The Effect of Darapladib on Plasma Lipoprotein-Associated Phospholipase A2 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

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Objectives
This study examined the effects of darapladib, a selective lipoprotein-associated phospholipase A2 (Lp-PLA2) inhibitor, on biomarkers of cardiovascular (CV) risk.

Background
Elevated Lp-PLA2 levels are associated with an increased risk of CV events.

Methods
Coronary heart disease (CHD) and CHD-risk equivalent patients (n = 959) receiving atorvastatin (20 or 80 mg) were randomized to oral darapladib 40 mg, 80 mg, 160 mg, or placebo once daily for 12 weeks. Blood samples were analyzed for Lp-PLA2 activity and other biomarkers.

Results
Baseline low-density lipoprotein cholesterol (LDL-C) was 67 ± 22 mg/dl. Plasma Lp-PLA2 was higher in older patients (≥75 years), in men, in those taking atorvastatin 20 mg, at LDL-C ≥70 mg/dl or high-density lipoprotein cholesterol (HDL-C) <40 mg/dl, or in those with documented vascular disease (multivariate regression; p < 0.01). Darapladib 40, 80, and 160 mg inhibited Lp-PLA2 activity by approximately 43%, 55%, and 66% compared with placebo (p < 0.001 weeks 4 and 12). Sustained dose-dependent inhibition was noted overall in both atorvastatin groups and at different baseline LDL-C (≥70 vs. <70 mg/dl) and HDL-C (<40 vs. ≥40 mg/dl). At 12 weeks, darapladib 160 mg decreased interleukin (IL)-6 by 12.3% (95% confidence interval [CI] −22% to −1%: p = 0.028) and high-sensitivity C-reactive protein (hs-CRP) by 13.0% (95% CI −28% to +5%; p = 0.15) compared with placebo. The Lp-PLA2 inhibition produced no detrimental effects on platelet biomarkers (P-selectin, CD40 ligand, urinary 11-dehydrothromboxane B2). No major safety concerns were noted.

Conclusions
Darapladib produced sustained inhibition of plasma Lp-PLA2 activity in patients receiving intensive atorvastatin therapy. Changes in IL-6 and hs-CRP after 12 weeks of darapladib 160 mg suggest a possible reduction in inflammatory burden. Further studies will determine whether Lp-PLA2 inhibition is associated with favorable effects on CV events. (SB-480848 in Subjects With Coronary Heart Disease; NCT00269048) (J Am Coll Cardiol 2008;51:1632–41) © 2008 by the American College of Cardiology Foundation

Residual risk of cardiovascular (CV) events persists even in patients with aggressively controlled risk factors (1). These findings suggest the presence of additional modifiable mechanisms of the underlying atherosclerotic process. To this end, atherosclerosis initiation, its progression, and the transition to acute coronary syndromes (e.g., due to plaque

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rupture) are accompanied by focal inflammation in lesion-prone areas (2). In addition, elevated levels of circulating inflammatory biomarkers have been reported in several populations at risk (3,4). Recent clinical trials further implied that achieving lower levels of high-sensitivity C-reactive protein (hs-CRP) in conjunction with aggressive low-density lipoprotein cholesterol (LDL-C)-lowering (<70 mg/dl) are associated with reduced progression of coronary atheroma and fewer CV events (5,6). Nevertheless, statin trials offer only indirect evidence of the link between inflammation and clinical events because of the primary benefit of concomitant lipid-lowering (7). Other treatments that are proven to reduce CV events lack consistent anti-inflammatory effects in the clinical setting (e.g., aspirin or angiotensin-converting enzyme inhibitors) (8). Thus, the causal role of inflammation in the progression of atherosclerotic vascular disease and CV events remains to be proven by pharmacologic interventions that specifically target the inflammatory cascade.

Lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}) is an emerging biomarker of CV risk that is pharmacologically modifiable and therefore well positioned to address novel mechanisms of atherosclerotic vascular disease. Lipoprotein-associated phospholipase A\textsubscript{2} is produced by inflammatory cells involved in atherogenesis (macrophages, T cells, mast cells), is predominantly bound to atherogenic lipoproteins, and accumulates in human atherosclerotic lesions (9). Although this enzyme has been referred to as platelet-activating factor-acetylhydrolase (PAF-AH), Lp-PLA\textsubscript{2} exhibits much broader substrate specificity (10,11). In particular, Lp-PLA\textsubscript{2} rapidly degrades polar phospholipids present in oxidized LDL-C, releasing downstream products such as lysophosphatidylcholine species and oxidized nonesterified fatty acids (12). These products of the Lp-PLA\textsubscript{2} reaction exhibit a wide range of pro-inflammatory and pro-apoptotic effects in experimental settings (9). In this context, Lp-PLA\textsubscript{2} could be proposed as the enzyme that links oxidized LDL-C with atherosclerosis progression and plaque vulnerability. This hypothesis is supported by studies that suggest increased risk of CV events with elevated levels of circulating Lp-PLA\textsubscript{2}, the presence of high expression of Lp-PLA\textsubscript{2} in apoptotic macrophages in high-risk human coronary lesions, and the ability to alter the phenotype of inflammatory cells with Lp-PLA\textsubscript{2} inhibition in vitro (13-18). The view of Lp-PLA\textsubscript{2} as a pro-atherogenic enzyme, however, has not been uniformly accepted, because of contradictory results of Lp-PLA\textsubscript{2} overexpression in preclinical models of atherosclerosis, a theoretical concern that inhibition of Lp-PLA\textsubscript{2} might lead to increase in platelet activation, and conflicting data with a genetic variant of Lp-PLA\textsubscript{2} in Japanese subjects (9,19).

In this study, we sought to investigate whether darapladib (SB-480848), a selective Lp-PLA\textsubscript{2} inhibitor, produces sustained inhibition of plasma Lp-PLA\textsubscript{2} activity in CV patients treated aggressively with atorvastatin. In addition, the effects of darapladib on several CV biomarkers of risk and safety were examined.

**Methods**

**Study design.** The multicenter, randomized, double-blind, placebo-controlled, parallel-group study that was conducted in 110 sites in 15 countries from November 2005 to June 2006 (a list of investigators is provided in the Online Appendix). This dose-ranging study evaluated the ability of darapladib to produce sustained inhibition of plasma Lp-PLA\textsubscript{2} activity in subjects with stable coronary heart disease (CHD) or CHD-risk equivalent receiving concomitant atorvastatin therapy. Those subjects not currently receiving statin therapy first received open-label atorvastatin 20 mg for 2 weeks. All eligible subjects were initially randomized to double-blind atorvastatin 20 or 80 mg once daily (Fig. 1). After 4 weeks, subjects who tolerated atorvastatin therapy and achieved LDL-C levels of ≤115 mg/dl were then randomized to concomitant administration of darapladib 40 mg, 80 mg, 160 mg, or placebo once daily for 12 weeks. After discontinuing darapladib or placebo, subjects continued on double-blind atorvastatin for an additional 2 weeks. Ethics committees approved the protocol, and all subjects provided written informed consent before enrollment in the study.

**Study subjects.** Subjects ages 18 to 80 years with stable CHD or CHD-risk equivalent (defined as diabetes mellitus requiring hypoglycemic medication; carotid stenosis >50%; prior carotid surgery or stenting; peripheral arterial disease; or a cluster of risk factors resulting in 10-year risk for coronary events >20% according to Framingham Risk Score) were eligible for screening. Main exclusion criteria were CV event or vascular procedure within the preceding 6 months, contraindications to double-blind atorvastatin therapy, serum triglycerides >400 mg/dl, elevated liver function tests (alanine transaminase, aspartate transaminase, alkaline phosphatase, or total bilirubin >1.5 × ULN), hemoglobin subtype A1c >10%, chronic inflammatory diseases, inadequately controlled hypertension (>160 mm Hg systolic or >100 mm Hg diastolic), renal disorder (serum creatinine >2.5 mg/dl), severe congestive heart failure (New York Heart Association functional class III or IV), and prolonged QTc interval (>440 ms for men or >450 ms for women).

**Assessments.** Blood assessments were carried out in the fasting state at baseline (before randomization to darapladib or placebo) and at 4 and 12 weeks of dosing with darapladib or placebo. These included total cholesterol, high-density lipoprotein-cholesterol (HDL-C), calculated LDL-C, tri-
glycerides, and routine safety labs. In addition, plasma Lp-PLA2 activity, inflammatory biomarkers, and platelet-related biomarkers were measured at baseline and at 4 and 12 weeks approximately 24 h after dosing with darapladib or placebo. Plasma Lp-PLA2 activity and platelet-related biomarkers were also measured 2 weeks after discontinuing darapladib or placebo. Supine vital signs were collected at baseline, 4, 8, and 12 weeks, and 2 weeks after discontinuing darapladib or placebo; a physical examination and electrocardiogram were also performed at baseline and at 4 and 12 weeks.

