Short Communication

Significance of platelet-activating factor acetylhydrolase in patients with non-insulin-dependent (type 2) *diabetes mellitus*

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Received: November 15, 2002; Accepted: December 10, 2002

Abstract

Background: Non-insulin dependent *diabetes mellitus* (NIDDM) represents an independent risk factor for cardiovascular diseases (CVD), being characterized by a continuous low-grade inflammation and endothelial activation state. Plasma platelet - activating factor - acetylhydrolases (PAF-AHs) are a subgroup of Ca²⁺-independent phospholipase A₂ family (also known as lipoprotein-associated phospholipases A₂) that hydrolyze and inactivate the lipid mediator platelet-activating factor (PAF) and/or oxidized phospholipids. This enzyme is considered to play an important role in inflammatory diseases and atherosclerosis. The present study aims to investigate the relations between the levels of PAF-AH activity and LDL-cholesterol / HDL-cholesterol (LDL-ch / HDL-ch) ratio in NIDDM patients as compared to controls. **Methods:** serum PAF-AH activity was measured in 50 patients with dyslipidemia, in 50 NIDDM patients and in 50 controls (normal lipid and glucose levels). Total cholesterol, LDL-ch, HDL-ch, triglyceride and blood glucose were determined in all subjects. **Results:** All NIDDM patients display hiperlipidemia, with increased LDL-ch and triglyceride levels. There is a significant correlation between LDL-ch levels (especially LDL-ch / HDL-ch ratio) and PAF-AH activity in dyslipidemic and NIDDM patients. **Conclusion:** Diabetic and dyslipidemic patients have an increased plasma PAF-AH activity correlated with their LDL-ch levels and mainly with LDL-ch / HDL-ch ratio. Plasma PAF-AH high levels appear to be important as a risk marker for endothelial dysfunction in patients with NIDDM.

Keywords: platelet-activating factor acetylhydrolase • non-insulin-dependent *diabetes mellitus*
• endothelial cell dysfunction

Introduction

The human plasma platelet-activating factor acetylhydrolases (PAF-AHs) (EC 3.1.1.47), also known

as lipoprotein-associated phospholipases A₂, represent a unique group of phospholipases A₂ (PLA₂s), exhibiting unusual substrate specificity toward platelet-activating factor (PAF) (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) and/or specialized (including oxidized) phospholipids that are structurally similar to PAF [reviewed in 1, 2]. PAF-AHs are mainly associated with low-density lipoproteins

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(LDL). Recently, plasma paraoxonase-1 has been identified as a high-density lipoprotein (HDL) - associated esterase, which exhibits PAF-AH-like activity [3]. Monocytes, macrophages, T lymphocytes and mast cells release PAF-AH in plasma [4]. PAF-AHs contain the catalytic triad (serine, aspartate and histidine) characteristic for phospholipases, but unlike other phospholipases have marked selectivity for phospholipids with short acyl chains in the sn-2 position [5]. One of the physiological functions of plasma PAF-AH is to degrade oxidized phospholipids in circulating lipoproteins [6]. Kosaka *et al.* investigated more than 3000 healthy Japanese and found that serum PAF-AH activity was higher in men, but this activity increased with age especially in women [7]. Plasma PAF-AH deficiency, due to missense (G (994)--> T) in exon 9 of the PAF-AH gene, is associated with atherosclerotic occlusive disease in Japan [8], suggesting a protective role of PAF-AH activity. However, dual consequences of plasma PAF-AHs activities have been reported:

1. decrease of the proinflammatory mediator PAF and degradation of PAF-like oxidized phospholipids, protecting against atherogenic processes, and
2. production of oxidized free fatty acids (ox-FFA) and lysophosphatidyl choline (LPC), compounds that are highly effective inflammatory mediators, capable of attracting monocytes and exacerbating the atherogenic process. Ox-FFA released by PAF-AH have not yet been structurally characterized and their biological action is not fully defined. In contrast, a great number of papers are referring to the pro-atherogenic and pro-inflammatory actions of LPC [9].

Among the risk factors for endothelial dysfunction, type 2 or non-insulin-dependent *diabetes mellitus* (NIDDM), including the insulin resistance, plays a special role as an independent risk factor, with increased incidence in endothelial dysfunction, associated with increased incidence in cardiovascular diseases (CVDs), having multiple complications and bad prognostic. Atherosclerotic lesions occur in diabetic patients at an earlier age, with severe clinical manifestations and poor outcome, strongly suggesting that CVD pathogenesis is directly influenced by the diabetic state. Several longitudinal observational studies have demonstrated a relationship between glycemic control and the development of CVDs [10]. Hyperglycemia might contribute to endothelial cell dysfunction in NIDDM in different ways:

- causes glycosylation of proteins in a process

that induce cross-linking of collagen and other extracellular matrix proteins in the arterial wall; advanced glycation end products (AGEs) modify LDL-cholesterol, prolonging its half-life and producing changes in the artery, that render it more susceptible to atherosclerosis;

- induces activation of protein kinase C with a proliferative effect, and
- increases intracellular oxidative stress.

PAF production and its subsequent processing by PAF-AH appear to be highly regulated events. It is conceivable that the consequences of hyperglycemia, affecting lipid metabolism, may interact with PAF-AH activity and the pro-atherogenic effect of its products, LPC and ox-FFA. We tried to find out the variation of PAF-AH activity, in patients with one risk factor (dyslipidemia) and in patients with two risk factors (dyslipidemia and NIDDM), as compared to healthy subjects.

