

Low activity of platelet-activating factor acetylhydrolase associated with plasma high-density lipoprotein as a predictor for carotid atherosclerosis in patients with arteriosclerosis obliterans

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Abstract

Platelet-activating factor (PAF) is a potent proinflammatory lipid mediator that is implicated in atherogenesis. Platelet-activating factor acetylhydrolase (PAF-AH) is a Ca^{2+} -independent phospholipase A₂ that is secreted mainly from monocytes/macrophages, including those in atheroma. In human plasma, PAF-AH is associated primarily with low-density lipoprotein (LDL), particularly with small dense LDL particles, while a small proportion of the enzyme is associated with high-density lipoprotein (HDL). The role of PAF-AH in atherosclerotic diseases is controversial. In several studies, plasma PAF-AH represents an independent risk factor for atherosclerotic vascular disease. However, genetic PAF-AH deficiency is also known as a risk factor. The aim of this study was to evaluate the possible relationship between PAF-AH activity, which is correlated with its protein levels, and intima-media wall thickness (IMT) in a sample of arteriosclerosis obliterans (ASO) patients and healthy controls. Plasma PAF-AH activity, HDL-PAF-AH activity, and biochemical markers [total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, apolipoprotein A-I, apolipoprotein B, apolipoprotein E, and lipoprotein(a)] were determined from ASO patients and healthy controls (all subjects gave written informed consent). Atherosclerosis markers (ankle brachial pressure index [ABI] and IMT) were also determined in ASO patients. The patients were then classified normal (IMT < 1.1 mm) or thick IMT (1.1 mm \leq IMT < 2 mm) or plaque formed (focal IMT \geq 2.0 mm) or occlusion. Ankle brachial pressure index was negatively correlated with triglycerides ($r = -0.50$, $p < 0.05$). IMT was negatively correlated with HDL-PAF-AH activity ($r = -0.57$, $p < 0.01$). HDL-PAF-AH activity predicted IMT most strongly, followed by lipoprotein(a), HDL-cholesterol, and total plasma PAF-AH activity in multi-variate analysis. Carotid artery atherosclerosis was related to reduction in HDL-PAF-AH activity. This result was possible that a positive relationship between atherosclerosis and low HDL-PAF-AH activity, and that some of the anti-atherogenicity of HDL is related to its PAF-AH activity.

Key words

platelet-activating factor acetylhydrolase, high-density lipoprotein, arteriosclerosis obliterans, intima-media thickness

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Introduction

Platelet-activating factor (PAF) is a potent proinflammatory lipid mediator that is implicated in atherogenesis¹⁾. In plasma, PAF is hydrolyzed and inactivated by PAF acetylhydrolase (PAF-AH) (EC 3.1.1.47).

PAF-AH is a Ca^{2+} -independent phospholipase A_2 (MW: 45.4 kDa) that is secreted mainly from monocytes/macrophages, including those in atheroma²⁾. In human plasma, PAF-AH is associated primarily with low-density lipoprotein (LDL), particularly with small dense LDL particles³⁾, while a small proportion of the enzyme is associated with high-density lipoprotein (HDL). Plasma PAF-AH is also named lipoprotein-associated phospholipase A_2 . The mean plasma PAF-AH and HDL-PAF-AH concentrations were reported as $1.30 \pm 0.55 \mu\text{g/ml}$ and $0.14 \pm 0.07 \mu\text{g/ml}$, respectively, in healthy subjects in Japan⁴⁾. On the other hand, the measurement distribution of plasma PAF-AH and HDL-PAF-AH activities were 200–800 IU/l and 75–200 IU/l⁴⁾. Both ratios differ in concentration and an activity value. However, this activity value uses [1-myristoyl-2-(p-nitrophenylsuccinyl)phosphatidylcholine] for the substrate, and differs from the measured value of this study.

PAF-AH exhibits an α/β hydrolase conformation and has broad substrate specificity toward lipid esters containing short acyl chains. Thus, PAF-AH can hydrolyze short-chain diacylglycerols and triacylglycerols (TG), but also displays phospholipase A_2 activities as well as transacetylase activity. Among these, the Ca^{2+} -independent phospholipase A_2 activity of PAF-AH has been principally studied. Indeed, PAF-AH has a marked preference for phospholipids with short-chain moieties at the sn-2 position and, with the exception of PAF, can hydrolyze proinflammatory and proatherogenic oxidized phospholipids produced by peroxidation of phosphatidylcholines containing an sn-2 polyunsaturated fatty acyl residue.