**Biochemical parameters.** The Lp-PLA2 activity was measured in plasma by a colorimetric assay as previously described (20). The assay characteristics included intra-assay precision of 1.7% and inter-assay precision of 4.8%. In a subset of subjects, Lp-PLA2 activity also was measured by a radiometric assay with [3H]-platelet-activating factor as a substrate (21). The assay (Quest Diagnostics, Lyndhurst, New Jersey) included intra-assay precision: low 3.0%, mid 4.5%, high 2.2% and interassay precision: low 6.5%, mid 7.4%, high 8.8%. Details regarding the analysis of the remaining biomarkers are provided online.

**Statistical analyses.** The primary efficacy end point was sustained inhibition of plasma Lp-PLA2 activity measured as the change from 4 to 12 weeks at trough levels of darapladib. The primary comparison was based on 2-sided 95% confidence intervals (CIs) for the change in log-transformed Lp-PLA2 activity from week 4 to week 12 for each darapladib group. Sample size was calculated with an SD for change in log-transformed Lp-PLA2 activity values of 0.34 from a previous study, an equivalence limit of 0.883 to 1.132 (equivalent to ± 0.124 on the log scale), and 90% power. With these assumptions, 196 subjects/group were required, without adjustment for multiplicity or atorvastatin treatment group. Thus, assuming a 15% dropout rate, a minimum of 920 subjects (or 230/treatment group) were planned to be randomized.

Secondary end points included dose-response of darapladib over log-transformed Lp–PLA2 activity and changes in inflammatory biomarkers and platelet-related biomarkers. Primary and secondary end points of changes in Lp-PLA2 activity and inflammatory and platelet-related biomarkers were all assessed with analysis of covariance. Treatment group and atorvastatin doses (20 and 80 mg) were included in all statistical models, with baseline values also included for inflammatory and platelet-related biomarkers. Interactions for atorvastatin level by treatment group were tested at the 10% significance level. Correlations were assessed with Pearson product moment correlation coefficients.

**Results**

**Study population and baseline characteristics.** Figure 1 depicts subject flow through the study. A total of 1,410 subjects were screened for eligibility, 1,082 were assigned to double-blind atorvastatin, and 964 subjects were subsequently randomized to darapladib or placebo. Five of these subjects did not receive any dose of darapladib or placebo; therefore 959 subjects were included in the analyses. The exposure to darapladib or placebo was 81 ± 13 days (mean ± SD).

Baseline characteristics of the subject population are presented in Table 1. At study entry, 75% of subjects were
Levels of Lp-PLA2 activity were significantly higher in older subjects compared to younger subjects. Among subgroups of subjects randomized to placebo or the darapladib 40, 80, or 160 mg, the observed inhibition of Lp-PLA2 activity was sustained at approximately 43%, 55%, and 66% for darapladib 40, 80, and 160 mg, respectively. This inhibition corresponded to achieved levels of Lp-PLA2 activity (nmol/min/ml) at 12 weeks as follows: placebo: 124 (95% CI 120 to 128), darapladib 40 mg: 68 (95% CI 65 to 71), darapladib 80 mg: 56 (95% CI 53 to 60), and darapladib 160 mg: 43 (95% CI 40 to 45). After darapladib discontinuation (15 ± 4 days), levels of Lp-PLA2 activity returned toward baseline although they remained lower. In a subset of subjects (n = 323), the enzyme inhibition was confirmed with radiometric assay of Lp-PLA2 activity that reproduced the shape of dose response. As shown in Online Figure 1, excellent Pearson correlations between the colorimetric and radiometric assays were found for the percent inhibition from baseline to week 12 (r = 0.97, p < 0.0001) and between achieved levels of Lp-PLA2 activity at week 12 (r = 0.96, p < 0.0001). Lipoprotein-associated phospholipase A2 mass was also measured in a subset of subjects (n = 228). Darapladib reduced Lp-PLA2 mass at week 12 compared with placebo (p < 0.001 for all doses) but without a dose response (9.6%, 12.9%, and 9.3% reductions by darapladib 40, 80, and 160 mg, respectively).

