Plasma and Lp(a)-associated PAF-acetylhydrolase activity in uremic patients undergoing different dialysis procedures

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Plasma and Lp(a)-associated PAF-acetylhydrolase activity in uremic patients undergoing different dialysis procedures.

Background. Platelet-activating factor (PAF) is a potent inflammatory mediator associated with several physiopathological conditions, including renal diseases. PAF is degraded to the inactive metabolite lyso-PAF by PAF-acetylhydrolase (PAF-AH), which is considered as a potent anti-inflammatory and anti-atherogenic enzyme associated with lipoproteins. In this study, we evaluated the plasma- and lipoprotein(a) [Lp(a)]-associated PAF-AH activity in relationship to plasma lipid parameters and Lp(a) isoform size in patients with mild/moderate chronic renal failure (CRF), as well as in hemodialysis (HD) and chronic ambulatory peritoneal dialysis (CAPD) patients.

Methods. We studied 74 patients undergoing maintenance HD, 44 patients undergoing CAPD, 56 patients with mild/moderate CRF, and 98 healthy subjects whose lipid profile, as well as plasma and high-density lipoprotein (HDL)-associated PAF-AH activity, was determined. Moreover, the effect of Lp(a) plasma levels on the distribution of PAF-AH among plasma lipoproteins, as well as the specific activity and kinetic properties of PAF-AH on two different Lp(a) isoforms, was measured in each studied group.

Results. The plasma PAF-AH activity in all studied groups was significantly higher than in controls, and the increase was more profound in CAPD patients. The HDL-associated PAF-AH activity, expressed per milliliter of plasma, was similar among all studied groups; however, when it was expressed as either per milligrams of HDL cholesterol or per milligrams of plasma apolipoprotein (apo) AI, the PAF-AH activity was significantly higher in all patient groups compared with controls. All patient groups had significantly elevated plasma Lp(a) levels, which altered the distribution of PAF-AH among the plasma lipoproteins compared with that observed in subjects with very low plasma Lp(a) levels (<8 mg/dl). Additionally, in each studied group, the specific activity as well as the apparent Km and Vmax values of the 19K4 apo(a) isoform were significantly higher (P < 0.01) compared with the values of the 23K4 isoform. However, the specific activity, as well as the Km and Vmax, values on either the 19K4 apo(a) isoform or the 23K4 isoform, was significantly higher in CAPD patients compared with the other three groups.

Conclusions. Plasma PAF-AH activity is increased in uremic patients. This elevation is more profound in CAPD patients, who also exhibit a more atherogenic lipid profile and more pronounced alterations in the specific activity and the kinetic constants of Lp(a)-associated PAF-AH.

Platelet-activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent phospholipid mediator in inflammatory and allergic reactions. PAF is considered to be significantly involved in several physiopathological conditions and may have pivotal roles in several syndromes or diseases, including renal diseases [1–3]. It has been shown that PAF is produced in the healthy kidney by endothelial, mesangial, and renal medullary cells [4–6]. Studies in experimental animals and in humans indicate that PAF may be an important mediator of renal damage, suggesting that its production and action in the kidney may be dysregulated in disease states [3]. PAF has a short half-life time in plasma and is degraded to the inactive metabolite lyso-PAF by PAF-acetylhydrolase (PAF-AH; EC 3.1.1.47), which catalyzes the hydrolysis of the esterified acetate at the sn-2 position [7]. PAF-AH also hydrolyzes phospholipids containing oxidatively fragmented residues at the sn-2 position [8]. Such phospholipids are formed during the oxidative modification of low-density lipoprotein (LDL) and are thought to play key roles in the inflammatory reactions and particularly in vascular inflammation and atherosclerosis [9]. Thus, PAF-AH could be a potent anti-inflammatory and antiatherogenic enzyme [10]. Human plasma PAF-AH is associated with lipoproteins, especially with LDL and high-density lipoprotein (HDL) [11, 12]. Changes in plasma PAF-AH have been reported in various disorders, including renal diseases [2, 13]. In fact, increased plasma PAF-AH activity has been found in patients with primary glomerulonephritis and normal re-

Key words: platelet-activating factor, PAF-acetylhydrolase, lipoproteins, renal failure, uremia, inflammation.

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nal function [14], whereas it has recently been reported that it was unaltered in patients with renal failure undergoing hemodialysis (HD) [15, 16].

