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Secretory Phospholipase A2 and Lipoprotein-Associated Phospholipase A2 as Biomarkers of Cardiovascular Disease and Therapeutic Targets

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Atherosclerosis is not only a lipid-driven disease but it is an intricate process that also involves the simultaneous and combined effect of inflammatory and immunological factors. A substantial body of peer-reviewed studies has validated the cardiovascular risk predictive value of a variety of inflammatory markers including two members of the phospholipase A2 (PLA2) superfamily, the type IIA secretory PLA2 (sPLA2) and the lipoprotein-associated phospholipase A2 (Lp-PLA2). In animal, pathological and epidemiological studies, the increased levels of these two PLA2 have been related to an increase in complex coronary artery lesions and an increase in major cardiovascular clinical events. Therefore, inhibition of these enzymes has become the focus of research in the last decennium. Novel pharmacological inhibitors of those enzymes emerge as promising therapeutical options for treating patients with coronary artery disease (CAD).

The type IIA secretory PLA2 (*sPLA2*) is a Ca^{2+}-dependent enzyme expressed in hepatocytes, macrophages, platelets, and vascular smooth muscle cells. The catalytic function of this enzyme is related to the hydrolysis of the sn-2 acyl group of glycerophospholipids with further liberation of fatty acids and lysophospholipids, all playing an important role in the biosynthesis of lipid mediators such as platelet activating factor (PAF), leukotrienes, prostaglandins, and eicosanoids. Possible atherogenic mechanisms of sPLA2 include its effects on lipoproteins, which results in the release of various lipid mediators at the site of lipoprotein retention in the arterial wall that in turn may trigger local inflammatory cellular responses. Furthermore, in arterial tissue, sPLA2 may also directly modify LDL particles to become more atherogenic and may increase the affinity of apolipoprotein B-100 on LDL to glycosaminoglycans and proteoglycans. sPLA2 is also implicated in the production of isoprostanes which exhibit strong mitogenic activity and induce platelet aggregation and vasoconstriction. High sPLA2 concentrations may predict CAD events in patients with stable coronary artery disease and unstable angina, and all-cause mortality in patients with acute myocardial infarction. Raised sPLA2 concentration and activity is also associated with increased risk of incident CAD events in apparently healthy men and women. Recently it was also shown that sPLA2 mass and activity, may be predictive of secondary cardiovascular events in patients with CAD.

It is well established that substituted indoles, 6,7-benzoindoles and indolizines are potent inhibitors of sPLA2. Among them *varespladib* methyl (1-H-indole-3-glyoxamide; A-002; Anthera Pharmaceuticals, San Mateo, CA) is an oral selective sPLA2 inhibitor. The effect of this inhibitor on enzyme concentration and on plasma

lipoproteins in patients with stable CAD was evaluated in the *PLASMA* (Phospholipase Levels and Serological Markers of Atherosclerosis) study, a Phase II, randomized, double-blind, placebo controlled parallel arm dose-response study. A total of 393 patients were randomly assigned to receive either placebo (n=79) or one of four doses of A-002; 50 mg [n=79], 100 mg [n=80], 250 mg [n=78], or 500 mg [n=77] twice daily), for 8 weeks. The primary endpoint was the change in sPLA2 concentration or activity from baseline to week 8. Mean sPLA2 concentration fell by 86.7% in the overall active treatment group and by 48% in the placebo group (p <0·0001). The reductions in sPLA2 concentration in the A-002 groups were dose dependent (ranging from 69.2% in the 50 mg group to 95.8% in the 500 mg group) and differed significantly from placebo (p <0.0001 for all doses). The results of this study show that A-002 reduces sPLA2 concentration in humans thus it might be an effective anti-atherosclerotic agent. The effect of this inhibitor on sPLA2 levels and other inflammatory markers of cardiovascular risk as well as on the incidence of myocardial injury is currently under investigation in clinical studies (*PLASMA II, FRANCIS-ACS* and *SPIDER-PCI*).

Lipoprotein-associated phospholipase A2 (*Lp-PLA2*) is a calcium-independent PLA2 that is predominantly synthesized by macrophages. In plasma, Lp-PLA2 is bound to LDL and HDL lipoproteins, with a greater affinity for the polar surface of LDL particles, particularly small dense LDL. Lp-PLA2 is produced and secreted by inflammatory cells involved in atherogenesis primarily monocyte-derived macrophages. Lp-PLA2 rapidly degrades oxidatively modified phospholipids leading to formation of the proinflammatory and cytotoxic products lysophosphatidylcholine and oxidized free fatty acids. Lp-PLA2 staining in pathologic intimal thickening plaques is nearly absent; whereas in complex lesions such as thin-cap fibroatheromas and ruptured plaques, an intense Lp-PLA2 expression within necrotic cores and surrounding macrophages including those in the fibrous cap is observed. This may imply that derived cytotoxic compounds from Lp-PLA2 play important role in plaque vulnerability. These observations, therefore, suggest that Lp-PLA2 inhibition may favorably affect rupture-prone lesions.

Many epidemiological studies have shown that increased concentrations of Lp-PLA2 predict future cardiovascular events. In a recent prospective, population-based survey of the epidemiology and pathogenesis of atherosclerosis (*Bruneck* study), the factors that influence plasma levels of Lp-PLA2 and the prognostic value of this enzyme for cardiovascular disease were investigated. Subjects with incident cardiovascular disease (CVD) (cardiovascular death, myocardial infarction, stroke, and transient ischemic attack) had higher levels of Lp-PLA2 activity (884±196 versus 771±192 mmol/min/L, P <0.001). Increased Lp-PLA2 activity was significantly related to incident CVD [age- and sex-adjusted hazard ratio (95%CI) 2.9 (1.6–5.5); third versus first tertile group; P <0.001] and with vascular mortality but not with non-CVD mortality.

Recent clinical studies have demonstrated that Lp-PLA2 inhibition by azetidinones, a class of selective Lp-PLA2 inhibitors that target the active-site serine residue of the enzyme, such as darapladib, may represent promising therapeutical options for treating patients with CVD. *Darapladib* [40, 80, and 160mg inhibits Lp-PLA2 activity approximately 43, 55, and 66% compared with placebo (P <0.001 at week 12). In the *IBIS-2* (Integrated Biomarkers and Imaging Study-2) trial, a multicenter, randomized, double-blind, placebo-controlled study in 330 patients with angiographically confirmed CAD, darapladib halted the increase in the necrotic core volume compared with placebo without affecting total atheroma volume. Lp-PLA2 inhibition may represent a new approach for the treatment of atherosclerosis, however, the benefit of this intervention need to be established by on-going event-driven outcomes trials such as the *STABILITY* (Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy) trial.

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