**Lipid measurements and the effects of darapladib.** Treatment with darapladib did not modify total cholesterol, LDL-C, HDL-C, or triglyceride levels at week 4 (not shown) or at week 12 as compared with placebo (Table 3). Hence, the observed changes in Lp-PLA2 activity levels in subjects taking darapladib cannot be attributed to changes in lipoprotein carriers of this enzyme.

We explored whether atorvastatin dose modified the effect of darapladib on change in Lp-PLA2 activity at week 12 and found no significant interaction (p = 0.60). We also explored whether baseline LDL-C or HDL-C levels influenced inhibition of Lp-PLA2 activity over 12 weeks. There was no interaction between baseline LDL-C (<70 mg/dl vs. ≥70 mg/dl) or HDL-C (<40 mg/dl vs. ≥40 mg/dl) and the change in Lp-PLA2 activity at 12 weeks for all darapladib doses (p = 0.12 and p = 0.13, respectively). Within the darapladib 160 mg group, achieved levels of Lp-PLA2 activity were similar at 12 weeks at different levels of dose.
baseline LDL-C (<70 mg/dl [n = 94] vs. ≥70 mg/dl [n = 51]: 42 nmol/min/ml [95% CI 39 to 45 nmol/min/ml] vs. 42 nmol/min/ml [95% CI 38 to 48 nmol/min/ml]; p = NS). Similarly, at different levels of baseline HDL-C, 160 mg dose of darapladib produced similar achieved levels of Lp-PLA2 activity (<40 mg/dl [n = 110] vs. <40 mg/dl [n = 35]: 40 nmol/min/ml [95% CI 38 to 44 nmol/min/ml] vs. 48 nmol/min/ml [95% CI 41 to 55 nmol/min/ml]; p = NS).

**Effects of darapladib on biomarkers of CV risk.** At baseline, hs-CRP was low (geometric mean 1.17 mg/l [interquartile range 0.6 to 2.5]), owing to intensive background atorvastatin therapy and stable CV status of the enrolled subject population. Nonetheless, treatment with darapladib 160 mg (n = 161) produced a 20.2% (95% CI −31% to −8%) reduction in hs-CRP at week 12 compared with baseline (p = 0.003; within group comparison) and a 13.0% (95% CI −18% to +5%) reduction from baseline compared with placebo (p = 0.15; between groups comparison) (Table 4). When response to darapladib was analyzed post hoc according to quartiles of baseline Lp-PLA2 activity, darapladib 160 mg (n = 38) significantly reduced hs-CRP in the highest quartile (reduction of 43.2% [95% CI 64% to 11%] relative to placebo [n = 43] from 1.17 to 0.79 mg/l in the darapladib 160 mg group vs. 1.26 to 1.45 mg/l in the placebo group, p = 0.013).

In regard to interleukin-6 (IL-6), the greatest reduction was again observed with darapladib 160 mg (n = 150) resulting in 21.5% (95% CI −28% to −14%) reduction from baseline (p < 0.001; within group comparison) and 12.3% (95% CI −22% to −1%) when compared with placebo (p = 0.028; between groups comparison) (Table 4). When response to darapladib was analyzed post hoc according to quartiles of baseline Lp-PLA2 activity, darapladib 160 mg (n = 39) reduced IL-6 in the highest quartile by 20.5% (95% CI 38% to 3%) compared with placebo (n = 42) from 2.67 to 2.12 ng/l versus 2.56 to 2.62 ng/l (p = 0.08). These findings are not likely attributable to treatment-induced changes in body weight, because it remained stable over the course of the study. There were no significant changes in plasma myeloperoxidase and matrix metalloproteinase-9 in any treatment groups (Table 4).

Theoretical concern that inhibition of Lp-PLA2 activity might adversely affect platelet activity prompted us to...
Baseline and week 12 data are presented as geometric means. Within group comparisons depict changes from baseline. Between group comparisons indicate changes from baseline relative to placebo. Within group comparisons were analyzed with analysis of covariance with baseline value, atorvastatin level, and treatment included as covariates. Values in parenthesis are 95% CI. *p < 0.05; †p < 0.005.

Abbreviations as in Tables 1 and 2.

