Significant association between lower pulse pressure and increasing levels of a novel type of phospholipid

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ABSTRACT. The aim of this study was to analyze the association between pulse pressure and a novel type of phospholipid with solubility similar to that of lysophosphatidic acid (LPA), designated as AP, which was reported to be elevated during ischemia. In this cross-sectional study, 416 hypertensive patients and 252 controls aged between 35 and 70 years were enrolled consecutively. Fasting blood samples were extracted for assays of phospholipids and other biomarkers. Compared to controls, the hypertensive patients had higher levels of both LPA [odds ratio (OR) = 3.83] and AP (OR = 4.30). Changes in blood pressure did not affect the levels of LPA or AP. However AP, but not LPA, levels were significantly higher in patients with lower or higher pulse pressure

(Pearson χ^2 = 11.239, P = 0.001). For patients whose pulse pressure was ≤60 mmHg, plasma levels of AP were significantly negatively correlated with pulse pressure. However, this was not observed for LPA and nine other biomarkers, including lipoproteins. Plasma levels of AP increased in hypertensive patients with higher or lower pulse pressure. Thus, attention should be paid to the possibility of cerebral ischemia in hypertensive patients when they have abnormal pulse pressure, especially for those with relatively low pulse pressure.

Key words: Hypertension; Lysophosphatidic acid; Pulse pressure; Ischemia; Novel phospholipid (AP)

INTRODUCTION

Hypertension is a disease with high prevalence that can cause serious medical conditions, such as cardiovascular events and stroke (both ischemic and hemorrhagic stroke). Controlling patients' blood pressure is one of the most important methods for preventing both cardiovascular and cerebral vascular events (Millar et al., 1999). Compared with systolic pressure and diastolic pressure, pulse pressure is relatively less studied. However, pulse pressure is also a strong risk factor for coronary events (Millar et al., 1999; Millar and Lever, 2000). Some studies have also indicated the significance of pulse pressure in relation to vascular stiffness (Mokhtari et al., 2008; Toprak et al., 2009); however, the biochemical mechanism underlying these relationships remains unclear. In the last two decades, several studies have emerged showing that lysophospholipids play important roles in thrombus formation, atherosclerosis, and so on (Smyth et al., 2008; Pamuklar et al., 2009). Lysophosphatidic acids (LPAs) are released from activated blood platelets, and can be used for future active blood platelets (Gerrard and Robinson, 1989; Eichholtz et al., 1993; Haserück et al., 2004; Khandoga et al., 2008). Siess et al. (1999) found that LPA accumulated in atherosclerotic plaques, and was the primary platelet-activating lipid. The amount of LPA within human carotid atherosclerotic lesions is the highest in the lipid-rich core, the region that is the most thrombogenic and the most prone to rupture (Siess et al., 1999; Rother et al., 2003).

In the course of a previous study on the biological effects of LPA, we found a novel type of phospholipid (abbreviated as "AP") with solubility similar to LPA. This phospholipid was very sensitive to cerebral ischemia (Sun et al., 2002; Yao et al., 2004). Since a lower pulse pressure might be more closely related to impaired hemodynamics and reduced cardiac output (Voors et al., 2005), and LPA was reported to be involved in the etiology of hypertension (Xu et al., 2003), we conducted the present study to investigate the relationship between blood pressure (systolic, diastolic, and pulse pressure) and the levels of LPA and AP.

MATERIAL AND METHODS

Patient selection

This cross-sectional study was conducted from March 1, 2007 to December 29, 2008. Written informed consent was obtained from all of the participants. The study protocol was ap-