Lipoprotein(a) [Lp(a)] is an LDL-like particle distinguished from LDL by the presence of apolipoprotein(a) [apo(a)], a high molecular mass glycoprotein that shows remarkable size polymorphism, which is attached to apo B-100 through disulfide linkage [17, 18]. Apo(a) is highly homologous to the plasma protease zymogen plasminogen, and thus, it has been suggested that Lp(a) may promote thrombogenesis by inhibiting fibrinolysis [19, 20]. High plasma Lp(a) levels are considered to be an independent risk factor for atherosclerosis in both Caucasian and Oriental populations [21, 22]. In recent years, several reports have shown that serum Lp(a) concentrations are increased in patients with end-stage renal disease (ESRD) and that the Lp(a) serum concentration is an independent factor contributing to the risk of atherosclerotic disease, which is the major cause of morbidity and mortality in both HD and chronic ambulatory peritoneal dialysis (CAPD) patients [23–27].

As it has been shown previously [28, 29], Lp(a) is enriched in PAF-AH, which is mainly associated with the apo B rather than the apo(a) moiety of the lipoprotein particle. We have also suggested that the apo(a) moiety of Lp(a) affects the kinetic properties of the enzyme, as the specific activity and apparent $K_m$ and $V_{max}$ values are significantly decreased as the apo(a) isoform size increased [28]. Furthermore, using a gradient ultracentrifugation method, we have shown that in healthy subjects, Lp(a) alters the enzyme distribution between LDL and HDL in a concentration-dependent fashion [28].

To the best of our knowledge, there is a paucity of data concerning PAF-AH activity in total plasma and in Lp(a) of uremic patients undergoing dialysis. We undertook this study in order to evaluate plasma PAF-AH activity in patients with mild/moderate chronic renal failure (CRF), as well as in HD and in CAPD patients and its association with lipid abnormalities, and to clarify, in this clinical context, Lp(a)-associated PAF-AH activity, as well as the Lp(a) isoform-dependent effect on the enzyme-specific activity and kinetic constants.

**METHODS**

**Study population**

Seventy-four patients undergoing maintenance HD, 44 patients undergoing CAPD, 56 patients with mild/moderate CRF (serum creatinine 1.8 to 8 mg/dl, and creatinine clearance 10 to 60 ml/min), and 98 subjects selected from individuals receiving medical check-up at our hospital who were free from any illness by history, physical examination, and routine laboratory data (control group) were studied. Population characteristics are shown in Table 1. The HD schedule was for four hours three times per week using cuprophane or hemophan hollow fiber dialyzers and acetate ($N = 6$) or bicarbonate ($N = 68$) dialysate containing 2 g/liter glucose. CAPD patients performed four exchanges per day with twoliter solutions of 1.86 or 3.86% glucose, depending on the individual need for ultrafiltration. All patients had been free of peritonitis at least three months preceding blood sampling. The method of replacement therapy was the initial treatment in most patients of both groups. Patients with a different method of replacement at the onset of dialysis had to be on the present therapy for at least six months before their inclusion in the study. The adequacy of the dialytic treatment was evaluated by the $Kt/V$ ratio, which ranged from 1.2 to 1.4 for HD patients and from 1.9 to 2.0 (weekly) in CAPD patients. Patients with mild/moderate CRF did not have significant proteinuria (urine protein $<1$ g/24 hr). The renal diagnosis for all patients was chronic pyelonephritis in 28 patients, chronic glomerulonephritis in 88 patients, hypertensive nephropathy in 6 patients, and polycystic kidney disease in 9 patients; the origin of CRF was unknown in the remaining patients. Patients and controls with a known family history of primary hyperlipidemia, excessive alcohol consumption, diabetes mellitus, obesity (body mass index $>30$ kg/m$^2$), liver disease, systemic illnesses, or other metabolic or endocrine disorders were excluded from the study. Patients received no other medication except polyvitamins, calcitriol, phosphate binders, and iron. In hypertensive patients, angiotensin-converting enzyme inhibitors, calcium channel blockers, or both were also given. In some CRF patients, frusemide 20 to 60 mg/day per os was also administered. Thirty-one HD patients and 22 CAPD patients were on erythropoietin treatment.

Blood samples were taken in patients and controls after a 14-hour overnight fast for the determination of serum lipid parameters, serum albumin, prealbumin, creatinine, and PTH levels, as well as plasma PAF-AH activity. In HD patients, fasting blood was obtained before dialysis, whereas in 30 HD patients, blood was also taken immediately after dialysis for the determination of PAF-AH activity. In CAPD patients, fasting venous blood was drawn without interruption of CAPD. In patients with CRF, proteinuria was determined on 24-hour urine samples, and creatinine clearance was also calculated. In dialysis patients, the residual renal function estimated by the average of the residual creatinine and urea clearances was almost negligible ($<5.0$ ml/min).