Table 3  Effect of Darapladib on Lipid Parameters

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Darapladib 40 mg</th>
<th>Darapladib 80 mg</th>
<th>Darapladib 160 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>n = 184</td>
<td>n = 167</td>
<td>n = 164</td>
<td>n = 162</td>
</tr>
<tr>
<td>Baseline</td>
<td>145 ± 28</td>
<td>141 ± 28</td>
<td>143 ± 31</td>
<td>140 ± 27</td>
</tr>
<tr>
<td>12 weeks</td>
<td>144 ± 28</td>
<td>143 ± 32</td>
<td>142 ± 31</td>
<td>141 ± 28</td>
</tr>
<tr>
<td>Within group comparison</td>
<td>-0.7 (−3.2 to 1.8)</td>
<td>+1.9 (−2.2 to 5.9)</td>
<td>-1.3 (−5.1 to 2.6)</td>
<td>+1.3 (−1.9 to 4.5)</td>
</tr>
<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>+1.6 (−2.8 to 6.1)</td>
<td>-0.6 (−5.1 to 3.9)</td>
<td>+0.9 (−3.6 to 5.3)</td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td>n = 181</td>
<td>n = 166</td>
<td>n = 159</td>
<td>n = 160</td>
</tr>
<tr>
<td>Baseline</td>
<td>69 ± 23</td>
<td>67 ± 21</td>
<td>68 ± 21</td>
<td>67 ± 21</td>
</tr>
<tr>
<td>12 weeks</td>
<td>66 ± 23</td>
<td>65 ± 25</td>
<td>66 ± 23</td>
<td>64 ± 22</td>
</tr>
<tr>
<td>Within group comparison</td>
<td>-3.2 (−5.2 to −1.2)*</td>
<td>-2.6 (−5.8 to −0.7)</td>
<td>-2.6 (−5.4 to 0.2)</td>
<td>-2.4 (−5.1 to 0.3)</td>
</tr>
<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>+0.1 (−3.4 to 3.6)</td>
<td>+0.6 (−3.0 to 4.1)</td>
<td>+0.2 (−3.3 to 3.8)</td>
</tr>
<tr>
<td><strong>HDL-C</strong></td>
<td>n = 183</td>
<td>n = 167</td>
<td>n = 164</td>
<td>n = 162</td>
</tr>
<tr>
<td>Baseline</td>
<td>50 ± 13</td>
<td>50 ± 13</td>
<td>49 ± 13</td>
<td>49 ± 13</td>
</tr>
<tr>
<td>12 weeks</td>
<td>50 ± 12</td>
<td>51 ± 12</td>
<td>48 ± 12</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>Within group comparison</td>
<td>+0.04 (−1.0 to +1.1)</td>
<td>+0.9 (−0.2 to +1.9)</td>
<td>-0.2 (−1.3 to +1.0)</td>
<td>+1.4 (0.3 to +2.5)*</td>
</tr>
<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>+0.9 (−0.5 to +2.2)</td>
<td>-0.6 (−2.0 to +0.7)</td>
<td>+1.0 (0.3 to +2.4)</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>n = 184</td>
<td>n = 167</td>
<td>n = 164</td>
<td>n = 162</td>
</tr>
<tr>
<td>Baseline</td>
<td>127 ± 61</td>
<td>118 ± 48</td>
<td>135 ± 80</td>
<td>126 ± 70</td>
</tr>
<tr>
<td>12 weeks</td>
<td>142 ± 65</td>
<td>137 ± 67</td>
<td>145 ± 98</td>
<td>137 ± 73</td>
</tr>
<tr>
<td>Within group comparison</td>
<td>+14.2 (+7.3 to +21.2)*</td>
<td>+18.8 (+11.4 to +26.3)*</td>
<td>+10.5 (+3.2 to +24.2)</td>
<td>+10.9 (+2.0 to +19.8)*</td>
</tr>
<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>2.0 (−10.5 to +14.5)</td>
<td>−1.6 (−14.2 to +11.0)</td>
<td>−3.7 (−16.4 to +8.8)</td>
</tr>
</tbody>
</table>

Baseline and week 12 data are presented as means ± SD (mg/dl). Within group comparisons depict changes from baseline. Between group comparisons indicate changes from baseline relative to placebo. Within group comparisons (mg/dl) mean and 95% CI were analyzed using paired t test. Between group comparisons (mg/dl) mean and 95% CI were analyzed with analysis of covariance with baseline value, atorvastatin level, and treatment included as covariates. All subjects on concomitant atorvastatin 20 or 80 mg. Similar results were obtained comparing week 4 of treatment. †p < 0.005.

Abbreviations as in Tables 1 and 2.

