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Community-based statins and advanced carotid plaque: Role of CD163 positive macrophages in lipoprotein-associated phospholipase A_2 activity in atherosclerotic plaque

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ABSTRACT

Background and aims: Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), an enzymatic inflammatory biomarker primarily bound to low-density lipoprotein cholesterol, is associated with an approximate twofold increased risk of cardiovascular disease and stroke. Despite indications that circulating Lp-PLA_2 is sensitive to statins, it remains largely unknown whether statin usage exerts local effects on Lp-PLA_2 expression at the site of atheromatous plaque.

Methods: Carotid plaques (n = 38) were prospectively collected from symptomatic (n = 18) and asymptomatic (n = 20) patients with (n = 20) or without (n = 18) documented statin history. In all cases, endarterectomy was performed where the primary stenosis was removed in an undisturbed manner. Serial cryosections of the presenting lesion were assessed histologically for macrophages, Lp-PLA_2, and cell death (apoptotic index).

Results: Symptomatic lesions exhibited less calcification, with greater inflammation characterized by increased expression of $CD68^+$ and $CD163^+$ macrophage subsets, and Lp-PLA_2. Symptomatic plaques also exhibited greater necrotic core area and increased apoptosis, as compared with asymptomatic lesions. In contrast, statin treatment did not appear to influence any of these parameters, except for the extent of apoptosis, which was less in statin treated as compared with statin naïve lesions. Overall, Lp-PLA_2 expression correlated positively with necrotic core area, $CD68^+$ and $CD163^+$ macrophage area, and cell death. Finally, *in vitro* assays and dual immunofluorescence staining confirmed CD163-expressing monocytes/macrophages are also a major source of Lp-PLA_2.

Conclusions: Statin treatment has no effect on local atherosclerotic lesion Lp-PLA2 activity, therefore, the addition of anti-inflammatory treatments to further decrease macrophage Lp-PLA_2 expression in atherosclerotic lesions may reduce lesional inflammation and cell death, and prevent necrotic core expansion and lesion progression.

Abbreviations: Hb, hemoglobin; Hp, haptoglobin; Hb:Hp, hemoglobin:haptoglobin complex; IEL, internal elastic lamina; LDL, low-density lipoprotein; Lp-PLA_2, lipoprotein-associated phospholipase A_2; Lyso-PC, lysophosphatidylcholine; Mhem, hemoglobin-stimulated macrophages; ORO, oil red O; oxLDL, oxidized low-density lipoprotein; TIA, transient ischemic attack.

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1. Introduction

Arterial inflammation is an essential component of the pathogenesis of atherosclerosis contributing to the complications of acute coronary syndromes and stroke. The basis for inflammation is thought to be driven by a myriad of complex interactions principally involving oxidized low-density lipoprotein (oxLDL) where products from these processes have emerged as potential markers of cardiovascular risk. Recent studies suggest that molecular pathways implicating oxLDL could contribute to inflammation through the release of bioactive metabolites produced by a family of phospholipase A_2 enzymes. Elevated levels of circulating lipoprotein-associated phospholipase A_2 (Lp-PLA_2) confirmed by both mass and activity have emerged as a moderate and consistent risk marker for the future development of cardiovascular disease [1–3]. Moreover, circulating Lp-PLA_2 is increased in patients with high-grade carotid stenosis and may serve as an independent marker of unstable plaque [4]. These fundamental observations have led to the hypothesis that Lp-PLA_2 could serve as an independent predictor of cardiovascular risk.

In humans, Lp-PLA_2 primarily circulates in active form bound to the apoB fraction of LDL cholesterol and other circulating lipoproteins [5]. The enzyme is characterized by its ability to specifically hydrolyze oxidized phospholipids in LDL to generate two potent pro-inflammatory mediators, lysophosphatidylcholine (lyso-PC) and oxidized non-esterified fatty acids (oxNEFA) [6]. Lyso-PC for example serves as an effective chemoattractant for monocytes, resulting in foam cell accumulation within the artery wall [7]. In addition to the circulating enzyme, Lp-PLA_2 is expressed in human coronary plaques and is particularly abundant in lesional macrophages and foam cells surrounding the necrotic core region, in advanced lesions prone to rupture [8,9]. Translational evidence that Lp-PLA_2 contributes to inflammatory responses was shown in a diabetic hypercholesterolemic porcine model of atherosclerosis where selective inhibition of Lp-PLA_2 by darapladib resulted in a significant decrease in relevant markers of macrophage activation as well as limited expansion of the plaque necrotic core [10]. Clinically, direct inhibition of Lp-PLA_2 by darapladib has failed to reduce cardiovascular outcomes in patients with stable coronary heart disease (STABILITY 10A) and acute coronary syndrome (SOLID-TIMI 52 10B). However, lyso-PC products generated by Lp-PLA_2 have been found in abundance in carotid plaques and were associated with increased levels of proinflammatory cytokines suggesting a significant role of lyso-PCs in plaque inflammation and lesion stability [11]. Thus in essence, arterial Lp-PLA_2 derived locally from macrophages and foam cells might be considered more proatherogenic than actual circulating levels of Lp-PLA_2.