**Analytical methods**

Serum cholesterol and triglycerides were determined by enzymatic colorimetric assay using an RA-1000 analyzer (Technicon Instruments, Tarrytown, NY, USA), whereas HDL cholesterol was determined enzymatically.
in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using the Friedewald formula [30] if fasting triglyceride levels were less than 400 mg/dl. Serum apo AI and apo B were measured by immunonephelometry with the aid of a Beckman array analyzer (Beckman Instruments, Palo Alto, CA, USA). Lp(a) was measured using a monoclonal anti-Lp(a) antibody technique by the enzyme immunoassay Macra Lp(a) (Terumo Medical Corporation Diagnostic Division, Elkton, MD, USA). Serum and urine creatinine were measured by the Jaffe method and serum albumin by the bromocresol green method. Serum prealbumin was measured by immunonephelometry, and serum parathyroid hormone (PTH) levels were measured by radioimmunometric assay [24, 25].

**Apolipoprotein(a) phenotyping**

Apolipoprotein(a) isoform size was determined by agarose gel electrophoresis in 1.5% sodium dodecyl sulfate (SDS)-agarose gels followed by immunoblotting, according to the method of Kamboh, Ferrell, and Kottke [31], as modified by Doucet et al [32]. Isoform size was expressed as number of kringle 4 (K4) repeats by using a standard with five different apo(a) isoforms (35, 27, 23, 19, and 14 K4) obtained from Immuno AG (Innsbruck, Austria).

**Fractionation of plasma lipoproteins**

Plasma lipoproteins were fractionated by density gradient ultracentrifugation in a Beckman L7-65 ultracentrifuge at 40,000 r.p.m., 15°C for 24 hours, using a type SW 41 rotor as described previously [28]. After ultracentrifugation 28 fractions of 400 μl were collected by successive aspiration from the meniscus downward. All fractions were analyzed for their cholesterol and Lp(a) content as well as for their PAF-AH activity.

**Lipoprotein(a) isolation**

Three subjects from each patient group and controls presenting with a single apo(a) isoform band of either 19 K4 or 23 K4 and with plasma Lp(a) levels higher than 20 mg/dl were selected in order to isolate Lp(a) and study the kinetic properties of PAF-AH. Lp(a) was isolated by density gradient ultracentrifugation followed by gel filtration chromatography on Sepharose 6B column, as described previously [28]. To avoid any oxidation, Lp(a) was prepared in the presence of 0.3 mM ethylenediaminetetraacetic acid (EDTA). Furthermore, Lp(a) preparations were stored in the presence of EDTA at 4°C under nitrogen and were used within two weeks. The absence of oxidation in our Lp(a) preparations was confirmed by agarose gel electrophoresis (Hydragel Lipo+Lp(a) kit; Sebia), as well as by the thiobarbituric acid reactive substances (TBARS) assay [33]. The Lp(a) preparations were essentially free of any other lipoprotein or albumin as evaluated by both nondenaturing 2 to 16% gradient polyacrylamide gel electrophoresis (PAGE) [34] and by gradient 5 to 19% SDS-PAGE, followed by immunoblotting using sheep polyclonal anti-apo(a) and anti-apo B-100 antibodies and revealed by chemiluminescence. The lipid content of Lp(a), that is, cholesterol, triglycerides, and phospholipids, was analyzed by enzymatic methods using BioMerieux kits (Marcy l’Etoile, France) and the protein content by the bicinchoninic acid method. Lipoprotein mass was calculated as the sum of all lipid and protein components. Lp(a) was stored under nitrogen and used within two weeks after preparation.

**Platelet-activating factor-acetylhydrolase assay**

Platelet-activating factor-AH activity was measured by the trichloroacetic acid (TCA) precipitation procedure [12]. A [3H]-PAF solution with a concentration of 1 mM (specific activity 2000 cpm/nmol) containing PAF (hexadecyl; Bachem, Zurich, Switzerland), and 1-O-hexadecyl-2-[3H-acetyl]-sn-glycero-3-phosphocholine (10 Ci/
mmol; DuPont-New England Nuclear, Boston, MA, USA), 1 mmol/liter, prepared as previously described [12], was used as a substrate for PAF-AH. The enzyme activity in plasma or HDL was measured as already mentioned [35]. PAF-AH activity was also determined in 25 μl of each gradient fraction and on Lp(a) using 10 μg of Lp(a) protein [28]. The reaction was performed for 10 minutes at 37°C by adding 10 μl of [3H]-PAF (100 μmol/liter final concentration). PAF-AH activity was expressed as nmol PAF degraded per minute per ml of plasma or per mg HDL cholesterol or per mg apo AI. Lp(a)-associated PAF-AH activity was expressed per mg of Lp(a) protein or mg of Lp(a) mass. The kinetic properties of Lp(a)-associated PAF-AH were evaluated in each isoform using 2.5 to 100 μl [3H]-PAF final concentration as described [28]. The apparent K_m and V_max values of the enzyme were calculated using the Lineweaver-Burk representation of the data.