PAF-AH is produced by inflammatory cells of myeloid origin. Plasma PAF-AH is associated with circulating atherogenic lipoproteins such as small dense LDL and lipoprotein(a) [Lp (a)], and is highly

expressed in diseased vessels⁵⁾.

The role of PAF-AH in atherosclerotic disease is controversial. In several studies, plasma PAF-AH is an independent risk factor for atherosclerotic vascular disease^{6,7)}. However, genetic PAF-AH deficiency is also reported to be a risk factor⁸⁾.

Carotid artery intima-media wall thickness (IMT) is a clinical sign of early atherosclerotic disease and is regarded as an early marker of morphological changes of the vessel wall. Vascular remodeling of the carotid artery with IMT is an important predictive factor for cardiovascular disease.

In general, patients with an ankle brachial pressure index (ABI) of less than 0.9 are considered to have arteriosclerosis obliterans (ASO). The prevalence of ASO was 2.7% in male Japanese subjects of mean age 66 years⁹⁾. Among the atherosclerotic risk factors, age and total cholesterol (T-CHOL) seem to be the most important risk factors of ASO⁹⁾.

Risk factors may vary among different types of atherosclerosis: age alone for renal atherosclerosis; age and plasma LDL cholesterol (LDL-C) for abdominal aortic sclerosis; age, LDL-C, and HDL cholesterol (HDL-C) for iliac atherosclerosis; and HDL-C and diabetes mellitus for coronary artery disease in familial hypercholesterolemia¹⁰⁾.

The aim of the present study was to evaluate the possible relationship between PAF-AH activity, which is correlated with its protein levels⁴⁾, and IMT as an early marker of carotid atherosclerosis in groups of ASO patients and healthy controls.

Methods

1. Study population

The study population comprised 22 male patients (aged 56–85 years) and 14 healthy men (aged 51–64 years). Patients in a stabilized period (53 ± 33 months) after an operation for ASO were outpatients of Kanazawa Medical Center. The study was approved by the National Hospital Organization, Kanazawa Medical Center Ethics Committee (No.H16-3, 2004.7.). All subjects gave written informed consent.

2. Clinical procedures

This cross-sectional study investigated the relation of atherosclerosis and plasma PAF-AH activity in ASO patients. Using the ABI-form BP-203RPE Automatic Arteriosclerosis Measurement System (Colin, Japan), ABI was measured before operation for ASO, and carotid ultrasonography after the operation was used as an index of atherosclerosis. The ultrasound evaluation from B-mode imaging of carotid arteries was performed with high-resolution ultrasonography (Toshiba SSA-340A with an 8.0-MHz linear-array probe). IMT was determined as the maximum far-wall measurement on bilateral examination of the common carotid artery, the carotid bifurcation, and the internal carotid artery in all patients. Patients were then classified normal ($IMT < 1.1$ mm) or thick ($1.1 \text{ mm} \leq IMT < 2 \text{ mm}$) or plaque formed (focal $IMT \geq 2.0$ mm) or occlusion. Patients were deemed to have hypertension if they were already being treated with antihypertensive

agents. Diabetes mellitus was defined if they were currently using insulin or oral hypoglycemic agents. No current smoker was found, but previous history was not investigated. Venous blood samples were collected in non-fasting state during 9-12 am. Plasma and serum samples were stored at -80°C without thawing until they were used for assays.

3. Laboratory procedures

Plasma PAF-AH activity was determined using $10 \mu\text{l}$ plasma by a commercial colorimetric assay (Cayman Chemical Co.), with 2-thio-PAF as substrate according to the manufacturer's directions; enzymatic hydrolysis of the acetyl thioester bond was detected with 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 405 nm (Figure 1). The PAF-AH activity is expressed in $\text{nmol min}^{-1} \text{ ml}^{-1}$. The intra-assay coefficient of variation was 3.2%. The linear range of this assay is shown in Figure 2.