Table 4  Effect of Darapladib on Inflammatory Biomarkers

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Darapladib 40 mg</th>
<th>Darapladib 80 mg</th>
<th>Darapladib 160 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hs-CRP (mg/l)</strong></td>
<td>n = 177</td>
<td>n = 166</td>
<td>n = 161</td>
<td>n = 161</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.09</td>
<td>1.14</td>
<td>1.03</td>
<td>1.28</td>
</tr>
<tr>
<td>12 weeks</td>
<td>1.06</td>
<td>1.02</td>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td>Within group comparison</td>
<td>-3.3 (−15 to +10)</td>
<td>-10.5 (−22 to +3)</td>
<td>-1.5 (−17 to +17)</td>
<td>-20.2 (−31 to −8)*</td>
</tr>
<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>-6.0 (−22 to +13)</td>
<td>-0.3 (−18 to +20)</td>
<td>-13.0 (−18 to +5)</td>
</tr>
<tr>
<td><strong>IL-6 (ng/l)</strong></td>
<td>n = 171</td>
<td>n = 149</td>
<td>n = 146</td>
<td>n = 150</td>
</tr>
<tr>
<td>Baseline</td>
<td>2.45</td>
<td>2.57</td>
<td>2.35</td>
<td>2.73</td>
</tr>
<tr>
<td>12 weeks</td>
<td>2.28</td>
<td>2.17</td>
<td>2.17</td>
<td>2.14</td>
</tr>
<tr>
<td>Within group comparison</td>
<td>-6.9 (−15 to +2)</td>
<td>-15.5 (−23 to −7)*</td>
<td>-7.3 (−16 to +2)</td>
<td>-21.5 (−28 to −14)*</td>
</tr>
<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>-7.8 (−18 to −4)</td>
<td>-2.3 (−12 to +10)</td>
<td>-12.3 (−22 to −1)</td>
</tr>
<tr>
<td><strong>MPO (pmol/l)</strong></td>
<td>n = 179</td>
<td>n = 165</td>
<td>n = 162</td>
<td>n = 163</td>
</tr>
<tr>
<td>Baseline</td>
<td>508</td>
<td>558</td>
<td>524</td>
<td>588</td>
</tr>
<tr>
<td>12 weeks</td>
<td>515</td>
<td>552</td>
<td>530</td>
<td>586</td>
</tr>
<tr>
<td>Within group comparison</td>
<td>1.4 (−6 to +10)</td>
<td>-1.1 (−9 to +8)</td>
<td>1.1 (−9 to +11)</td>
<td>-0.3 (−10 to +10)</td>
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<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>1.6 (−9 to +14)</td>
<td>0.9 (−10 to +13)</td>
<td>4.7 (−6 to +17)</td>
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<tr>
<td><strong>MMP-9 (µg/l)</strong></td>
<td>n = 178</td>
<td>n = 165</td>
<td>n = 161</td>
<td>n = 163</td>
</tr>
<tr>
<td>Baseline</td>
<td>471</td>
<td>505</td>
<td>448</td>
<td>475</td>
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<tr>
<td>12 weeks</td>
<td>479</td>
<td>495</td>
<td>421</td>
<td>506</td>
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<tr>
<td>Within group comparison</td>
<td>2.0 (−9 to +13)</td>
<td>-2 (−13 to +11)</td>
<td>-6 (−17 to +5)</td>
<td>6.0 (−6 to +20)</td>
</tr>
<tr>
<td>Between groups comparison</td>
<td>N/A</td>
<td>-0.8 (−14 to +15)</td>
<td>-9.8 (−22 to +4)</td>
<td>4.8 (−9 to +21)</td>
</tr>
</tbody>
</table>

Baseline and week 12 data are presented as geometric means. Within group comparisons depict changes from baseline. Between group comparisons indicate changes from baseline relative to placebo. Within group comparisons were analyzed using paired t test. Between group comparisons were analyzed with analysis of covariance with baseline value, atorvastatin level, and treatment included as covariates. Values in parenthesis are 95% CI. *p < 0.05; †p = 0.028.

Abbreviations as in Tables 1 and 2.

equire the effects of darapladib on several biomarkers associated with enhanced platelet aggregation. There was no evidence for the increase in any of the measured platelet biomarkers when analyzed for the changes from baseline (i.e., within group comparison) or in comparison with placebo (i.e., between groups comparison) at week 4 (not shown) and week 12 of treatment with darapladib (Table 5).