Statistical analysis

Values are expressed as mean ± SD, except for age and Lp(a), which are expressed in terms of median and range. A comparison of continuous variables was performed by unpaired two-tailed t-test, as well as by one-way analysis of variance with multiple Scheffe-type comparisons. Because of the highly skewed distribution of CRF patients, the nonparametric Mann–Whitney U-test and the Kruskal–Wallis test were applied to discriminate for differences of these parameters between groups. The distribution of plasma PAF-AH activity was tested for normality using the Kolmogorov Smirnov test. Because the PAF-AH activity values did not significantly differ from normal distribution in all studied groups, one-way analysis of variance (with Fisher’s least significant difference method for multiple comparisons) was used to compare PAF-AH activity among groups. The same procedure was used to assess the differences in specific activity and kinetic constants of PAF-AH associated with two different apo(a) isoforms. Correlations between PAF-AH activity and covariates were estimated using Pearson’s correlation coefficients. Spearman’s rank correlation coefficients were used to evaluate the degree of association between Lp(a) and other values. Pearson’s χ² test was used to assess the difference in prevalence between the studied groups. Significance levels were set at 0.05 in all cases.

RESULTS

Patients’ lipid profile

As shown in Table 2, all patient groups had significantly increased serum triglyceride and decreased HDL cholesterol and apo AI levels compared with controls. The atherogenic risk ratio of total cholesterol/HDL cholesterol was also significantly higher in all patient groups in comparison with normal subjects. Interestingly, CAPD patients exhibited significantly higher serum total cholesterol, LDL cholesterol, and apo B levels compared with the other patient groups or controls. Moreover, the atherogenic risk ratio of total cholesterol/HDL cholesterol was significantly higher in this patient group compared with HD or CRF patients. All patient groups had increased serum Lp(a) concentrations when compared with controls (Table 2). Although CAPD patients exhibited higher serum Lp(a) levels than HD and CRF patients, the differences were not statistically significant. In none of the patients’ groups were serum Lp(a) concentrations correlated with the other lipid parameters or age or with serum creatinine, albumin, prealbumin, or PTH levels. Additionally, no correlation between serum Lp(a) concentrations and dialysis duration was present in either dialysis group.

Plasma and HDL-associated PAF-AH activity

As shown in Figure 1, the plasma PAF-AH activity in all patient groups was higher than controls (P < 0.005 for CRF and HD before dialysis and P < 0.001 for CAPD patients). Additionally, the enzyme activity in CAPD patients was significantly higher compared with either HD or CRF patients (P < 0.001 for both comparisons), whereas no difference was observed between HD and CRF patients. The enzyme activity also studied in 30 HD patients immediately after dialysis revealed no difference compared with that observed before dialysis (68.8 ± 13.6 vs. 64.4 ± 15.6 nmol/ml/min, respectively, P = NS). No significant differences in the plasma PAF-AH activity were observed in HD patients using cuprophane compared with those using hemophane hollow fiber dialyzers (data not shown). The PAF-AH activity in controls, HD, and CRF patients was correlated with total cholesterol, LDL cholesterol, and apo B levels (Table 3). Additionally, in all patient groups, the enzyme activity was correlated with the triglyceride levels (Table 3). Interestingly, in CAPD patients, no correlation was observed between the plasma PAF-AH activity and total cholesterol, LDL cholesterol, or apo B levels, whereas an inverse correlation with HDL cholesterol and apo AI levels was noticed (Table 3). PAF-AH activity was not significantly correlated with Lp(a) levels in all studied groups, although the correlation coefficient values in all patient groups were higher compared with controls (Table 3). No correlation was found between the enzyme activity and the mean blood pressure values in all studied groups (data not shown). Finally, the plasma enzyme activity in CRF patients was not correlated with plasma creatinine levels or with creatinine clearance values (Table 3).

The HDL-associated PAF-AH activity, expressed per milliliter of plasma, was similar among all studied groups (6.2 ± 3.2 nmol/ml plasma/min for controls, 6.6 ± 1.6 for HD patients, 6.7 ± 1.1 for CAPD, and 6.6 ± 1.4 for CRF
activity in the study population. PAF-AH activity was determined by higher in subjects with Lp(a) plasma levels 31 to 51 mg/dl, Kruskal-Wallis test.

is expressed as median value (range). The difference in the four groups was analyzed by one-way ANOVA, except for Lp(a) differences that were analyzed by

protein (a); HD, hemodialysis; CAPD, chronic ambulatory peritoneal dialysis; CRF, chronic renal failure. Values represent mean

6

(6.0 (0.8–68) 18.4 (0.8–147) 21.3 (0.8–105) 17.5 (0.8–102)

<0.001 (Kruskal-Wallis)