For the determination of HDL-PAF-AH activity,

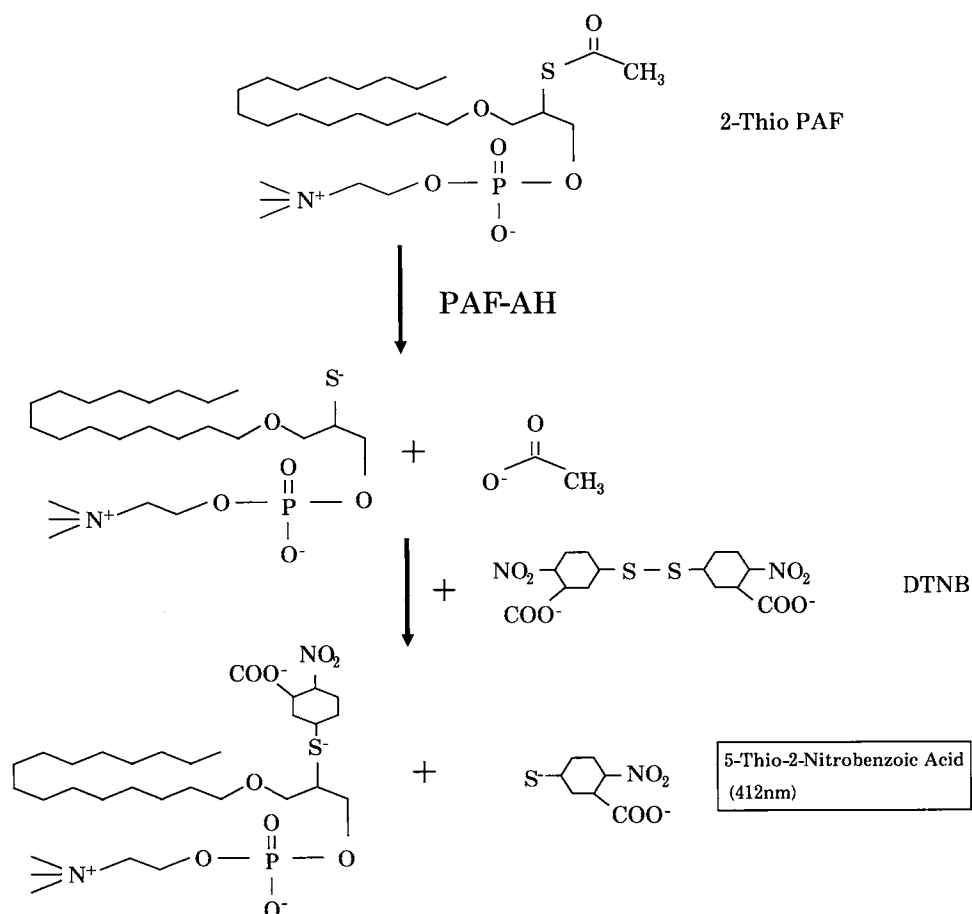


Figure 1. Measurement of PAF-AH activity

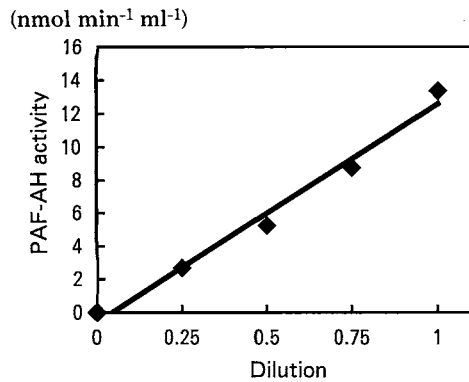


Figure 2. Linear range of PAF-AH activity assay

apo B containing lipoproteins were precipitated by phosphotungstate and $MnCl_2$ using reagents from a commercial kit for the measurement of HDL-C (Daiichi Pure Chemicals, Tokyo, Japan). The intra-assay coefficient of variation was 5.0%.

Non HDL-PAF-AH activity, including that of LDLs, was calculated by the difference between plasma PAF-AH and HDL-PAF-AH activities. Serum concentrations of T-CHOL, TG, HDL-C⁽¹⁾, LDL-C⁽²⁾, apolipoprotein A-I (Apo A-I)⁽³⁾, apolipoprotein B (Apo B)⁽³⁾, apolipoprotein E (Apo E)⁽³⁾, and Lp (a) were determined in a Hitachi 7170 automated analyzer using commercial kits (Sysmex and Daiichi Pure Chemicals and Eiken Chemicals Denka Seiken).

