Inhibition of Lipoprotein-Associated Phospholipase Activity by Darapladib
Shifting Gears in Cardiovascular Drug Development: Are Antiinflammatory Drugs the Next Frontier?

S. Matthijs Boekholdt, MD, PhD; Robbert J. de Winter, MD, PhD; John J.P. Kastelein, MD, PhD

Over the past 3 decades, we have witnessed in the Western world a remarkable reduction in morbidity and mortality resulting from coronary heart disease (CHD). For example, in Finland, the dramatic decrease in CHD mortality is estimated to be ≈50%, at least one third of which can be accredited to a reduction in cholesterol levels in the general population. An even more impressive reduction in the incidence of cardiovascular events has been observed among patients with prevalent CHD, as can be deduced from a comparison between the control arm of one of the earliest statin trials, the Scandinavian Simvastatin Survival Study (4S), and the aggressive lipid-lowering arm of one of the latest intervention trials, the Treating to New Targets (TNT) study. Major cardiovascular events occurred in 36.2% of patients in the placebo arm of 4S and in 8.7% of patients in the intensive lipid-lowering arm of TNT, an astounding 76% relative risk reduction that occurred in the last 15 years. Among patients with type 2 diabetes, another highly prevalent and high-risk population, a similarly impressive risk reduction was achieved with an intensified multifactorial intervention. However, a cursory glance at the event rates in the experimental arms of these trials alerts us to the fact that even under current state-of-the-art treatment, cardiovascular events are still common. This leaves us with a large and unmet medical need to develop new strategies to combat atherosclerotic vascular disease, and this quest often starts with the identification of novel cardiovascular risk factors as potential targets. In the mid 1990s, inflammation was introduced as a novel dimension in atherogenesis. Since then, we have seen an impressive range of publications about inflammatory molecules as novel risk markers, most prominently C-reactive protein, myeloperoxidase, and lipoprotein(a). Lipoprotein-associated phospholipase A2 (Lp-PLA2). The development of small molecules specifically targeted against inflammatory mediators can be seen as a new phase in cardiovascular drug development. Nevertheless, at the outset of this quest, we have to be realistic in our expectations; the fact that a compound has antiinflammatory properties does not automatically translate into a cardiovascular risk reduction, as we have recently witnessed. A good example is succinobucol, a molecule that has antiinflammatory properties and reduces circulating inflammatory biomarkers. Succinobucol also was shown to attenuate atherosclerosis in animal models and to reduce coronary atherosclerosis as quantified by intravascular ultrasound (IVUS) in humans. However, succinobucol was recently shown not to reduce the incidence of cardiovascular events among people with prevalent CHD.

Lp-PLA2 Biology and Epidemiology
An interesting target for cardiovascular drug development is Lp-PLA2, an enzyme that may act in the twilight zone between lipoprotein metabolism and inflammation. Lp-PLA2 is produced by macrophages and is bound primarily to various lipoproteins, including low-density lipoprotein (LDL) and lipoprotein(a). Lp-PLA2 has been detected in atherosclerotic plaque, particularly in plaques with a necrotic core and in ruptured plaques. Lp-PLA2 catalyzes the hydrolysis of the center (sn-2) ester bond of phospholipids. This activity also can be targeted at oxidized phospholipids, which are abundantly present on oxidized LDL particles in the atherosclerotic plaque. The products of this activity are oxidized fatty acids and lysophosphatidyl choline, a molecule with a range of potentially atherogenic effects, including chemotraction of monocytes, increased expression of adhesion molecules, and inhibition of endothelial nitric oxide production. In addition, lysophosphatidyl choline is cytotoxic to both monocytes-macrophages and vascular smooth muscle cells. Some experimental evidence suggests that inhibition of Lp-PLA2 may be atheroprotective, but evidence for the opposite effect also has been observed. For instance, Lp-PLA2 inhibition diminished the rise of lysophosphatidyl choline levels that usually occurs on LDL oxidation, and it reduced the ensuing apoptosis of monocytes-macrophages. In contrast, experimentally augmented expression of Lp-PLA2 reduced spontaneous atherosclerosis in apolipoprotein E-deficient mice. In addition, the effect of Lp-PLA2 activity in the arterial wall may be different from its effect in the circulation. When oxidized phospholipids enter the circulation, they are mostly scavenged by lipoprotein(a) and can subsequently be degraded by Lp-PLA2 activity. The prod-
uct lysophosphatidyl choline may then be transferred to albumin, where it is relatively innocuous. Thus, in plasma, Lp-PLA2 appears to play a beneficial role in the incapacitation of potentially toxic oxidized phospholipids.