Table 2. Serum lipid parameters in patients and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control population (N = 98)</th>
<th>HD patients (N = 74)</th>
<th>CAPD patients (N = 44)</th>
<th>CRF patients (N = 56)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-C mg/dl</td>
<td>201 ± 31</td>
<td>198 ± 40</td>
<td>238 ± 42*</td>
<td>206 ± 36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL-C mg/dl</td>
<td>52 ± 12</td>
<td>42 ± 12*</td>
<td>37 ± 7*</td>
<td>43 ± 14*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL-C mg/dl</td>
<td>124 ± 29</td>
<td>117 ± 34</td>
<td>154 ± 38*</td>
<td>127 ± 31</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total-C/HDL-C</td>
<td>3.9 ± 1.0</td>
<td>4.7 ± 1.5*</td>
<td>6.4 ± 1.7*</td>
<td>4.8 ± 1.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides mg/dl</td>
<td>121 ± 51</td>
<td>208 ± 72*</td>
<td>212 ± 64*</td>
<td>174 ± 73*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apo AI mg/dl</td>
<td>154 ± 25</td>
<td>131 ± 24*</td>
<td>125 ± 14*</td>
<td>134 ± 24*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apo B mg/dl</td>
<td>125 ± 31</td>
<td>132 ± 28</td>
<td>161 ± 47*</td>
<td>137 ± 32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lp(a) mg/dl</td>
<td>6.0 (0.8–68)</td>
<td>18.4 (0.8–147)</td>
<td>21.3 (0.8–105)</td>
<td>17.5 (0.8–102)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations are: Total-C, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; Apo AI, apolipoprotein AI; Apo B, apolipoprotein B; Lp(a), lipoprotein (a); HD, hemodialysis; CAPD, chronic ambulatory peritoneal dialysis; CRF, chronic renal failure. Values represent mean ± sd, except for Lp(a), which is expressed as median value (range). The difference in the four groups was analyzed by one-way ANOVA, except for Lp(a) differences that were analyzed by Kruskal-Wallis test.

Significant difference: a Control vs. HD, b control vs. CAPD, c CAPD vs. either HD or CRF, d control vs. CRF, as assessed by multiple comparison (Scheffe type)

< P < 0.01 compared to control population (Mann-Whitney U test)

< P < 0.001 compared to control population (Mann-Whitney U test)

PAF-AH activity on different Lp(a) isoforms

Lipoprotein(a) isoforms with 19 K4 repeats or 23 K4 repeats were isolated from three subjects of each group who had plasma Lp(a) levels higher than 20 mg/dl, whereas the Lp(a) mass as well as the PAF-AH specific activity and kinetic constants were determined on each isoform. No oxidation was observed in each Lp(a) preparation, as its electrophoretic mobility in agarose gels was

Fig. 1. Plasma platelet-activating factor-acetylhydrolase (PAF-AH) activity in the study population. PAF-AH activity was determined by the TCA precipitation method, by using 50 μl of plasma (diluted 1:50 vol/vol with HEPES buffer) as the source of the enzyme. Data are expressed as means ± sd. *P < 0.05 compared with controls; **P < 0.001 compared with controls; †P < 0.001 compared with hemodialysis (HD) as well as chronic renal failure (CRF).

patients). It is important to note that the HDL-associated PAF-AH activity when expressed as either per milligram of HDL cholesterol or per milligram of plasma apo AI was significantly higher in all patient groups compared with controls (Fig. 2). Also, similar results for the enzyme activity in plasma or in HDL were obtained when the patients receiving calcitriol, angiotensin-converting enzyme inhibitors, calcium channel blockers, or frusemide were excluded from the statistical analysis.

Effect of Lp(a) on the distribution of PAF-AH activity among plasma lipoproteins after gradient ultracentrifugation

Plasma from six subjects of each studied group with Lp(a) levels <8 mg/dl and from six subjects of each group with Lp(a) levels ranging from 31 to 51 mg/dl was fractionated into 28 fractions by density gradient ultracentrifugation. Lp(a) was not detectable in any gradient fraction or it was migrated as a narrow band in fractions 12 to 15 (d = 1.064 to 1.095) in the plasma of subjects with Lp(a) levels <8 mg/dl. By contrast, in plasma with Lp(a) levels 31 to 51 mg/dl, it was migrated in fractions 9 to 17 (d = 1.050 to 1.100 g/ml) independently of the apo(a) isoform size, as we have previously reported [28]. A representative curve of the lipoprotein profile obtained is shown in Figure 3A. No difference in the PAF-AH activity (expressed as a percentage of the total activity recovered in all gradient fractions) was observed among all studied groups for the same range of Lp(a) concentrations (Fig. 3B). By contrast, the enzyme activity distributed in fractions 9 to 17 was significantly higher in subjects with Lp(a) plasma levels 31 to 51 mg/dl, as compared with those with Lp(a) levels <8 mg/dl (P < 0.04 for all groups; Fig. 3B). In plasma with Lp(a) levels <8 mg/dl, the fractions 1 to 8 [very low density lipoprotein (VLDL) plus the major part of LDL] contained 19.5 ± 3.2% of total enzyme activity in all studied groups and the fractions 18 to 28 (the major part of HDL plus proteins) contained 33.4 ± 5.2%. Finally, in plasma with Lp(a) levels 31 to 51 mg/dl, the fractions 1 to 8 contained 20.1 ± 5.3% of total enzyme activity in all studied groups, and the fractions 18 to 28 contained 24.6 ± 4.8%, which was significantly lower (P < 0.04) compared with the same fractions from plasma with Lp(a) levels <8 mg/dl.
Table 3. Correlations between plasma PAF-AH activity and lipid parameters