proved by the institutional Ethics Committee of the First Affiliated Hospital of Chinese Army General Hospital, where 270 subjects were consecutively admitted in outpatient clinics. The age of subjects ranged from 35 to 70 years, and all had primary hypertension and/or a history of primary hypertension. Some patients had already received medical treatment, including angiotensin-converting enzyme inhibitors, diuretics, and so on. At the time of enrolment, their blood pressures were measured over the brachial artery of the elbow. The same day, LPA, AP, and other clinical diagnostic markers, such as triglyceride (TG), low-density lipoprotein (LDL), total cholesterol (t-CHO), and the like, were assayed. Exclusion criteria included severe liver, kidney, or lung disease, untreated thyroid disease, acute myocardial infarction (within 6 months), acute stroke (within 6 months), hematopathy, acute infections, tumors, severe gynecological diseases, immunization (within 3 months), immunological system diseases, rheumatism, erythematous lupus, allergosis, serious depression, and other severe diseases. For females in their menstruation period, the assays for the levels of LPA and AP were postponed until after their period. Volunteer non-hypertensive subjects were also selected as controls during the same time by the same experienced clinicians. The non-hypertensive subjects were subjected to neurological examinations, and were confirmed to definitely have no hypertension or hypertension history. The exclusion criteria of the controls were similar to those of the cases.

Measurement of blood pressure

Hypertension was diagnosed according to World Health Organization diagnostic criteria. Blood pressure was measured with a sphygmomanometer. The cuff pressure at which auscultatory (Korotkow) sounds were first heard was defined as the systolic pressure. The diastolic pressure was defined as the cuff pressure at which the Korotkow sounds become muffled. The detailed procedures were as follows: after enrollment, the subjects were told to measure their blood pressure in the two consecutive days between 9:00 and 10:30 am For each day, they were asked to rest for 15 min before taking the measurement, and then their blood pressure was taken 3 times, with a 5-min interval between each measurement. The final value for blood pressure was defined as the average value of the measurements over the 2 days. Pulse pressure is defined as the difference between the systolic pressure and the diastolic pressure (Eichholtz et al., 1993). We temporarily defined the normal range for pulse pressure to be between 40 and 60 mmHg (Ganong, 1979) because of the lack of consolidated criteria.

Laboratory measurements

Blood samples were collected in the morning after the participants had fasted for at least 8 h. Plasma lipids, such as TG, LDL, high-density lipoprotein, t-CHO, as well as the prothrombotic marker, D-dimer, blood glucose, and the like were measured routinely by enzymatic methods or immunoassay in the Clinical Laboratory Test Center of the hospital. Details of the assay for AP and LPA are as follows: 4 mL venous blood was drawn from each participant into commercially available anticoagulant tubes (Beijing TF Co.) in the morning. The lipid extraction reagents were also purchased from Beijing TF Co. (LPA packaged reagents). Whole blood was centrifuged at 8000 g for 10 min. Then, 1 mL platelet-poor plasma was obtained from the supernatant. Lipid extraction was performed at 0° to 4°C to minimize damage to ester bonds. The phospholipid extrac-

tion from plasma was performed according to previously published methods (Kolarovic and Fournier, 1986; Baker et al., 2001), with slight modifications. After the separation of the two phases, we discarded the lower phase. Hydrogen chloride was added to the remaining upper phase to adjust the pH to 2.0. The lipids were extracted once again as described above. The resulting pooled organic extract was AP. A 50-mL fraction of the pooled organic extract was used to assay for AP. The remaining extracts were dried *in vacuo*. Each sample was resuspended in 0.3 mL chloroform:methanol:water:28% NH_4OH (250:100:15:0.3, v/v), and was immediately filtered through a 3 µm Econosphere 50 x 4.6 mm silica column (Alltech Associates; Deerfield, IL, USA). LPAs were eluted with a mobile phase of chloroform:methanol:water:28% NH_4OH (250:100:15:0.3, v/v) at 0.5 mL/min. After elution, the LPA and AP concentrations were quantified by measuring the inorganic phosphorus component using colorimetric assays.

Definitions and nomenclature

The cut-off value for AP was 0.474 mM and it was 3.2 µM for LPA. Chronic kidney disease (CKD) was defined as kidney damage for ≥3 months, as defined by structural or functional abnormalities of the kidney, with or without a decreased glomerular filtration rate (GFR). In this study, we only enrolled subjects with stage 3 or lower CKD, that is, GFR ≥ 30 mL/min per 1.73 m^2.

Statistical analysis

Comparisons of data were performed according to the nature of variables. For two samples of continuous variables, the Student *t*-test was used, whereas for categorical variables, the chi-squared test was used. Bivariate linear correlation analysis and binary logistic regression analysis were performed to investigate the relationship of AP with pulse pressure and other factors. All statistical analyses were performed with the SPSS software package for Windows version 13.0. The statistical significance level was set at $P < 0.05$.

