Circulating Levels of Secretory Type II Phospholipase A_2 Predict Coronary Events in Patients with Coronary Artery Disease

Kiyotaka Kugiyama, MD; Yasutaka Ota, MD; Keiji Takazoe, MD; Yasushi Moriyama, MD; Hiroaki Kawano, MD; Yuji Miyao, MD; Tomohiro Sakamoto, MD; Hirofumi Soejima, MD; Hisao Ogawa, MD; Hideki Doi, MD; Seigo Sugiyama, MD; Hirofumi Yasue, MD

Background—The circulating levels of secretory nonpancreatic type II phospholipase A_2 ($sPLA_2$) are increased in various chronic inflammatory diseases and the increase in the levels correlates with the disease severity. $sPLA_2$ may possibly play a role in atherogenesis and is highly expressed in atherosclerotic arterial walls that are known to have inflammatory features. Thus, this study prospectively examined whether circulating levels of $sPLA_2$ may have a significant risk and prognostic values in patients with coronary artery disease (CAD).

Methods and Results—Plasma levels of $sPLA_2$ were measured in 142 patients with CAD and in 93 control subjects by a radioimmunoassay. The $sPLA_2$ levels had a significant and positive relations with serum levels of C-reactive protein, a marker of systemic inflammation, and with the number of the traditional coronary risk factors associated with individuals. Multivariate logistic regression analysis showed that higher levels of $sPLA_2$ (>246 ng/dL; 75th percentile of $sPLA_2$ distribution in controls) were a significant and independent risk factor for the presence of CAD. In multivariate Cox hazard analysis, the higher levels of $sPLA_2$ were a significant predictor of developing coronary events (ie, coronary revascularization, myocardial infarction, coronary death) during a 2-year follow-up period in patients with CAD independent of other risk factors, including CRP levels, an established inflammatory predictor.

Conclusions—The increase in circulating levels of $sPLA_2$ is a significant risk factor for the presence of CAD and predicts clinical coronary events independent of other risk factors in patients with CAD; these results may reflect possible relation of $sPLA_2$ levels with inflammatory activity in atherosclerotic arteries. **(*Circulation*. 1999;100:1280-1284.)**

Key Words: atherosclerosis ■ coronary disease ■ lipids ■ prognosis ■ risk factors

Phospholipases A_2 (PLA_2) are ubiquitous enzymes that hydrolyze the *sn*-2-acyl bond of phospholipids of cell membrane and lipoproteins and yield free fatty acids and lysophospholipids, precursors of various proinflammatory lipid mediators including leukotrienes, eicosanoids, prostaglandins, and platelet-activating factors.[1–3] Several studies showed that secretory nonpancreatic type II phospholipase A_2 ($sPLA_2$) might importantly contribute to the pathogenesis of various inflammatory diseases.[2,3] Recently, $sPLA_2$ was found to be highly expressed in human atherosclerotic arterial walls,[4–6] where the inflammatory process is known to have a pathogenetic role.[7–9] The lipid products generated through $sPLA_2$ and their related products, including modified LDLs, participate in the development of atherosclerosis and play a significant role in the pathogenesis of coronary artery disease (CAD).[8–12] There is growing evidence[2,3,13,14] that the circulating levels of $sPLA_2$ are increased in various inflammatory diseases and that the levels reflect severity of these diseases, including rheumatic arthritis, inflammatory bowel diseases, and sepsis. Thus, it is expected that the circulating levels of $sPLA_2$ may also be increased in patients with CAD. In this study, we prospectively examined whether the circulating levels of $sPLA_2$ may have a significant risk and prognostic values in patients with CAD.

Methods

Study Patients

This study enrolled 196 consecutive patients with CAD who underwent elective and diagnostic cardiac catheterization for chest pain or ischemic changes in ECG in Kumamoto University Hospital. The patients with CAD who were admitted for the planned revascularization therapy were not eligible for this study. All patients had angiographic documentation of organic stenosis of >70% of at least one major coronary artery. Forty-nine patients were excluded because they had one of the following exclusion criteria: myocardial infarction, major surgery and trauma, and serious infectious diseases within previous 4 weeks; malignancies; and chronic inflammatory diseases, including rheumatoid arthritis, osteoarthritis, and inflammatory bowel disease. Ultimately, 147 (75%) of the 196 patients were included in this study. Patients' characteristics are shown in Table 1.

Received March 12, 1999; revision received June 10, 1999; accepted June 22, 1999.

From the Department of Cardiovascular Medicine, Kumamoto University School of Medicine, Kumamoto, Japan.