<table>
<thead>
<tr>
<th>Lipid parameter</th>
<th>Correlation coefficient</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.46 \textsuperscript{c}</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.12</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.51 \textsuperscript{c}</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.17</td>
</tr>
<tr>
<td>Apo AI</td>
<td>−0.13</td>
</tr>
<tr>
<td>Apo B</td>
<td>0.61 \textsuperscript{c}</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>−0.007</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td></td>
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</table>

Abbreviations are: PAF-AH, platelet-activating factor acetylhydrolase; HD, hemodialysis; CAPD, chronic ambulatory peritoneal dialysis; CRF, undialyzed patients with mild/moderate chronic renal failure.

\textsuperscript{a}P < 0.05
\textsuperscript{b}P < 0.001
\textsuperscript{c}P < 0.0001

not different than that of the Lp(a) band obtained after plasma electrophoresis of the same subject. Additionally, the TBARS values were 0.9 ± 0.3 nmol of malondialdehyde equivalents per mg of Lp(a) protein. The Lp(a) mass was not different between the two isoforms in all studied groups (0.6 ± 0.2 mg/ml plasma). As shown in Table 4, the PAF-AH specific activity, as well as the apparent K\textsubscript{m} and V\textsubscript{max} values of the 19 K4 apo(a) isoform, in each studied group, was significantly higher compared with the values of the 23 K4 isoform of the same group (P < 0.01 for all comparisons). It is also important to note that the specific activity as well as the K\textsubscript{m} and V\textsubscript{max} values on either the 19 K4 apo(a) isoform or the 23 K4 isoform in CAPD patients were significantly higher compared with the values of the same apo(a) isoform in the other patient groups as well as in controls (Table 4).

**DISCUSSION**

In this study, we showed that the plasma PAF-AH activity is increased in patients with renal failure with or without renal replacement therapy. This elevation in the enzyme activity was more pronounced in patients undergoing CAPD. All patient groups had significantly higher plasma levels of Lp(a), which alter the distribution of PAF-AH among the plasma lipoproteins. The specific activity and kinetic constants of PAF-AH associated with Lp(a) of two different isoforms with 19 K4 repeats or 23 K4 repeats were significantly higher in CAPD patients compared with the other patient groups or with controls, whereas no difference was observed between controls and HD or CRF patients.

Changes in plasma PAF-AH activity have been reported in various diseases [13], although in most of these studies, the mechanisms underlying the reported alterations in the enzyme activity are not clear yet. Especially in renal failure, only recently was it reported that the plasma PAF-AH activity is unaltered in patients with CRF who received HD [16]. Unlike these results, our study showed that HD patients exhibit significantly higher enzyme activity compared with controls. Furthermore, PAF-AH activity in patients with mild/moderate CRF was not different from that of HD patients, whereas no correlation between serum creatinine levels or creatinine clearance values and plasma PAF-AH activity was observed in this patient group. Thus, these results provide evidence that the degree of the renal dysfunction does not influence the plasma PAF-AH activity and further confirm the observation of other studies indicating that the kidney is not a major source of the plasma enzyme pool [14, 15]. Our study also shows that the plasma PAF-AH activity is significantly higher in CAPD patients compared with HD or CRF patients, suggesting an association between plasma PAF-AH activity and dialysis procedure.