Considering 80% of Lp-PLA_2 in human plasma is bound to LDL cholesterol, one would assume that statin therapy would inherently lower circulating levels of this enzyme. Consistent with this hypothesis, the anti-atherogenic effects of statins have been partially linked to a reduction of circulating Lp-PLA_2 activity and mass in plasma [12,13]. Despite indications that circulating Lp-PLA_2 is sensitive to statins, it remains largely unclear whether statin usage has any effect on the Lp-PLA_2 expression at the site of atheromatous plaque, or if there is an association with vulnerable plaque stabilization.

In the present study, we assessed the influence of documented long-term statin treatment used by independent community physicians on the composition of advanced atherosclerotic plaques of carotid arteries. The study is unique considering we implemented a rigorously defined representative sampling of tissues that allows pathologic interrogation of carotid lesion morphology, as it existed in the patient. The overall results suggest that statin therapy provides suboptimal control in respect to plaque phenotype and supports a rationale for instituting additional anti-inflammatory therapy to reduce macrophages producing Lp-PLA_2 to further improve clinical outcomes.

2. Materials and methods

Human carotid plaques (n = 38) were prospectively collected from symptomatic and asymptomatic patients where the primary stenotic portion was removed by endarterectomy in an undisturbed manner. In all cases, community physicians had treated about half the patients with statins, while the remaining half was untreated by statins or other lipid altering drugs. In total, there were 18 symptomatic (statin-naïve = 8 and statin-treated = 10) and 20 asymptomatic (statin-naïve = 10 and statin-treated = 10) cases (Fig. 1). Serial cryosections at three independent levels, including the presenting lesion (most advanced plaque) and adjacent lesions, were selected for histomorphometric analysis and immunohistochemistry where CD68 (pan-macrophage marker), CD163 (hemoglobin scavenger molecule), Lp-PLA_2, apolipoprotein B, and cell death (apoptotic index) were evaluated (Fig. 1). The details of the sectioning methodology are available in Supplementary Materials. The study protocol was approved by the Institution for using de-identified pathological specimens as an exempted study.

3. Results

3.1. Histologic plaque morphologies

A total of 114 histologic sections from 38 carotid lesions were evaluated. The most frequent plaque morphology was fibroatheroma in both symptomatic and asymptomatic plaques, irrespective of the presence of statin treatment (Fig. 2). Plaque rupture was more commonly observed in symptomatic plaques, whereas healed rupture was equally frequent among groups. Fibrocalcific plaque and/or calcified nodule were more common in asymptomatic plaques. Representative histologic images from symptomatic and asymptomatic plaques with or without statin treatment are shown in Fig. 3.

3.2. Comparisons between statin-treated and statin-naïve plaques

There were no significant differences in risk factors between the statin-treated (n = 20) and statin-naïve (n = 18) patients, except that previous history of coronary artery disease was more frequent in the statin group ((70%) compared with 22% for the statin naïve) (Table 1). Serum cholesterol levels were available in 15 patients where total cholesterol levels were significantly lower in statin-treated as compared with statin-naïve patients; however, other lipid profiles did not differ significantly between groups (Table 1). There were no significant differences in area measurements (internal elastic lamina [IEL], lumen, plaque, stenosis, necrotic core, and calcification) between the statin-treated and the statin-naïve patients. Similarly, no differences were observed for areas of the plaque occupied by CD68 and CD163 positive macrophages, apoB, and expression of Lp-PLA_2 (Table 1). The extent of apoptosis measured by DNA fragmentation staining (apoptotic index), however, was the only variable found significantly less in the statin-treated patients as compared with the statin-naïve patients.

Among 20 statin-treated lesions, seven patients were receiving high potency statins (atorvastatin and rosuvastatin) while the remaining 13 were on standard statin therapy (pravastatin, lovastatin, and simvastatin). The duration of statin treatment ranged from 6 months to 13 years (5.3 ± 5.1 years) and was not significantly different between the high potency and the standard statins. Neurological symptoms were more frequent in patients treated