4. Statistical analyses

Data were expressed as mean \pm SD. Changes in concentrations were analyzed by the t-test. Relationships between different parameters and

plasma PAF-AH activity, HDL-PAF-AH activity, ABI, and IMT were assessed using Pearson's correlation coefficient. The chi-square test was used to assess the difference in prevalence between the studied groups. Stepwise regression analysis was performed to assess factors that predict IMT. As for stepwise regression analysis, linear analysis of the measured value of IMT was performed using the backward elimination method. $p < 0.05$ was regarded as statistically significant. All tests were performed with Stat View software (version 5.0; SAS Institute, Japan).

Results

1. Clinical characteristics of the study population

The clinical characteristics of the study population are shown in Table 1 (healthy controls vs ASO patients). T-CHOL, TG, LDL-C, plasma PAF-AH activity, and HDL-PAF-AH activity were not significantly different between the healthy controls and the ASO patients. However, ASO patients exhibited significantly lower HDL-C at baseline compared with healthy controls.

2. Relationship between plasma PAF-AH activity, HDL-PAF-AH activity, and biochemical markers

The correlation of plasma PAF-AH activity or HDL-PAF-AH activity with biochemical markers is shown in Table 2. Plasma PAF activity was negatively correlated with HDL-C, but positively correlated with T-CHOL, TG, LDL-C, and Apo-B.

Table 1. Clinical characteristics of the study population. Values represent the mean \pm SD.

	Healthy controls	ASO patients
Number of subjects (M/F)	14 (14/0)	22 (22/0)
Age (years)	57 \pm 5	73 \pm 7*
T-CHOL (mg/dl)	196 \pm 25	185 \pm 31
TG (mg/dl)	142 \pm 59	130 \pm 60
LDL-C (mg/dl)	108 \pm 24	106 \pm 26
HDL-C (mg/dl)	55.4 \pm 11.2	44.4 \pm 11.7*
Plasma PAF-AH activity (nmol min ⁻¹ ml ⁻¹)	15.7 \pm 3.6	15.6 \pm 4.5
HDL-PAF-AH activity (nmol min ⁻¹ ml ⁻¹)	6.2 \pm 0.9	6.7 \pm 1.4
HDL/Plasma ratio in PAF-AH activity	0.41 \pm 0.08	0.46 \pm 0.13

*: p -value < 0.01 significant difference from healthy controls.

T-CHOL: Total cholesterol, TG: Triglycerides, LDL-C: LDL cholesterol, HDL-C: HDL cholesterol

Table 2. Correlation coefficient (r) of plasma PAF-AH activity or HDL-PAF-AH activity with biochemical markers (n = 22)

	Plasma PAF-AH activity	HDL-PAF-AH activity
T-CHOL	0.27	0.10
HDL-C	-0.31	0.21
LDL-C	0.48**	0.08
TG*	0.04	-0.12
Lp (a)*	-0.18	0.07
Apo A-I	-0.22	0.22
Apo B	0.47**	0.10
Apo E	0.30	0.28

* : Log-transformed.

** : p-value < 0.05.

Lp (a): lipoprotein(a), Apo A-I: apolipoprotein A-I, Apo B: apolipoprotein B, Apo E: apolipoprotein E

In particular, there was a positive relationship between LDL-C, Apo-B, and plasma PAF-AH activity ($r = 0.48$, $p < 0.05$; $r = 0.47$, $p < 0.05$, respectively). The relationship between HDL-PAF-AH activity and T-CHOL, TG, HDL-C, and LDL-C was not significant.

3. Relationship between plasma PAF-AH activity, HDL-PAF-AH activity, and atherosclerosis markers

The clinical characteristics of ASO patients classified by carotid ultrasonography are shown in Table 3 (normal, thick IMT, plaque formed, occlusion).

Patients with thick IMT exhibited significantly

higher T-CHOL, LDL-C, and Apo B at baseline compared with those with plaque formed, but were not significantly different from normal.