It is well documented that CRF is a clinical state of impaired immunoreactivity with high susceptibility to infections [36]. Several studies have demonstrated the presence of inflammatory mediators in an ongoing acute-phase reaction in CRF patients not requiring dialysis, as well as in patients with ESRD undergoing HD or CAPD [37, 38]. In this context, an association between severe renal disease (that is, CRF, primary glomerulonephritis) and increased PAF production leading to elevated plasma PAF levels has been reported, whereas PAF may have a prominent role in renal glomerular pathology [14, 15]. Thus, the increase in plasma PAF-AH activity found in this study may represent a protective response to the inflammatory actions of PAF. Alternatively, it has been previously shown that the plasma PAF-AH activity is increased in both clinical and experimental hypertension [39, 40]. Because PAF is a strong hypotensive agent [41], the increase in plasma PAF-AH activity may well be part of a regulatory counter-response mechanism in arterial blood pressure homeostasis. However, according to our results, the plasma PAF-AH activity was not related to
Fig. 2. High density lipoprotein (HDL)-associated PAF-AH activity expressed (A) per mg plasma HDL cholesterol, and (B) per mg plasma apolipoprotein AI (apo AI) in the study population. PAF-AH activity was determined by the TCA precipitation method by using 50 μl of the HDL-containing supernatant after treatment of plasma with magnesium-dextran sulfate (diluted 1:5 vol/vol with HEPES buffer) as the source of the enzyme. Data are expressed as means ± sd. *P < 0.05 compared with controls.

blood pressure values in all studied groups. Additionally, the more profound increase in the plasma PAF-AH activity in CAPD patients can not be explained by this assumption, as there were no significant differences in the mean blood pressure values among the patient groups.

Indeed, in this study, we do not provide experimental data for the mechanisms responsible for the increased PAF-AH activity in the plasma of our patients. However, it is known that the major sources of the plasma enzyme are monocyte-derived macrophages and liver cells [42, 43]. The secretion of PAF-AH from macrophages is suppressed in the presence of various cytokines, such as tumor necrosis factor-α and interleukin-1β [44], which are increased in the plasma of patients with renal failure [37]. Thus, it is unlikely that the increased PAF-AH activity in the plasma of these patients is due to enhanced secretion from macrophages. It has been reported that PAF stimulates the synthesis and secretion of PAF-AH from the liver [45]. Consequently, because of the increased PAF production in renal failure, it is more likely that the liver may be the main source of the increased PAF-AH in plasma of these patients. Additionally, in patients with renal failure and especially in CAPD patients, a stimulation of hepatic synthesis of proteins as well as of lipoproteins is observed [46]. This pronounced

Fig. 3. (A) Representative curve of the lipoprotein profile obtained after density gradient ultracentrifugation of plasma. (B) Distribution of PAF-AH activity in gradient fractions 9 to 17 (d = 1.050 to 1.100 g/ml) containing the Lp(a). Ultracentrifugation was performed using a type SW 41 rotor at 40,000 r.p.m., 15°C for 24 hours, and PAF-AH activity was determined by the TCA precipitation method, by using 25 μl of gradient fractions as the source of the enzyme. Data are expressed as means ± sd.
hepatic secretion of proteins may also explain the significantly higher enzyme activity in CAPD patients compared with CRF or with HD patients. Because it has been hypothesized that the secretion of the enzyme occurs independently of the secretion of lipoprotein particles and then it associates with them [47], a direct increased synthesis of PAF-AH might be the underlying mechanism of the increased enzyme activity in this population.

The HDL-associated enzyme activity expressed per mg of HDL cholesterol or mg of apo AI was significantly higher in the patient groups compared with controls, suggesting an enrichment of HDL with PAF-AH activity. However, when the enzyme activity was expressed per ml of plasma, it was similar among all groups, because of the lower HDL cholesterol levels observed in the patient groups compared with controls. It has been suggested that the HDL-associated paraoxonase and PAF-AH activities work in concert to detoxify the lipid peroxides formed on LDL during oxidation, thus providing HDL with an anti-atherogenic potency [48]. The increased HDL-associated PAF-AH activity found in our patients could counteract the reduced paraoxonase activity observed by some investigators in such patients, which could result in a decreased HDL antioxidant capacity [16, 49].

All patient groups exhibited a moderate hypertriglyceridemia, which is a characteristic feature of uremic dyslipidemia [50]. Our results show that the PAF-AH activity is positively correlated with plasma triglyceride levels in all patient groups but not in controls. This association between PAF-AH and serum triglycerides has already been observed, although the biochemical mechanism(s) for this correlation has not been yet elucidated [51, 52]. Although PAF-AH activity has been found on VLDL [12, 53], an elevation in plasma VLDL concentration does not increase the plasma PAF-AH activity, an indication suggesting that any correlation between plasma PAF-AH activity and triglyceride levels is unlikely to reflect a direct causal association between these two parameters [51]. Accordingly, our density gradient ultracentrifugation experiments showed no increase in the enzyme activity associated with fractions 1 to 8 in all patient groups compared with controls. Like previous studies, a strong correlation between plasma PAF-AH activity and LDL cholesterol levels was found in all groups except for CAPD patients. It has been suggested that the correlation observed between these two parameters reflects a process that is probably the rate of LDL clearance, which influences both of these variables [51]. This could also explain the positive correlation between PAF-AH activity and apo B levels revealed in our study. The absence of such a correlation in CAPD patients is intriguing and may be related to a more pronounced derangement of lipid metabolism characterizing this dialysis procedure. In fact, the elevated hepatic lipoprotein secretion consequent upon protein losses in the dialysis fluid and increased carbohydrate absorption via the peritoneum is of paramount importance for the development of dyslipidemia in these patients [46]. Thus, an increased LDL synthesis rather than a retarded LDL clearance is the main mechanism responsible for the increased LDL levels in CAPD patients [46], leading to the absence of correlation between PAF-AH activity (dependent on the rate of LDL removal) and LDL cholesterol levels.