Correspondence to Kiyotaka Kugiyama, MD, PhD, Department of Cardiovascular Medicine, Kumamoto University School of Medicine, Honjo 1-1-1, Kumamoto City, Japan 860-8556. E-mail kiyo@gpo.kumamoto-u.ac.jp

Circulation **is available at http://www.circulationaha.org**

TABLE 1. Baseline Characteristics of Study Patients and Controls

	Control Subjects (n=93)	Patients With Coronary Artery Disease (n=142)
Age, y	60 (50, 69)	62 (52, 66)
Male sex (%)	63 (68)	99 (70)
Smoking (%)	40 (42)	65 (46)
Diabetes mellitus (%)	9 (10)	53 (37)†
Hypertension (%)	26 (27)	66 (46)†
Total cholesterol, mg/dl	198±39	201±38
HDL-cholesterol, mg/dl	50±14	41±11†
LDL-cholesterol, mg/dl	121±38	131±35*
Triglycerides, mg/dl	118 (77, 173)	118 (88, 181)
BMI, kg/m^2	23±3	24±3
No. of risk factors	1.3±1.0	2.2±1.1†
CRP, mg/dL	0.24 (0.08, 0.25)	0.25 (0.09, 0.56)‡
Previous MI (%)	...	54 (38)
Unstable angina (%)	...	34 (24)
Peripheral artery disease (%)	...	10 (7)

This study initially included 147 patients with coronary artery disease. Five patients with coronary artery disease were lost to follow-up and were excluded. Values in parentheses represent median (25th and 75th percentiles) except where noted.

BMI indicates body mass index; CRP, C-reactive protein; and MI, myocardial infarction. *P<0.05, †P<0.01 vs control subjects (χ^2 test or unpaired *t* test). ‡P=0.02 vs control subjects (Mann-Whitney *U* test).

This study also enrolled 124 consecutive control subjects who underwent cardiac catheterization for atypical chest pain during the same study period as the patients with CAD. These control subjects were studied to evaluate $sPLA_2$ as a risk factor differing between patients with CAD and non-CAD patients. The control subjects had angiographically normal coronary arteries (<10% stenosis), normal left ventriculography, and no clinical evidence of coronary artery spasm and syndrome X. Of these subjects, this study finally included 93 age- and sex-matched control subjects who did not have any of the same exclusion criteria as described above for the patients with CAD. Written informed consent was obtained from all patients and subjects before the study. This study was in agreement with the guidelines approved by the ethics committee at our institution.

Biochemical Measurements

Venous blood was taken from all of the study patients and control subjects after an overnight fast. Blood samples, anticoagulated with EDTA or citrate, were immediately centrifuged at 3000 rpm at 4°C for 10 minutes. The plasma was aliquoted and stored at −80°C until analyzed. Levels of immunoreactive $sPLA_2$ in EDTA-plasma were measured by a radioimmunoassay using a monoclonal antibody developed against membrane-associated PLA_2, which was purified from human spleen and was identical with type IIA PLA_2 purified from rheumatoid arthritic synovial fluid (Shionogi Pharmaceutical Ltd., Osaka, Japan).[15,16] This monoclonal antibody had no detectable cross-reactivity with human pancreatic PLA_2 (type IB).[14,15] The radioimmunoassay gave a linear response in a range from 78 to 5000 ng/dL of $sPLA_2$.[14,15] The interassay and intra-assay coefficients of variation were <8%.[14,15] The plasma levels of the immunoreactive $sPLA_2$ had a significant correlation with the calcium-dependent PLA_2 activity in the citrated plasma (ρ=0.923, P<0.0001, n=88 by Spearman's rank correlation test), a result which is compatible with that in previous reports.[13,14] Serum levels of C-reactive protein (CRP) were measured in all of the control subjects and the patients with CAD using an N Latex CRP immunodetection kit (Dade Behring).[14,15] Serum levels of total cholesterol, triglycerides, and HDL-cholesterol were measured by the enzymatic methods,[17] and LDL-cholesterol levels were calculated as previously described.[17]

