary plaques. The reduction in hs-CRP at 24 weeks of treatment should be viewed as a preliminary finding. Even in individuals with stable coronary disease, hs-CRP levels vary considerably over time,[22] and the elevated levels that are seen after an ACS decrease both over time and with statin therapy.[23] Assessing the effect of an intervention under these changing conditions is difficult, and these results must be confirmed in a larger trial.

Table 5. Patients With Change in No. of Lesions on CT According to Treatment Group

	Placebo (n=18)	VIA-2291 25 mg (n=14)	VIA-2291 50 mg (n=15)	VIA-2291 100 mg (n=13)	All VIA-2291 Groups (n=42)
Patients with increase in No. of lesions, n (%)	5 (27.8)	1 (7.1)	0 (0)	1 (7.7)	2 (4.8)*
Patients with no change in No. of lesions, n (%)	13 (72.2)	13 (92.9)	15 (100)	12 (92.3)	40 (95.2)

*$P=0.01$ vs placebo.

In addition, this is one of the first studies using coronary CT to evaluate the effects of an antiatherogenic medication. The findings with respect to change in number of lesions and change in noncalcified plaque volume should be interpreted

Table 6. Adverse Events Among Patients Assigned to Placebo and VIA-2291

	Placebo (n=52) n (%)	VIA-2291 25 mg (n=49) n (%)	VIA-2291 50 mg (n=46) n (%)	VIA-2291 100 mg (n=44) n (%)	VIA-2291 Total (n=139) n (%)
Angina pectoris	5 (9.6)	3 (6.1)	8 (17.4)	7 (15.9)	18 (12.9)
Noncardiac chest pain	4 (7.7)	5 (10.2)	3 (6.5)	4 (9.1)	12 (8.6)
Extremity pain	4 (7.7)	7 (14.3)	4 (8.7)	5 (11.4)	16 (11.5)
Headache	6 (11.5)	5 (10.2)	5 (10.9)	5 (11.4)	15 (10.8)
Fatigue	5 (9.6)	4 (8.2)	6 (13.0)	7 (15.9)	17 (12.2)
Dizziness	5 (9.6)	1 (2.0)	3 (6.5)	7 (15.9)	11 (7.9)
Epistaxis	2 (3.8)	1 (2.0)	3 (6.5)	7 (15.9)	11 (7.9)
Contusions	7 (13.5)	5 (10.2)	2 (4.3)	4 (9.1)	11 (7.9)
Back pain	3 (5.8)	3 (6.1)	5 (10.9)	0	8 (5.8)
Increased ALT	3 (5.8)	5 (10.2)	1 (2.2)	1 (2.3)	7 (5.0)
ALT ≥3×ULN	1 (1.9)	5 (10.2)	0	0	5 (3.6)
Nasopharyngitis	4 (7.7)	1 (2.0)	5 (10.9)	0	6 (4.3)
Dyspnea	2 (3.8)	1 (2.0)	5 (10.9)	1 (2.3)	7 (5.0)
Hb decrease ≥1 g/dL	3 (5.8)	5 (10.2)	3 (6.5)	4 (9.1)	12 (8.6)
Patients with any SAE	5 (9.6)	4 (8.2)	3 (6.5)	7 (15.9)	14 (10.1)
Study drug-related SAE	0	0	0	0	0

ULN indicates upper limit of normal; ALT, alanine aminotransferase; Hb, hemoglobin; and SAE, serious adverse event.

cautiously because many patients were excluded from this analysis. Logistical reasons were the main determinant of which patients entered the MDCT substudy, and neither their selection nor the exclusions for technical reasons are likely to be affected by important biases. ACS patients with noncalcified plaques may be different than those without, and the results in the former groups might not be applicable to the latter. The ultimate significance of leukotriene reduction, hs-CRP lowering by 5-LO inhibition, or changes in atherosclerotic plaque imaging can be demonstrated only in a large patient outcomes study in which vascular events are assessed in patients treated with VIA-2291.

Sources of Funding

The study was funded by VIA Pharmaceuticals.

Disclosures

Dr Tardif holds the Canadian Institutes of Health Research and Pfizer chair in atherosclerosis.

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CLINICAL PERSPECTIVE

Acute coronary syndromes are caused by rupture of an atherosclerotic plaque in a coronary artery, and inflammation is thought to play a major role in plaque instability. Leukotrienes are a class of proinflammatory signaling molecules involved in arterial wall inflammation, and 5-lipoxygenase is a key regulatory enzyme in the biosynthesis of leukotrienes. This double-blinded, randomized study examined the safety and efficacy of the 5-lipoxygenase inhibitor VIA-2291 in 191 patients treated for 12 weeks after an acute coronary syndrome. The primary study end point, whole blood stimulated leukotriene LTB4 at trough drug level, was reduced at all 3 VIA-2291 dose levels, with approximately 80% inhibition in >90% of patients in the highest dose group (100 mg daily). No serious adverse events were considered related to study drug. A subset of patients underwent a 64-slice coronary CT scan at baseline and after 24 weeks of treatment. New coronary plaques were observed in 5 of 18 (27.8%) placebo patients and 2 of 42 (4.8%) VIA-2291–treated patients ($P=0.01$). A reduction in noncalcified plaque volume at 24 weeks was observed in VIA-2291–treated groups in the 34 of these 60 patients in whom this end point was analyzable ($P<0.01$). We conclude that VIA-2291 reduces leukotriene production at 12 weeks after an acute coronary syndrome. Preliminary data from the CT substudy suggest that such a reduction in leukotriene production may have a favorable effect on atherosclerosis at 24 weeks; however, this requires confirmation in a larger study.