RESULTS

The baseline characteristics of hypertensive patients and non-hypertensive subjects are shown in Table 1. Clinical and laboratory data, including age, gender, diabetes mellitus, atherosclerosis, hypercholesterolemia, and smoking status, were not significantly different between these two groups. None of the controls had high blood pressure or histories of hypertension.

Correlation analysis between LPA and some risk factors in hypertensive patients was conducted. The Pearson correlation coefficients were: age, r = 0.063, P = 0.200; diabetes history, r = 0.010, P = 0.839; LDL, r = 0.091, P = 0.064; tCHO, r = 0.131, P = 0.008; TG, r = 0.085, P = 0.083; smoking history, r = -0.132, P = 0.008; CKD, r = 0.073, P = 0.137.

Hypertensive patients had higher levels of AP and LPA than controls. Table 2 shows the comparison results including the odds ratios (ORs) with 95% confidence intervals (CI). The number of individuals with AP ≥ 0.474 mM (cut-off value) was significantly higher in the hypertensive patient group than in the control group. The mean value of AP was also significantly larger in the patient group. The results of LPA were similar to those of AP.

Table 1. Baseline characteristics of hypertensive patients and controls.

	Hypertensive patients	Controls	Pearson χ^2	P
N	416	252		
Demographic				
Age (mean ± SD, years)	66.1 ± 12.9	64.8 ± 15.8		0.247*
Male	201 (48.3%)	132 (52.4 %)	1.037	0.309
Risk factors				
Diabetes mellitus	43 (10.3%)	17 (6.9%)	2.905	0.116
Atherosclerosis**	59 (14.1%)	29 (11.6%)	0.982	0.322
Smoking	65 (15.7%)	33 (12.9%)	0.802	0.370
Hypercholesterolemia	57 (13.6%)	23 (9.1%)	3.116	0.078
Chronic kidney diseases	30 (7.2%)	7 (2.8%)	5.896	0.015
Hypertension‡	416 (100%)	0 (0%)		
Treated by ACEIs or ARBs	140 (33.7%)			
Treated by β-blockers	99 (23.7%)			
Treated by CCBs	119 (28.5%)			
Treated by diuretics	72 (17.3%)			

Data are reported as number with percent in parentheses. *Student *t*-test is used, t = 1.158. Values are compared by the 2 x 2 chi-square test. ‡Pharmacological therapy may include combination of the following 2 or even 3 drugs. **Positive finding of artery examination by ophthalmoscope.

Table 2. Hypertensive patients have elevated levels of both AP and LPA Comparing with controls.

	Hypertensive patients	Controls	P	OR (95%CI)
Comparison of AP				
AP < 0.474 mM	194 (46.6%)	199 (79.0%)		
AP ≥ 0.474 mM	222 (53.4%)	53 (21.0%)		
Total	416	252	0.00†	4.30 (3.00-6.15)
AP (means ± SD mM)	0.550 ± 0.211	0.387 ± 0.158	0.00 (t = 10.59)‡	
Comparison of LPA				
LPA < 3.2 μM	185 (44.4%)	190 (75.5%)		
LPA ≥ 3.2 μM	231 (55.6%)	62 (24.5%)		
Total	416	252	0.00§	3.83 (2.71-5.41)
LPA (means ± SD μM)	4.04 ± 2.81	2.82 ± 1.83	0.00 (t = 6.15)‡	

Data are reported as number with percent in parentheses, unless otherwise indicated. †Compared by the 2 x 2 chi-square test. χ^2 = 67.74. ‡Compared by Student *t*-test. §Compared by the 2 x 2 chi-square test. χ^2 = 60.96.

Although hypertensive patients had higher levels of both phospholipids, the status of blood pressure measured during the study did not significantly affect the levels of LPA or AP. We divided the patients into 4 groups: 1) normal blood pressure (note: hypertension patients had been treated with medicine); 2) only systolic blood pressure elevated; 3) only diastolic pressure elevated; and 4) both systolic and diastolic pressure elevated. The results of the 4 x 2 chi-squared test and one-way analysis of variance (ANOVA) showed that there were no significant differences between any groups (Table 3).