Follow-Up Study

After laboratory samples and catheterization data were obtained, the 147 patients with CAD (1-vessel disease, 70 patients; 2-vessel disease, 34 patients; three-vessel disease, 27 patients; left main coronary stenosis, 16 patients) were followed up every month in hospital or with a clinic visit for a maximum of 24 months or until occurrence of one of the following clinical coronary events: recurrent or refractory angina pectoris requiring coronary revascularization by PTCA or CABG, nonfatal myocardial infarction, and cardiac death. Time from the day when blood sampling was performed to first coronary event was prospectively evaluated. Diagnosis of myocardial infarction was made by chest pain, appearance of new Q wave on the ECG, and elevation of creatine kinase enzyme to more than twice the upper limit of normal. Cause of death was determined from hospital records. For the study, revascularization therapy based only on angiographic data were not counted as a coronary event. All of the patients received standardized medical therapy. The patients with high extent of CAD (3-vessel disease or left main coronary stenosis) who were included in this study were also followed up without revascularization therapy immediately after the inclusion because of diffuse peripheral CAD, a high risk with the procedure, previous CABG or repeated PTCA, or unwillingness for the revascularization therapy. The attending physician and interventional cardiologists independent of this prospective study decided the need for and timing of revascularization.

Statistical Analysis

Because $sPLA_2$ levels were not distributed normally, results of $sPLA_2$ levels are expressed as median and range (25th and 75th percentiles) and nonparametric analyses were used. Mann-Whitney *U* test was used to evaluate difference in $sPLA_2$ levels between the 2 groups. Spearman's rank correlation test was used for relations of $sPLA_2$ levels with CRP levels and number of the coronary risk factors associated with individuals. To evaluate $sPLA_2$ levels as an independent risk factor differing between the patients with CAD and the control subjects, forward, stepwise, multiple logistic regression analysis was performed using the following factors as categorical covariates: smoking history (defined as smoking at least 10 cigarettes per day for ≥10 years), hypertension (≥140/90 mm Hg or requiring antihypertensive medication), diabetes mellitus (according to World Health Organization criteria,[18] hypercholesterolemia (≥220 mg/dL or the use of lipid-lowering medications), high LDL-cholesterol (≥130 mg/dL), low HDL-cholesterol (<35 mg/dL), and high CRP levels (>0.48 mg/dL, 90th percentile of the distribution of the CRP levels in the control subjects). Kaplan-Meier method (log-rank test) was applied in survival analysis according to the levels of $sPLA_2$. The predictive value for coronary events during the follow-up period was assessed by Cox proportional hazard analysis. The multivariate Cox analysis always included the following factors as categorical covariates: $sPLA_2$ levels, stenosis of the left main coronary artery, number of coronary arteries with stenosis, low left ventricular ejection fraction (LVEF) on baseline left ventriculography (< 50%), age (≥70 years), sex (male), smoking history, hypertension, diabetes mellitus, hypercholesterolemia, high LDL-cholesterol, and low HDL-cholesterol. Cutoff point (246 ng/dL) between higher and lower levels of $sPLA_2$ was arbitrarily defined as 75th percentile of the distribution of the $sPLA_2$ levels in the control subjects. In Cox hazard model and Kaplan-Meier analyses, $sPLA_2$ levels were divided into tertiles that were based on 90th and 75th percentiles of the distribution of the $sPLA_2$ levels in the control subjects. On scoring the number of coronary arteries with stenosis, stenosis of the left main coronary artery was counted as 2-vessel disease. Mean values of continuous variables with normal distribution and frequencies among subgroups were compared by unpaired *t* test and χ^2 analysis, respectively. Statistical significance was defined as P<0.05. The analyses were performed partly using SPSS Professional Statistics 6.1 for the Macintosh (SPSS Japan Inc).

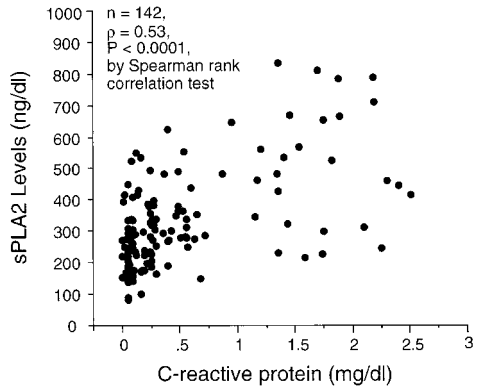

Figure 1. Relation between levels of $sPLA_2$ and C-reactive protein in patients with CAD.

Results

$sPLA_2$ as a Coronary Risk Factor

The distribution of the levels of $sPLA_2$ immunoreactivity in patients with CAD was skewed and shifted to lower levels with a median level of 286 (218 and 386) ng/dL, which was significantly higher than that in control subjects (191 [154 and 246] ng/dL) ($P<0.0001$). The levels of $sPLA_2$ had a significant and positive correlation with CRP levels (Figure 1). As shown in Figure 2, the levels of $sPLA_2$ were significantly related with the number of the traditional coronary risk factors associated with individuals. There was no significant association of higher $sPLA_2$ levels (>246 ng/dL, 75th percentile of the $sPLA_2$ distribution in controls) with each of the traditional coronary risk factors (age ≥70 years, smoking history, diabetes mellitus, hypertension, hypercholesterolemia, and low HDL-cholesterol).