Similarly, discontinuation of darapladib produced no significant effect on these measurements at follow-up (not shown).
Adverse events. There were no clinically important effects on vital signs, electrocardiograms, or laboratory data in the darapladib groups compared with placebo. Serious adverse events were reported in 3% in the placebo (n = 7) and darapladib 40 mg (n = 6) and 80 mg (n = 7) groups and 2% (n = 5) in the darapladib 160 mg group. These events were not concentrated in any particular organ system class and displayed no dose-related pattern. No deaths occurred after randomization to darapladib or placebo. The CV events were infrequent at 2% in the placebo group (n = 4), <1% in the darapladib 40 and 80 mg groups (n = 2 in both groups), and 1% in the darapladib 160 mg group (n = 3). The most commonly reported serious adverse event was angina at <1% in the placebo (n = 2) and darapladib 40 mg (n = 1) groups, none in the darapladib 80 mg group, and 1% (n = 3) in the darapladib 160 mg group.

A total of 3% (n = 33) of subjects prematurely withdrew from study drug due to an adverse event; 2% (n = 4) in the placebo group, 4% (n = 10) in the darapladib 40 mg group, 3% (n = 8) in the darapladib 80 mg group, and 5% (n = 11) in the darapladib 160 mg group. Within each organ system class, the incidence of adverse events leading to premature withdrawal was ≤1% across all treatment groups with the exception of a 3% (n = 6) incidence of premature withdrawals due to gastrointestinal events (mostly change in feces odor or diarrhea) in the darapladib 160 mg group. A higher incidence of odor- (mainly feces or urine) or taste-related events was reported in all darapladib treatment groups (33% to 36%) compared with placebo (21%) that did not lead to frequent premature withdrawals (≤1% incidence of withdrawals due to odor- or taste-related events across all treatment groups).

No cases of rhabdomyolysis or creatine kinase elevation >10× upper normal limit were reported. Elevation of alanine transaminase ≥3× upper limit of normal was noted in <1% (n = 1) in placebo, <1% (n = 2) in the darapladib 40 and 80 mg groups, and 1% (n = 3) in the darapladib 160 mg group.

Discussion

Key findings. This is the first report of chronic administration of an Lp-PLA2 inhibitor, darapladib, in CHD or CHD-risk equivalent patients in the setting of intensive statin therapy. There was a significant dose-dependent and sustained reduction of Lp-PLA2 activity with darapladib. The effect was independent of baseline LDL-C or HDL-C levels and background atorvastatin regimen. In addition, the results suggested a possible anti-inflammatory effect of the highest dose of darapladib treatment when applied to patients receiving atorvastatin therapy. In regards to safety, there were no adverse effects of darapladib on biomarkers of platelet activity and no major safety concerns emerged from this study.

Relationship between Lp-PLA2, lipoproteins, and treatment of dyslipidemia. Owing to its binding to carboxy-terminus of human apolipoprotein B, approximately 80% of circulating Lp-PLA2, measured as mass or activity, is associated with apolipoprotein B–containing lipoproteins (22,23). The remaining Lp-PLA2 is less firmly associated with phospholipid moiety of HDL-C and does not bind to apolipoprotein A-I. Higher Lp-PLA2 mass or activity are found in pro-atherogenic small dense LDL-C and electronegative LDL-C particles, as opposed to large buoyant LDL-C and electropositive subfractions, respectively (24–26). Consistent with this distribution of Lp-PLA2, our findings show strong correlations between Lp-PLA2 activity and LDL-C and inverse relationship with HDL-C in patients on intensive atorvastatin treatment. Prior studies have also shown that various hypolipidemic drugs (e.g., statins, feno-
Comparisons between Lp-PLA2 and other inflammatory markers. Oxidatively truncated phospholipids are substrates for Lp-PLA2 that promote formation of putative pro-inflammatory and pro-apoptotic products (9). The concentration of oxidized LDL-C within human atheroma is approximately 70-fold higher than in circulation; thus, the concentration of oxidized LDL-C within human atheroma is approximately 70-fold higher than in circulation; thus, the activity of Lp-PLA2 (mass or activity) in stable CV patients (29–31). Similarly, other lipid modifying drugs, such as ezetimibe and fenofibrate, modestly lower Lp-PLA2 (mass or activity) (32). This study shows that a specific Lp-PLA2 inhibitor, darapladib, produces substantial additional reductions in Lp-PLA2 activity when added to intensive atorvastatin therapy (up to 66%). This effect was largely independent of atorvastatin dose and preserved in clinically relevant strata of LDL-C and HDL-C values.