Although the status of blood pressure did not affect the levels of LPA or AP, this was not the case for pulse pressure. We divided the patients into two groups: group 1 had a medium range ($40 \leq PP < 60$ mmHg) of pulse pressure and group 2 had either higher or lower pulse pressure ($PP < 40$ or $PP \geq 60$). The 2 x 2 chi-squared test results showed that the AP level in group 1 was significantly lower than that of group 2 (Table 4). However, for LPA, this difference was not significant (data not shown). The results of the 3 x 2 chi-squared test for the three categories of pulse pressure ($PP < 40$, $40 \leq PP < 60$, and $PP \geq 60$ mmHg) showed a significant influence of plasma AP (χ^2 = 10.775, P = 0.005).

Table 3. Comparisons of plasma AP and LPA for hypertensive patients according to different categories of blood pressure.

	Normal BP	SP elevated	DP elevated	Both elevated
Comparison of AP*				
AP < 0.474 mM	53 (52.0%)	70 (44.3%)	4 (23.5%)	64 (46.0%)
AP ≥ 0.474 mM	49 (48.0%)	88 (55.7%)	13 (76.5%)	75 (54.0%)
Total	102	158	17	139
AP (means ± SD mM)	0.512 ± 0.298	0.551 ± 0.293	0.602 ± 0.316	0.525 ± 0.325
Comparison of LPA[†]				
LPA < 3.2 μM	50 (49%)	68 (43.0%)	7 (41.2%)	67 (48.2%)
LPA ≥ 3.2 μM	52 (51.0%)	90 (57.0%)	10 (58.8%)	72 (51.8%)
Total	102	158	17	139
LPA (means ± SD μM)	3.89 ± 1.88	4.28 ± 2.57	4.44 ± 2.79	4.47 ± 2.05

Data are reported as numbers with percent in parentheses unless otherwise indicated. BP = blood pressure; SP = systolic pressure; DP = diastolic pressure. *AP: comparison of Pearson numbers by the 2 x 4 chi-square, Pearson χ^2 = 5.098, P = 0.165; comparison of average values of AP among groups by one-way ANOVA F = 2.307; P = 0.076. [†]LPA: 2 x 4 chi-square test: Pearson χ^2 =1.358, P = 0.715; comparison of average values of LPA among groups by one-way ANOVA F = 1.403; P = 0.241.

Table 4. Influence of different categories of pulse pressure (PP) on plasma AP.

	40 ≤ PP < 60 mmHg	PP < 40 or PP ≥ 60 mmHg	OR (95%CI)	P
Comparison of number				
AP < 0.474 mM	90 (54.5%)	95 (37.8%)		
AP ≥ 0.474 mM	75 (45.5%)	156 (62.2%)		
Total	165	251	1.971 (1.323-2.936)	0.001*
Comparison of concentration				
AP (means ± SD mM)	0.464 ± 0.192	0.571 ± 0.218		0.000[†]
LPA (means ± SD μM)	3.65 ± 2.26	3.87 ± 2.37		0.346[‡]

*Chi-square test, Pearson χ^2 = 11.239. [†]Student *t*-test (t = 5.131). [‡]Student *t*-test (t = 0.943).

Bivariate linear correlation analysis showed that plasma AP levels were significantly negatively correlated with pulse pressure when PP ≥ 60 mmHg. However, LPA and the other 9 other widely used clinical markers, such as lipoproteins, glucose, and so on, were not significantly correlated with pulse pressure (Table 5).

Table 5. Correlation coefficient for the relationship between age and each of the biomarkers with pulse pressure.

Marker	N	Pearson's correlation coefficient	P
Age (years)	416	0.167	0.000*
AP (mM)	416	-0.208	0.000*
LPA (μM)	416	-0.070	0.154
t-CHO (mM)	404	-0.031	0.534
HDL (mM)	404	-0.076	0.127
LDL (mM)	404	0.081	0.104
TG (mM)	404	-0.025	0.616
Glucose (mM)	399	0.028	0.577
Lipoprotein (a) (g/L)	393	-0.065	0.220
Lipoprotein (b) (g/L)	390	0.061	0.229
D-dimer (μg/L)	377	-0.071	0.169
Uric acid (μM)	367	0.066	0.207

The correlation analysis was performed for hypertensive patients with pulse pressure ≤60 mmHg. *Correlation statistically significant.