Correlation of plasma PAF-AH activity or HDL-PAF-AH activity with atherosclerosis is shown in Table 4. ABI was negatively correlated with TG ($r = -0.46$, $p < 0.05$). IMT was negatively correlated with HDL-PAF-AH activity ($r = -0.57$, $p < 0.01$). We performed a stepwise multi-variate analysis of IMT as an atherosclerosis marker, with T-CHOL, TG, LDL-C, Lp (a), plasma PAF-AH activity, and HDL-PAF-AH activity. As a result, HDL-PAF-AH activity and Lp (a) were chosen and the multiple regression type of $Y = 3.33 - 331 X_1 + 0.54 X_2$ [Y : IMT, X_1 : HDL-PAF-AH activity, X_2 : log Lp (a), the goodness of fit was 43%] was obtained. (Table 5)

The relationship between carotid atherosclerosis classified by carotid ultrasonography and plasma PAF-AH activity and HDL-PAF-AH activity is shown in Figure 3. Carotid artery atherosclerosis was related to reduction in HDL-PAF-AH activity. A significant difference was found between normal and occlusion ($p < 0.05$), and between thick IMT and occlusion ($p < 0.05$).

4. Relationship between drug and plasma PAF-AH activity or HDL-PAF-AH activity

No effect of drugs was found between patients taking a specific drug and those not taking it,

Table 3. Clinical characteristics classified by carotid ultrasonography in ASO patients
Values represent the mean \pm SD, except for hypertension and diabetes mellitus.

	Normal	Thick IMT	Plaque formed	Occlusion
Number of subjects	5	9	5	3
Age (years)	72 \pm 11	73 \pm 6	74 \pm 3	69 \pm 10
BMI (kg/m ²)	24.1 \pm 3.3	22.3 \pm 1.3	20.5 \pm 3.0	22.4 \pm 2.0
Hypertension, n (%)	4 (80)	5 (56)	2 (40)	2 (67)
Diabetes mellitus, n (%)	0 (0)	2 (22)	2 (40)	0 (0)
T-CHOL (mg/dl)	172 \pm 37	206 \pm 28*	165 \pm 11	181 \pm 29
TG (mg/dl)	134 \pm 34	150 \pm 71	89 \pm 38	127 \pm 79
LDL-C (mg/dl)	94 \pm 33	120 \pm 26**	91 \pm 10	111 \pm 17
HDL-C (mg/dl)	39.0 \pm 10.0	48.1 \pm 15.1	45.6 \pm 9.3	40.3 \pm 1.5
Apo A-I (mg/dl)	111 \pm 20	133 \pm 34	122 \pm 19	115 \pm 6
Apo B (mg/dl)	87 \pm 29	98 \pm 16**	76 \pm 12	97 \pm 15
Apo E (mg/dl)	4.5 \pm 1.0	4.4 \pm 1.0	3.3 \pm 1.2	3.5 \pm 0.8
Lp (a) (mg/dl)	21.2 \pm 19.4	34.9 \pm 42.7	50.2 \pm 39.2	42.4 \pm 54.4

* : p-value < 0.01, significant difference from plaque formed.

** : p-value < 0.05, significant difference from plaque formed.

BMI: Body mass index

Table 4. Correlation coefficient (r) of plasma PAF-AH activity or HDL-PAF-AH activity with atherosclerosis marker (n = 22)

	ABI	IMT
T-CHOL	-0.16	-0.15
HDL-C	0.04	0.12
LDL-C	-0.07	0.02
Lp (a)*	-0.25	0.28
Apo A-I	-0.09	0.02
TG*	-0.46**	-0.28
Plasma PAF-AH activity	0.30	-0.38
HDL-PAF-AH activity	0.12	-0.57***

* : Log-transformed.
 ** : p-value < 0.05.
 *** : p-value < 0.01.

Table 5. Multiple stepwise regression analysis for determinant of IMT (n = 22)

Beta: standardized regression coefficients.

Parameters	Beta	p-value	F value
HDL-PAF-AH	-0.60	<0.005	11.8
Lp(a)*	0.33	0.08	3.5

*: Log-transformed.

(angiotensin II receptor blocker, calcium antagonist, α -blocking agent, anti-blood platelet tablet, prostaglandin, statin). The effects of statin on LDL-C, HDL-C, plasma PAF-AH activity, and HDL-PAF-AH activity were not significant in this population (there was no drug information for one patient) (Table 6).