Findings from epidemiological studies also are not consistent and equivocal. Genetic deficiency of Lp-PLA2, which is common in Japan, has been associated with an increased risk of CHD, stroke, and peripheral artery disease.\(^{12}\) Apparently in contrast, numerous population-based studies have shown that circulating Lp-PLA2 levels are positively associated with the risk of future cardiovascular risk. Among the first large prospective studies to investigate the association between Lp-PLA2 plasma levels and the risk of future cardiovascular risk was a posthoc analysis of the West of Scotland Coronary Prevention Study.\(^{13}\) Study participants with elevated Lp-PLA2 levels had an \(\approx 2\)-fold increased risk of future cardiovascular events that was independent of established cardiovascular risk factors. Although some population-based studies like the Women’s Health Study and the Atherosclerosis Risk in Communities study have suggested that this association was not independent of established cardiovascular risk factors, most other large studies like the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) Augsburg cohort and Rancho Bernardo have observed an association that is indeed independent of established cardiovascular risk factors. Studies among people with established CHD, including a German study and a Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction (PROVE-IT TIMI22) substudy, have confirmed these.\(^{14,15}\) In summary, biological and experimental evidence is not equivocal about the potentially proatherogenic and antiatherogenic effects of Lp-PLA2 activity and its inhibition. Epidemiological evidence suggests that complete deficiency of Lp-PLA2 is a risk factor for atherosclerotic disease, whereas in the normal range, higher levels are associated with elevated cardiovascular risk independently of established risk factors.

**Darapladib**

Darapladib is an agent specifically targeted to inhibit Lp-PLA2 activity. The first clinical study performed in humans was published recently.\(^{16}\) People with stable CHD or a CHD risk equivalent who were on aggressive lipid-lowering therapy with atorvastatin 20 or 80 mg were randomized to placebo or darapladib 40, 80, or 160 mg. Compared with placebo, darapladib treatment resulted in a dose-dependent decrease in Lp-PLA2 activity by up to 66% the 160-mg group. Despite the already modest baseline C-reactive protein levels, treatment with darapladib 160 mg resulted in additional lowering of C-reactive protein levels by 20%. Darapladib also reduced interleukin-6 levels, whereas myeloperoxidase and matrix metalloproteinase-9 levels were not affected. The study revealed no unexpected laboratory or clinical adverse events. We should bear in mind, however, that the effects of Lp-PLA2 inhibition on circulating biomarkers may not necessarily represent its consequences in the arterial wall. Therefore, a next step in the development of this intriguing novel drug would be the direct visualization of atherosclerotic plaques in the coronary arterial tree, and the study by Serruys and colleagues\(^{17}\) in this issue of *Circulation* represents exactly that.

The Integrated Biomarkers and Imaging Study-2 was an international, multicenter, randomized, double-blind, placebo-controlled study among patients with angiographically documented CHD. Patients were randomized to darapladib 160 mg or placebo once daily. After treatment for 12 months, participants underwent a repeat angiography and IVUS. The authors hypothesized that treatment with darapladib would have an effect on plaque deformability, composition, and size and chose not to use accepted IVUS methods of atherosclerosis regression such as change in percent atheroma volume or change in normalized total atheroma volume. The study used novel IVUS methodologies for the primary and secondary end points: IVUS-based palpography that measures the mechanical properties of the tissue during the cardiac cycle that shows high strain values when the dominant plaque type is fatty or fibrofatty and IVUS-based virtual histology that uses radiofrequency spectral analysis of the ultrasound backscatter signals to identify 4 different components of atherosclerotic plaque (fibrous, fibrofatty, dense calcium, and necrotic core). IVUS palpography has been validated in animals and humans.\(^{18}\) IVUS-based virtual histology tissue characterization has been validated against histology after carotid endarterectomy and directional coronary atherectomy and showed a predictive accuracy for necrotic core of 88%. Moreover, unstable lesions defined as IVUS virtual histology thin-cap fibroatheromas were significantly more prevalent in patients with acute coronary syndromes compared with patients with stable angina,\(^{21}\) although another study showed reverse associations.\(^{22}\) The coprimary end point of the study by Serruys and colleagues of change from baseline in high strain density did not differ significantly between treatment arms in the overall study but showed a significant reduction in high strain in the darapladib group when only patients with highly deformable plaque at baseline were analyzed. The treatment arms also did not differ with regard to the second coprimary end point, C-reactive protein levels during follow-up, or the secondary end point of total atheroma volume. Importantly, however, the other secondary end point, change in necrotic core size, was significantly lower in patients treated with darapladib; ie, necrotic core size remained unchanged in the darapladib group but increased among those treated with placebo. Sensitivity analyses suggested that this beneficial treatment effect was similar in various clinical subgroups as defined by established risk factors. Disappointingly, however, the investigators did not analyze whether the treatment effect was affected by circulating oxidized phospholipid levels, which may be the main driving force behind the proatherogenic effects of Lp-PLA2 activity. Because the concentration of oxidized LDL, an important source of oxidized phospholipids, is \(\approx 70\)-fold higher in plasma than in atherosclerotic plaque, the effects of Lp-PLA2 activity and Lp-PLA2 inhibition may have substantially different, and perhaps even opposing, effects in plasma compared with atherosclerotic plaque. The ultimate effect of darapladib will depend on the balance between these effects. Another important limitation of the present study is the fact that compared with recent
atherosclerosis regression studies, this study was relatively small and follow-up was short. Although the discordance between the IVUS palmpography findings, the IVUS radiofrequency results, and the changes in circulating inflammatory markers is disappointing, the prevention of necrotic core expansion with darapladib may represent the local effects on plaque composition that one would specifically hope to achieve with Lp-PLA2 inhibition. However, several recent examples have dramatically underlined that the extrapolation of surrogate markers of atherosclerosis to actual cardiovascular events is risky. Therefore, both the promising effects of Lp-PLA2 inhibition by darapladib as observed in this study and the use of these novel IVUS imaging modalities must await confirmation in larger event-driven trials before clinical relevance can be ascribed to them.

Disclosures
None.

References
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