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Effect of hypolipidemic drugs on lipoprotein-associated platelet activating factor acetylhydrolase Implication for atherosclerosis

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Abstract

Human plasma platelet activating factor acetylhydrolase (PAF-AH) is an enzyme associated mainly with the apolipoprotein B (apoB)-containing lipoproteins and primarily with low-density lipoprotein (LDL). A small proportion of enzyme activity is also associated with high-density lipoprotein (HDL). PAF-AH activity is essential for the metabolism of PAF and oxidized phospholipids, i.e. bioactive lipids that are involved in the pathophysiology of atherosclerosis. Thus, PAF-AH may play a significant role in atherogenesis. Accumulating data indicate that PAF-AH associated with HDL particles plays a predominantly antiatherogenic role. By contrast, the role of LDL-associated PAF-AH remains controversial. Dyslipidemia induces a significant increase in total plasma PAF-AH activity and alters the enzyme distribution between proatherogenic apoB- and antiatherogenic apo AI-containing lipoproteins by increasing the PAF-AH activity associated with apoB-containing lipoproteins. The decreased rate of LDL removal from the circulation and the abnormal catabolism of triglyceride-rich lipoproteins play important roles in these abnormalities. Atorvastatin or fenofibrate therapy can restore, at least partially, the dyslipidemia-induced alterations in plasma PAF-AH by increasing the ratio of HDL-PAF-AH to plasma PAF-AH (or to LDL-cholesterol) levels, which may represent an important antiatherogenic effect of these hypolipidemic drugs.

Keywords: Atherosclerosis; Dyslipidemia; Lipoproteins; PAF-acetylhydrolase; Atorvastatin; Fenofibrate

1. Introduction

PAF-AH is an enzyme that exhibits an α/β hydrolase conformation and has broad substrate specificity towards lipid esters containing short acyl chains. Among the various activities, the Ca^{2+}-independent phospholipase A_2 activity of PAF-AH has been principally studied. Indeed, PAF-AH has marked preference for phospholipids with short chain moieties at the *sn*-2 position and, in addition to the potent proinflammatory lipid mediator PAF, it can hydrolyze proinflammatory and proatherogenic oxidized phospholipids [1].

In normolipidemic human plasma PAF-AH is associated mainly with the apoB-containing lipoproteins and primarily with LDL. A small proportion of circulating enzyme activity (less than 20%) is associated with HDL. Within these lipoprotein pools, the enzyme preferentially associates with small-dense LDL and with the very high-density lipoprotein-1 (VHDL-1) subfraction, alternatively denoted as HDL-3c [2]. Among LDL subspecies, the PAF-AH activity in normolipidemic plasma is positively correlated only with the enzyme activity associated with dense LDL subfractions, thus further supporting the preferential association of PAF-AH with small-dense LDL particles [3]. The distribution of PAF-AH between LDL and HDL can be influenced by the presence of lipoprotein (a) (Lp(a)) when plasma levels of this lipoprotein exceed 30 mg/dL [4]. It has been shown that Lp(a) contains several-fold greater PAF-AH activity compared with LDL when assayed at equimolar protein concentrations [4,5].

The primary sources of circulating PAF-AH constitute cells of hematopoietic origin and primarily macrophages

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Abbreviations: HDL, high-density lipoprotein; HomoFH, homozygous familial hypercholesterolemia; LDL, low-density lipoprotein; Lp(a), lipoprotein (a); NonFH, non-familial hypercholesterolemia; PAF-AH, platelet activating factor acetylhydrolase; VLDL + IDL, very low-density + intermediate-density lipoproteins.

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[6,7]. Although PAF-AH in plasma is associated with lipoproteins, their existence in plasma is not required for the enzyme activity. Since the cells of hematopoietic origin do not secrete lipoproteins, it seems that secretion of PAF-AH occurs independently of the secretion of lipoprotein particles; the enzyme subsequently associates with these particles in plasma. Consistent with this hypothesis, PAF-AH activity in plasma of individuals with HDL deficiency (Tangier disease) is higher than that of normal subjects [8], whereas individuals with abetalipoproteinemia have normal or slightly subnormal PAF-AH activity [9]. However, our recent studies have demonstrated that the lipoprotein metabolism as well as the lipoprotein plasma levels significantly influences the plasma PAF-AH activity. In this brief report, we present our resent results on the effect of hypolipidemic drugs on the plasma- and lipoprotein-associated PAF-AH activity in the most common types of dyslipidemia, primary hypercholesterolemia, combined hyperlipidemia, and primary hypertriglyceridemia.