Materials and methods

Subjects

The study was performed on three groups of subjects: a control group of 50 healthy subjects, a group of 50 patients with dyslipidemia and a group of 50 patients with both dyslipidemia and NIDDM. The average age of subjects was 49 years. All plasma samples were frozen and stored at -20°C until analysis.

Measurement of PAF-AH activity

Measurement of PAF-AH activity by the spectrophotometric method, described by Kosaka *et al.* [7, 11], was performed by using the Azwell Auto PAF-AH kit (Azwell Inc., Osaka) and a Pharmacia LKB-Ultaspec III spectrophotometer. *Principle of the method:* PAF-AH hydrolyzes the sn-2 position of substrate (1-myristoyl-2-(4-nitrophenyl succinyl) phosphatidylcholine) producing 4-nitrophenyl succinate, compound which is immediately degraded in aqueous solution and liberates 4-nitrophenol. The enzymatic activity is measured by the change in absorbance difference between 405nm and 505nm and is expressed as IU/L.

Fig. 1 Plasma PAF-AH activity (data are expressed as mean $\pm$ SD; $p < 0.05$).

Biochemical assays

Plasma glucose was assayed by the glucose oxidase method (using a kit from Human Gesselheit, Wiesbaden, Germany). Plasma triglycerides and total cholesterol were determined by enzymatic methods (kits from Boehringer Mannheim, Germany). HDL-cholesterol (HDL-ch) was measured by precipitation with $MgCl_2$ and dextran-sulphate. LDL-cholesterol (LDL-ch) was calculated using Friedewald's formula.

Results and discussion

The PAF-AH levels were significantly higher (mean value: 393.73 IU/L) in patients with both NIDDM and dyslipidemia, as compared to healthy subjects (mean value: 241.06 IU/L), and patients with dyslipidemia alone (mean value: 302.35 IU/L) (Fig.1). In the NIDDM group, we found that dyslipidemia was characterized by moderate hypertriglyceridemia (150-200 mg/dL), lower HDL-ch values (< 40 mg/dL) and similar LDL-ch values (170-200 mg/dL), as compared to non-diabetic, dyslipidemic patients, in accordance with data presented by others [10]. These results are reflected in LDL-ch/HDL-ch ratio. There was a good correlation ($r=0.54$) between PAF-AH activity and LDL-ch/HDL-ch ratio in patients with NIDDM (Fig. 2a). A similar correlation ($r=0.52$) was found between PAF-AH activity

and LDL-ch / HDL-ch ratio in patients with dyslipidemia (Fig. 2b) as compared to control (Fig. 2c).

Diabetic patients have been generally described as being under enhanced oxidative stress. Increased production and release of reactive oxygen species (ROS) is thought to be one of the key events in the pathogenesis of endothelial dysfunction and atherogenesis [12]. Glucose reacts non-enzymatically with primary amino groups in proteins, aminophospholipids such as phosphatidyl ethanolamine or phosphatidyl serine. Early reaction products (glucose-derived Schiff base; Amadori products) undergo a series of inter- and intra-molecular rearrangements, dehydration and oxidation-reduction reactions, to produce AGEs, promoting in this way fatty acid oxidation [13]. Formation of AGEs is one of the causes of endothelial dysfunction [14]. Activation of phagocytes through a specialized receptor for AGEs may also occur [15]. Protein glycation can lead to oxidative stress by direct release of $O_2^{\bullet-}$ and H_2O_2 . The titer of auto-antibodies against ox-LDL is elevated in NIDDM, suggesting a link between ROS produced by protein glycation, lipoprotein oxidation, and enhancement of immune inflammatory response *in vivo* [reviewed in 16]. ROS generated by NADH/NADPH oxidase contribute to LPC-induced activation of ERK1/2 and subsequent growth promotion in vascular smooth muscle cells. The ROS-dependent ERK activation by LPC seems to involve protein kinase C and Ras-dependent Raf-

Fig. 2

a. The relationship between PAF-AH activity and LDL-ch/HDL-ch ratio in patients with NIDDM (**a**), dyslipidemia (**b**), and in healthy subjects (**c**).

1 activation [17]. However, some benefic LPC-induced cellular response (i.e. up-regulation of endothelial nitric oxide synthase promoter) has been shown to involve phosphoinositide 3-kinase (PI3K) activation [18]. PI3K plays a central role in insulin-mediated metabolic function. Recent data support the role of dysfunctional PI3K recruitment/activation in the diabetes development, and PI3K activity appears to be suppressed in subjects with impaired fat metabolism, who display severe symptoms of hyperglycemia and insulin resistance [reviewed in 19]. On the other hand, it was recently shown that HDL have an antiinflammatory action, inhibiting endothelial cell expression of cytokine-induced leukocyte adhesion molecules *in vitro* [20].

The decreased HDL-ch level and increased LDL-ch/HDL-ch ratio in NIDDM patients, correlated with increased PAF-AH activity seem to work in the same direction, to exacerbate endothelial cell dysfunction.

Further work is needed to elucidate the condi-

tions determining the protective versus pro-atherogenic effects of PAF-AH activity. Nevertheless, we can conclude that assessment of circulating levels of PAF-AH may be significant in the evaluation of risk and prognostic for endothelial cell dysfunction, in patients with NIDDM.

Acknowledgements

This work has been supported by grant no. 164, "Viasan" National Program, under the patronage of the Romanian Academy of Medical Sciences.

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