Relationship between Lp-PLA2 and inflammatory biomarkers. Oxidatively truncated phospholipids are substrates for Lp-PLA2 that promote formation of putative pro-inflammatory and pro-apoptotic products (9). The concentration of oxidized LDL-C within human atheroma is approximately 70-fold higher than in circulation; thus, the comparisons between Lp-PLA2 and other inflammatory markers in plasma might not accurately reflect intratissue inter-dependency (33). In this context, it is not surprising that circulating Lp-PLA2 shows weak correlations with other inflammatory biomarkers, as shown in this and previous studies (34,35). Nonetheless, increasing levels of Lp-PLA2 have been reported to have additive effects with hs-CRP or oxidized phospholipid/apolipoprotein B ratio in predicting the risk of CV events (14,16,36). When assessing the effects of Lp-PLA2 inhibition on soluble inflammatory biomarkers, it is important to underscore that baseline hs-CRP levels were lower in this study compared with other investigations using intensive atorvastatin treatment and therefore might have minimized the opportunity to observe additional effects on this biomarker (5,6,37,38). Despite these caveats, a significant reduction in hs-CRP was noted in the highest darapladib group (160 mg) after 12 weeks of treatment (within group comparison). Interestingly, the darapladib 160 mg group also exhibited a significant reduction in IL-6 (within group and between groups comparisons). This finding is noteworthy, because IL-6 is a trigger for hepatic production of hs-CRP and it is less affected by background atorvastatin treatment (37,39).

Relationship between Lp-PLA2 and PAF. Initial descriptions of Lp-PLA2 focused on the ability of this enzyme to degrade PAF, a potent pro-inflammatory lipid mediator that is also implicated in platelet aggregation (10). However, responsiveness to PAF is not altered in Japanese subjects with a genetic variant in Lp-PLA2 (Val276Phe) that results in the absence of circulating enzyme (40). In addition, other clinical trials failed to show measurable benefit of recombinant human Lp-PLA2 in conditions suspected to be PAF-mediated (e.g., severe sepsis, asthma) (41,42). These observations together suggested that other extra- and intracellular enzymatic pathways are involved in hydrolysis of PAF, independent of Lp-PLA2 activity. To this end, studies identified several PAF inactivating enzymes, including lecithin-cholesterol acyl transferase and unrelated phospholipases (e.g., group X secretory PLA2 and intracellular PAF-AH [II]) (43,44). These findings provide mechanistic explanation for the lack of detrimental effect of darapladib on platelet biomarkers despite high levels of Lp-PLA2 inhibition. The importance of our findings is also underscored by the fact that several biomarkers were studied, including urinary 11-dehydrothromboxane B2, which is less affected by pre-analytical artifacts associated with the measurements of circulating markers (45).

Study limitations. There are several limitations of this study to be emphasized. First, the clinical relevance of the observed Lp-PLA2 inhibition with darapladib must await evidence linking Lp-PLA2 inhibition to a beneficial effect on clinical events. Nonetheless, marked inhibition of the enzyme activity in the presence of intensive background statin therapy is an important pre-requisite for testing this concept in high-risk CV patients. Second, our study cannot address the effects of Lp-PLA2 inhibition within atheroma that is postulated to be ultimately responsible for clinical benefits. It is important to underscore, however, that comparable exposures of darapladib inhibit intralaminar Lp-PLA2 activity in the clinical setting (46). Third, the effects on IL-6 and hs-CRP were modest and limited to the highest dose of darapladib. These results might be viewed as hypothesis-generating and will require replication in other studies.

Conclusions

Although treatment with statins has resulted in improved prognosis in primary and secondary prevention populations, high-risk individuals continue to experience recurrent CV events despite adequate treatment. It is postulated that inflammatory mediators not addressed by LDL-C lowering contribute to plaque instability and thrombotic events. The results of this study indicate that darapladib produces substantial inhibition of Lp-PLA2 activity in the presence of intensive statin therapy and suggest that such intervention might result in additional systemic anti-inflammatory effects. Future studies are required to determine whether chronic Lp-PLA2 inhibition will stabilize high-risk lesions and potentially reduce CV events.

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REFERENCES


For a supplementary figure and a list of the Darapladib Investigators, please see the online version of this article.