Significantly elevated plasma Lp(a) concentrations, a typical feature of uremic dyslipidemia [23–27], were observed in all patient groups. We have already stated that the distribution of PAF-AH among the LDL and HDL subfractions in plasma studied by density gradient ultracentrifugation is influenced by the plasma Lp(a) levels in an Lp(a) concentration-dependent fashion [28]. Our results confirm these observations showing that the presence of Lp(a) in plasma alters the distribution of PAF-AH among the plasma lipoproteins, as the PAF-AH activity in the density region of Lp(a) (fractions 9 to 17) is increased accompanied by a decrease in fractions 18 to 28 in subjects from all studied groups having plasma Lp(a) levels 31 to 51 mg/dl compared with those with Lp(a) levels <8 mg/dl. However, the distribution of PAF-AH activity in these gradient fractions was not different among the studied groups with the same range of plasma Lp(a) levels despite the significant differences in the plasma enzyme activity among these groups.

### Table 4. Specific activity and kinetic constants of PAF-AH associated with two different Lp(a) isoforms in patients with renal failure and controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>$K_v$, $\mu M$</th>
<th>$V_{max}$, mmol/mg protein/min</th>
<th>Specific activity, mmol Lp(a) mass/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19K4</td>
<td>23K4</td>
<td>19K4</td>
</tr>
<tr>
<td>Controls</td>
<td>45.9 ± 2.1</td>
<td>26.8 ± 0.6</td>
<td>29.3 ± 0.9</td>
</tr>
<tr>
<td>HD patients</td>
<td>45.1 ± 3.9</td>
<td>26.5 ± 1.7</td>
<td>29.6 ± 2.5</td>
</tr>
<tr>
<td>CRF patients</td>
<td>46.8 ± 2.2</td>
<td>25.5 ± 0.5</td>
<td>30.2 ± 1.6</td>
</tr>
<tr>
<td>CAPD patients</td>
<td>67.2 ± 3.3</td>
<td>37 ± 1.5</td>
<td>34.2 ± 1</td>
</tr>
</tbody>
</table>

Abbreviations are: HD, hemodialysis; CAPD, chronic ambulatory peritoneal dialysis; CRF, undialyzed patients with mild/moderate chronic renal failure. Values represent the mean ± sd from three different Lp(a) preparations. The kinetic constants in each preparation were measured in duplicate.

$^a$ $P < 0.05$

$^b$ $P < 0.001$ compared to the values of the other studied groups for the same isoform.
ingly, no correlation was observed between plasma PAF-AH activity and Lp(a) levels in all groups. An important observation of our study is that the specific activity, as well as the apparent $K_m$ and $V_{\text{max}}$ values of the 19K4 isoform in each studied group, was significantly higher compared with the values of the 23K4 isoform of the same group. This finding, which supports our previous observation concerning healthy subjects, shows that this phenomenon also exists in the Lp(a) isolated from patients with renal failure. Furthermore, our study provides evidence that the Lp(a) isolated from CAPD patients has significantly higher PAF-AH activity but displays a lower affinity for the substrate (higher $K_m$ values) compared with the Lp(a) of the same isoform size isolated from controls or the other patient groups. Recently, it was shown that oxidized Lp(a) but not oxidized LDL is found in the plasma of CAPD patients [54]. We have previously reported that during Lp(a) oxidation, the Lp(a)-associated PAF-AH activity is dramatically decreased with a simultaneous decrease in its apparent $V_{\text{max}}$ values [28]. Because our Lp(a) preparations obtained from CAPD patients were not deficient in enzyme activity, we may speculate that during its oxidation in plasma, Lp(a) loses some of the enzyme activity, which may be replenished by transfer from other lipoproteins, mainly from HDL. This suggestion may explain the alteration of the kinetic properties of the Lp(a)-associated PAF-AH observed in CAPD patients.

We conclude that plasma PAF-AH activity is increased in uremic patients. This elevation is more profound in CAPD patients who also exhibit a more atherogenic lipid profile and more pronounced alterations in the specific activity and the kinetic constants of Lp(a)-associated PAF-AH.

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