2. Effect of hypolipidemic drugs on PAF-AH activity in patients with primary hypercholesterolemia

Patients with primary hypercholesterolemia (Type IIA dyslipidemia) exhibit an elevation of total plasma- and LDL-associated PAF-AH activity whereas the HDL-associated enzyme activity (HDL-PAF-AH) is not significantly altered. The plasma enzyme activity is positively correlated with total plasma cholesterol, LDL-cholesterol, as well as apoB levels. The increase in the LDL-PAF-AH concerns the increase in enzyme activity associated with all LDL particles, i.e. the large, intermediate, and small-dense LDL particles. Importantly, the plasma PAF-AH activity and that specifically associated with LDL increase in parallel with the increase in the severity of hypercholesterolemia. Indeed, the highest levels are seen in homozygous familial hypercholesterolemia (HomoFH) and the lowest in the non-familial hypercholesterolemia (NonFH) patient subgroup [3]. As a consequence, an altered distribution of enzyme activity among apoB- and apo AI-containing lipoproteins is observed, which is characterized by a significant decrease in the ratio of the HDL-PAF-AH to the plasma enzyme activity. This reduction is proportional to the increase of the plasma LDL-cholesterol levels and consequently to the severity of the hypercholesterolemia, being more profound in HomoFH patients. These results suggest that one of the important factors which determines plasma levels of PAF-AH as well as the enzyme distribution among plasma lipoproteins is the rate of removal of LDL from the circulation, an observation which accords with previously published results by our group [10] and others [11].

Indeed, our results show that the higher plasma PAF-AH activity in patients with primary hypercholesterolemia is due (i) to the elevated number of LDL particles in the plasma of these patients, and (ii) to the preferential enrichment of dense LDL subfractions with PAF-AH activity. Both observations could be attributed to the lower rate of LDL clearance, which is more profound in the HomoFH and less in NonFH patients [12]. Consistent with this notion is the observation that among the LDL subspecies, PAF-AH is preferentially associated with small-dense LDL. Indeed, total plasma enzyme activity in all patient subgroups is positively correlated only with the PAF-AH activity associated with the dense LDL subspecies. These particles are more slowly removed from the circulation as compared to the larger LDL particles due to their reduced binding to cellular LDL receptor [13].

The dependence of plasma PAF-AH activity as well as the enzyme distribution between LDL and HDL on the LDL clearance rate is further supported by the effect of lipid lowering therapy with HMG-CoA reductase inhibitors (statins). Thus, atorvastatin therapy in these patients significantly reduces total plasma PAF-AH as well as the enzyme activity associated with all LDL subspecies and this is primarily due to the reduction of plasma LDL levels [14]. This observation is also supported by the positive correlation observed between the reduction of plasma LDL cholesterol levels and that of plasma PAF-AH activity. The reduction in plasma PAF-AH activity by atorvastatin cannot be attributed to a decrease in PAF-AH secretion from its main cellular sources, since atorvastatin does not reduce PAF-AH secretion from macrophages *in vitro* [14]. Atorvastatin therapy does not affect HDL-PAF-AH activity, however, due to the reduction in LDL cholesterol levels, the ratio of HDL-associated PAF-AH activity to LDL-associated enzyme activity or to LDL-cholesterol levels significantly increases after drug administration.