Discussion

The main findings of the present study are as follows. A negative relationship was shown between HDL-PAF-AH activity and IMT by multivariate analysis. Moreover, HDL-PAF-AH assay may be superior to total PAF-AH activity or HDL-

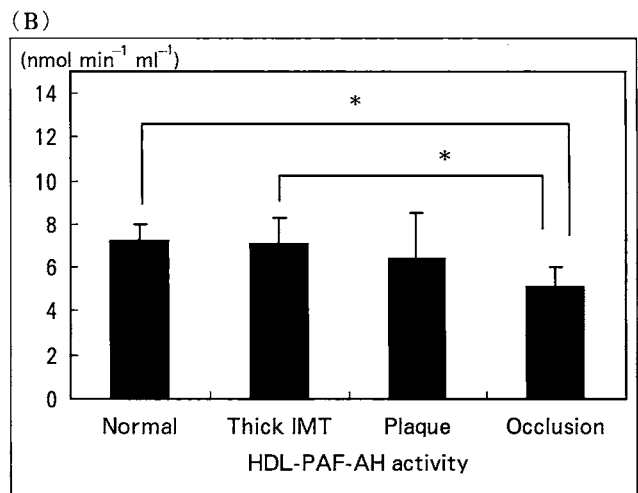
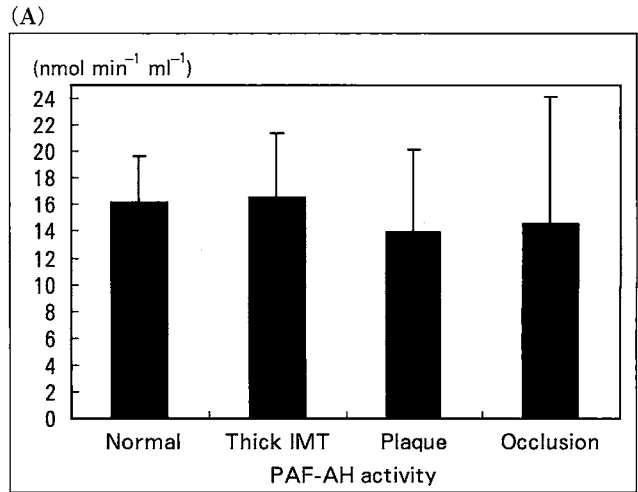


Figure 3. Atherosclerosis in carotid artery by ultrasound, and the relationship with PAF-AH activity (A) or HDL-PAF-AH activity (B)

Values represent the mean \pm SD.
 *: p-value < 0.05.

C as a marker for atherosclerosis.

A relationship between plasma PAF-AH activity and LDL-C and Apo-B was shown in this study as well as in several other studies^{14,15}. Change of plasma PAF-AH activity was dependent on LDL-C

Table 6. Effect of statin on LDL-C, HDL-C, plasma PAF-AH activity and HDL-PAF-AH activity

	Statin (+), (n=5)	Statin (-), (n=16)	p-value
LDL-C (mg/dl)	93 \pm 16	110 \pm 29	0.22
HDL-C (mg/dl)	47 \pm 13	44 \pm 12	0.64
plasma PAF-AH activity (nmol min ⁻¹ ml ⁻¹)	14.9 \pm 4.6	15.8 \pm 4.8	0.71
HDL-PAF-AH activity (nmol min ⁻¹ ml ⁻¹)	6.9 \pm 1.4	6.6 \pm 1.5	0.74
Non HDL-PAF-AH activity (nmol min ⁻¹ ml ⁻¹)	8.3 \pm 3.6	9.2 \pm 4.0	0.57

Values represent the mean \pm SD.
 All data were not significant.

and Apo-B. Plasma PAF-AH activity was considered to be a risk marker of atherosclerosis.

Plasma PAF-AH activity was comparable in control subjects and ASO patients. There are inconsistent results in studies on plasma PAF-AH activity as a marker of atherosclerosis. In the current study, the direct relationship of plasma PAF-AH activity or HDL-PAF-AH activity with ASO was negative. In contrast, previous reports showed elevated PAF-AH in atherosclerotic patients¹⁶⁾ and in patients with ischemic stroke¹⁷⁾.

In another study, there were no close correlations between brachial-ankle pulse wave velocity, ABI, or capacitive arterial compliance and fasting blood glucose, T-CHOL, TG, or body mass index¹⁸⁾. However, our study, revealed a relationship between TG and ABI. This may be explained by post-prandial TG increase in non-fasting samples.