To compare the effects of blood pressure and pulse pressure on AP, multivariate binary logistic regression was performed using the step-by-step backward method, with the probabilities for the stepwise method set to an entry level of P = 0.05 and a removal level of P = 0.1. The results showed that both systolic pressure and diastolic pressure were disregarded from further statistical analysis, and only pulse pressure was finally included in the regression equation: Log P = -0.399 + 0.859 pulse pressure, where P is the probability of AP elevation, and pulse pressure is defined as a binary categorical variable with a value of either 0 ($40 \leq$ PP < 60) or 1 (PP < 40 or PP ≥ 60).

This result indicated that only pulse pressure significantly influences the AP plasma level. Results of the chi-squared test showed that the logistic regression equation was statistically significant ($\chi^2 = 5.236$ and P = 0.022).

DISCUSSION

Although primary hypertension is a very common disease, its etiology is still not entirely clear. The relationship among systolic pressure, diastolic pressure, pulse pressure, and vascular events are relatively complex. However, according to the results of studies conducted to date, we can almost undoubtedly conclude that hypertension is one of the most important risk factors of vascular events. Elucidating the underlying mechanism will involve the combined study of basic medical science and clinical studies, including the study of new surrogate markers (Cohn, 2004). The LPA family has recently been recognized as important phospholipids with strong thrombogenic effects. In particular, AP is a promising surrogate marker for ischemia. The pathological production of AP theoretically involves the following steps. First, reactive oxygen species increase when the organ is in ischemia (Sowers, 2002). Then, the oxidative stress enhances the activities of phospholipase D and phospholipase A2 (Goto et al., 1988; Ito et al., 1997; Banno et al., 2001; Min et al., 2007). This activated enzyme catalyzes the breakdown of the chemical bonds of phospholipid molecules, and eventually produces AP.

Our current study shows that patients with either higher or lower pulse pressure have increased levels of AP. Petrie et al. (2009) proposed that pulse pressure is a reflection of the atherosclerotic burden on the one hand (high pulse pressure), and left ventricular dysfunction on the other hand (low pulse pressure), and that these two entities may counteract each other. Our result is consistent with this report. Furthermore, our results showed that there was a significantly negative correlation between AP and lower or higher pulse pressure. Based on the nature of AP described above, this result indicates that patients with a low pulse pressure would be more prone to ischemia. This deduction is based on current understanding of the nature of pulse pressure. Theoretically speaking, pulse pressure is positively proportional to heart stroke output, and is negatively proportional to aorta compliance. Therefore, a lower pulse pressure reflects a lower heart output when both the heartbeat and aorta compliance are relatively constant. Petrie et al. (2009) also proposed that pulse pressure might be closely related to stroke volume, and that low pulse pressure probably reflects decreased cardiac function (Voors et al., 2005). As a result of decreased heart stroke output accompanied by low pulse pressure, some organs, such as the brain, might ultimately manifest ischemia. Our current study showed that ischemia could be indicated by an elevated AP plasma level.

There is no clearly defined normal range of pulse pressure for clinical practice, despite the fact that there is ample evidence suggesting that pulse pressure plays an important role, at

least in cardiovascular events (Benetos et al., 1997; Verdecchia et al., 1998; Franklin et al., 1999). Voors et al. (2005) adopted 45 mmHg as the median value of pulse pressure. For our study, we temporarily defined the normal range of pulse pressure to be between 40 and 60 mmHg (Ganong, 1979); however, it should be noted that according to the Review of Medical Physiology, pulse pressure is normally about 50 mmHg. Therefore, this definition should be verified further.

AP levels varied among patients with either higher or lower pulse pressure. Physicians should pay attention to the possibility of (cerebral) ischemia in hypertensive patients with higher or especially lower pulse pressure. AP is a very promising marker for ischemic vascular diseases.

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Conflicts of interest

The authors declare no conflict of interest.

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