Similar to the atorvastatin effect on PAF-AH activity in patients with primary hypercholesterolemia is the effect of fenofibrate. Thus, fenofibrate decreases total plasma- and LDL-associated PAF-AH activity but it does not influence HDL-PAF-AH [15]. Like atorvastatin, the decrease in plasma LDL-cholesterol levels induced by fenofibrate could represent the major mechanism accounting for the drug-induced reduction in total plasma PAF-AH activity in these patients. This is supported by the strong positive correlation observed between the decrease in enzyme activity and the decrease in plasma LDL-cholesterol levels. However, unlike atorvastatin, fenofibrate does not influence the enzyme activity associated with the large and intermediate LDL particles, but preferentially reduces PAF-AH activity associated only with the small-dense LDL particles, suggesting that such reduction could primarily be due to the well-known drug effect on the transformation of small-dense LDL particles to the large buoyant LDL, i.e. particles that exhibit a higher rate of clearance from the circulation, compared with small-dense ones [13].

3. Effect of hypolipidemic drugs on PAF-AH activity in patients with combined hyperlipidemia

Similar to the results obtained for the total plasma- and LDL-associated PAF-AH activity in patients with primary hypercholesterolemia, are those obtained in patients with combined hyperlipidemia (Type IIB dyslipidemia). However, a significant increase in the subfraction of triglyceride-rich very low-density lipoprotein + intermediate-density lipoprotein (VLDL + IDL), in parallel to the increase in LDL-associated enzyme activity, is also observed in this patient group. This increase contributes to the increase in the total plasma enzyme activity, although to a much less extent compared to the LDL-associated PAF-AH. Furthermore, unlike in Type IIA patients, Type IIB patients exhibit reduced HDL-PAF-AH and plasma HDL-cholesterol levels, as well as increased plasma triglyceride levels compared with the normolipidemic subjects [15]. Importantly, the HDL-PAF-AH was negatively correlated to the plasma triglyceride levels. Thus, this patient group is also characterized by a decrease in the ratio of the HDL-PAF-AH to the plasma enzyme activity due to both the increase in LDL-associated PAF-AH and to the decrease in HDL-PAF-AH. Treatment of these patients with atorvastatin or fenofibrate results in the reduction of total plasma PAF-AH activity, which is mainly due to the reduction in LDL-associated PAF-AH, although a contributory role is played by the reduction in the VLDL + IDL-associated enzyme activity. The effect of these hypolipidemic drugs on PAF-AH activity associated with individual LDL subspecies is similar to that described for the Type IIA patients. Importantly, fenofibrate administration in Type IIB patient group induces a significant increase in HDL-PAF-AH, a phenomenon that is not observed in atorvastatin-treated patients [14,15]. This observation provides evidence that the metabolism of triglyceride-rich lipoproteins may affect the HDL-PAF-AH, a hypothesis that is further supported by the results obtained in patients with primary hypertriglyceridemia.

4. Effect of fenofibrate on PAF-AH activity in patients with primary hypertriglyceridemia

Like in the other types of dyslipidemia, patients with primary hypertriglyceridemia (Type IV dyslipidemia) exhibit significantly higher plasma PAF-AH activity than normolipidemic volunteers. However, in contrast to Types IIA and IIB dyslipidemic patients, the increased levels of triglyceride-rich lipoproteins may play a key role in this elevation. Indeed, the enzyme activity associated with LDL as well as with individual LDL subspecies, including small-dense LDL, is not altered in primary hypertriglyceridemia, whereas PAF-AH activity associated with the VLDL + IDL subfraction is significantly higher compared either to normolipidemic volunteers or to other dyslipidemic patient groups. Moreover, fenofibrate therapy does not affect LDL-cholesterol levels but significantly reduces the total plasma- and the VLDL + IDL-associated PAF-AH activity. Unlike other dyslipidemic patient groups, the fenofibrate-induced reduction in plasma enzyme activity in Type IV dyslipidemic patients is positively correlated with the reduction in plasma levels of apoE, which is primarily associated with the VLDL + IDL subspecies. Consequently, in primary hypertriglyceridemia, the plasma PAF-AH activity depends on the catabolism and the rate of clearance of the triglyceride-rich lipoproteins from the circulation.