It has been reported that plasma PAF-AH activity is comparable in normal and abnormal IMT groups^{19,20)}. In the current study, although no relationship was shown between plasma PAF-AH activity and IMT, we demonstrated a relationship between HDL-PAF-AH activity and IMT for the first time.

Previously, HDL-PAF-AH was found to increase in hyperlipidemia and diabetes⁴⁾. In other studies, significant reductions of plasma PAF-AH activity and increased HDL-PAF-AH activity were observed in patients undergoing fenofibrate treatment²¹⁾. Also, atorvastatin significantly reduced plasma PAF-AH activity²²⁾. In the current study, although a tendency of reduced plasma PAF-AH activity and increased HDL-PAF-AH activity was shown with statin use, the difference was not significant probably because of small sample size in present study.

Previously, HDL-PAF-AH was correlated with Apo A-I, but no correlation was found in the current study. The reasons were probably the relatively small sample number and the all-male population.

Recent studies suggest that the HDL-PAF-AH/total PAF-AH ratio is decreased in peritoneal dialysis patients²³⁾. Also, low HDL-PAF-AH activity was found in subclinical hypothyroidism patients²⁴⁾.

Oxidized lipids mediate inflammatory responses in the artery wall. HDL-associated enzymes such as PAF-AH, paraoxonase 1 (PON1), and lecithin: cholesterol acyltransferase (LCAT) can destroy these proinflammatory oxidized lipids, but are also inhibited by them. Excess oxidized lipids in ASO patients may inhibit PAF-AH expression in macrophages. It has been shown that human plasma PON1 hydrolyses PAF, suggesting that HDL-PAF-AH activity is due to PON1²⁵⁾. Therefore, PON1 and LCAT activities deserved analysis in future research in patients with low HDL-PAF-AH activity.

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閉塞性動脈硬化症患者における頸動脈硬化と高比重リポ蛋白分画血小板活性化因子アセチルヒドロラーゼ活性に関する研究

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要 旨

血小板活性化因子 (PAF) は動脈硬化に関係する 脂質メディエーターである。血小板活性化因子アセチルヒドロラーゼ (PAF-AH) は、動脈硬化巣において単球やマクロファージから主に分泌される。ヒトの血液中におけるPAF-AH (血漿 PAF-AH) のほとんどは低比重リポ蛋白 (LDL)、特にsmall dense LDLに存在しており、残りは高比重リポ蛋白 (HDL) に存在している。

動脈硬化症における PAF-AH の役割は一定の見解が示されていないのが現状である。血漿 PAF-AH は動脈硬化性の血管病の発症に独立した危険因子であると報告され、一方、遺伝性 PAF-AH 欠損症も危険因子であるとも報告されている。本研究の目的は閉塞性動脈硬化症 (ASO) 患者と健常者において血漿 PAF-AH 活性と動脈硬化の関係を評価することである。

同意書にて同意の得られた ASO 患者と健常者において、血漿 PAF-AH 活性、HDL-PAF-AH 活性と総コレステロール、トリグリセリド、HDLコレステロール (HDL-C)、LDLコレステロール、Apo A-I、Apo B、Apo E、Lp(a) を測定した。さらに、ASO 患者において動脈硬化の標識としてankle brachial pressure index (ABI) と内膜中膜複合体 (IMT) を測定した。IMTの結果から、ASO 患者は正常 (IMT < 1.1 mm)、IMT の肥厚 (1.1mm ≤ IMT < 2 mm)、プラークを形成 (IMT ≥ 2.0 mmを対象とした)、閉塞ありの 4 つにクラス分けした。その結果、ABIはトリグリセリドと負の相関関係にあった ($r = -0.50, p < 0.05$)。IMT はHDL-PAF-AH 活性と負の相関関係にあった ($r = -0.57, p < 0.01$)。多変量分析においてHDL-PAF-AH 活性は、Lp(a)、HDL-C、血漿 PAF-AH 活性より強くIMTを予測する因子であった。

頸動脈動脈硬化症はHDL-PAF-AH 活性の低下と関係が見いだされた。この結果はコレステロール逆転送能以外のHDLの抗動脈硬化性因子としてPAF-AH 活性の重要性を示唆するものである。