An important observation in this patient group is that the HDL-PAF-AH is significantly lower compared to normolipidemic subjects or to Type IIA patients, a phenomenon that is also observed in patients with combined hyperlipidemia. It is well known that both primary hypertriglyceridemia and combined hyperlipidemia are characterized by abnormal catabolism of triglyceride-rich lipoproteins and this metabolic defect significantly influences the plasma HDL levels, which are lower compared to those of normolipidemic volunteers [16]. Our recently published results suggest that the low HDL-PAF-AH observed in these patient groups reflects this metabolic defect. Furthermore, according to our results fenofibrate therapy leads to an increase in HDL-PAF-AH, which cannot be attributed to any drug effect on PAF-AH production and secretion by macrophages [15], but rather to the enzyme transfer from triglyceride-rich apoB-containing lipoproteins to HDL, during their enhanced lipolysis by lipoprotein lipase induced by fenofibrate [17]. This hypothesis may also explain the low baseline HDL-PAF-AH in patients with abnormal catabolism of triglyceride-rich lipoproteins (dyslipidemias of Types IIB and IV). The observation that elevation in HDL-PAF-AH concerns primarily the HDL-3c subfraction may suggest that this subfraction represents a better acceptor of PAF-AH from the triglyceride-rich apoB-containing lipoproteins during their degradation.

5. Plasma PAF-AH in atherosclerosis: the role of hypolipidemic therapy

PAF-AH may play a significant role in atherogenesis and cardiovascular disease due to its role in the metabolism of bioactive lipids, such as PAF and oxidized phospholipids [1]. However, the role of this enzyme in atherosclerotic disease remains a subject of controversy. Data from the WOSCOPS trial suggest that plasma levels of PAF-AH mass, which mainly reflects the LDL-associated enzyme, represent an independent risk factor for coronary artery disease [18]. In contrast, results from the Women's Health Study (WHS) suggest that plasma PAF-AH is not a strong predictor of cardiovascular risk in apparently healthy middle-aged women, over a mean follow-up of 3 years [19]. Nonetheless loss of plasma PAF-AH activity due to a $G^{994} \rightarrow T$ mutation in the PAF-AH gene may constitute a

genetic determinant of atherosclerotic disease in the Japanese population [20]. Despite conflicting observations concerning potential relevance of total plasma and LDL-PAF-AH to atherosclerotic disease, several lines of evidence suggest that HDL-PAF-AH, although present at low levels, may contribute to the antiatherogenic effects of this lipoprotein. Thus, adenoviral transfer of human plasma PAF-AH gene in apoE−/− mice significantly reduced macrophage adhesion and homing and inhibited injury-induced neointima formation and spontaneous atherosclerosis [21,22]. Considered together, the above findings support the assumption that HDL-PAF-AH may play an antiatherogenic role. As it is described above, a feature characteristic of all types of dyslipidemia is the alteration in the relative distribution of PAF-AH between proatherogenic apoB-containing lipoproteins and antiatherogenic apo AI-containing lipoproteins, resulting in a decrease in the ratio of HDL-PAF-AH to plasma PAF-AH (or to LDL-cholesterol) levels. Atorvastatin or fenofibrate therapy restores, at least partially, such an altered PAF-AH distribution, although these drugs act through different mechanisms. Thus, atorvastatin as well as fenofibrate may exert a beneficial antiatherogenic effect by improving the HDL potency against LDL oxidation and against the atherogenic biological effects of oxidized LDL.

6. Conclusion

Dyslipidemia induces a significant increase in total plasma PAF-AH activity and alters the enzyme distribution between proatherogenic apoB- and antiatherogenic apo AI-containing lipoproteins. The decreased rate of LDL removal from the circulation and the abnormal catabolism of triglyceride-rich lipoproteins play important roles in these abnormalities. Accumulating data indicate that PAF-AH activity associated with HDL particles plays a predominantly antiatherogenic role. By contrast, the role of LDL-associated PAF-AH remains controversial, possibly as a result of difficulty in dissecting the actions of the enzyme itself from that of the atherogenic dense LDL particle on which it is predominantly transported. Atorvastatin or fenofibrate therapy can restore, at least partially, the dyslipidemia-induced alterations in PAF-AH distribution by increasing the ratio of HDL-PAF-AH to plasma PAF-AH (or to LDL-cholesterol) levels, which may represent an important antiatherogenic effect of these hypolipidemic drugs.

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