The patients with CAD had significantly lower HDL-cholesterol levels, higher LDL-cholesterol levels, higher CRP levels, higher rates of diabetes mellitus and hypertension, and higher $sPLA_2$ levels compared to control subjects (Table 1). In multiple logistic regression analysis with forward stepwise selection, the higher levels of $sPLA_2$ (>246 ng/dL), diabetes mellitus, and hypertension were the variables differing significantly and independently between the patients with CAD and the control subjects, as shown in Table 2. The $sPLA_2$ levels were significantly higher in patients with unstable angina (the class B of Braunwald's classification) than stable angina (median [25th and 75th percentiles], 309 ng/dL [243, 461] versus 268 ng/dL,[217, 338], n= 34 and 108, respectively; $P=0.03$). The $sPLA_2$ levels were higher in patients with peripheral artery disease (diagnosed by angiography) than those without it (median [25th, 75th], 377 ng/dL [311, 583] versus 279 ng/dL, [217, 365], n=10 and 132, respectively; $P=0.02$).

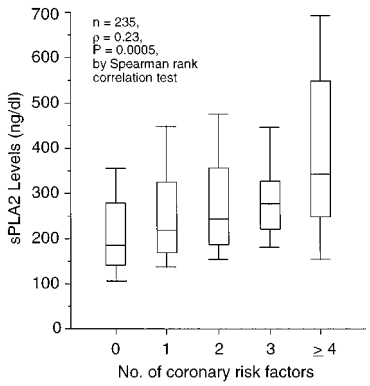

Figure 2. Box and whisker plots showing relation between $sPLA_2$ levels and the number of the traditional coronary risk factors associated with individuals, including age (≥70 years), histories of smoking, diabetes mellitus, hypertension, hypercholesterolemia (≥220 mg/dL), and low HDL-cholesterol (<35 mg/dL) in patients with CAD and control subjects. Lines within boxes represent median values; upper and lower lines of boxes, 75th and 25th percentiles, respectively; upper and lower bars outside of boxes, 90th and 10th percentiles, respectively. n=27 in 0 coronary risk factor, 62 in 1 risk, 65 in 2 risks, 51 in 3 risks, and 30 in ≥4 risks.

TABLE 2. Multiple Logistic Regression Analysis: Final Significant Variables Differing Between Patients with CAD and Control Subjects

Variables	β-Coefficient	SE	P	Odds Ratio	95% CI
$sPLA_2$>246 ng/dL	1.67	0.36	<0.00001	5.3	1.6–17.3
Diabetes mellitus	1.76	0.48	0.0002	5.8	1.1–30.1
Hypertension	1.08	0.37	0.004	3.0	1.3–6.6
Constant	−1.48	0.40	0.0002		

Forward, stepwise, multiple logistic regression analysis was performed using the following factors as categorical covariates: $sPLA_2$>246 ng/dL, smoking history, hypertension, diabetes mellitus, hypercholesterolemia, high levels of LDL-cholesterol, low levels of HDL-cholesterol, and high CRP levels (>0.48 mg/dL).

$sPLA_2$ as a Predictor of Coronary Events in Patients with CAD

All of the patients received the standard medical therapy consisting of a combination of calcium channel blockers (used in 82% of patients), β-blockers (41%), nitrates (70%), angiotensin-converting enzyme inhibitor (38%), aspirin (93%), and lipid-lowering drugs (34%) during the follow-up study. Seven patients with unstable angina and high extent of CAD had intravenous infusion of heparin and nitrates for several days (2 to 7 days) after the inclusion. Only 5 patients with CAD were lost to follow-up. The remaining 142 patients with CAD were followed for a mean duration of 17.2 months (range, 0.5 to 24 months). The patients with higher levels of $sPLA_2$ (>246 ng/dL, 95 patients) had 41 coronary events (14 PTCA, 13 CABG, 5 myocardial infarction, 9 coronary death) during the follow-up period, whereas the patients with lower levels (≤246 ng/dL, 47 patients) had 7 events (1 PTCA, 5 CABG, 1 coronary death) ($P<0.01$ in frequencies of coronary events between the 2 subgroups). There was no significant difference in the rates of each of the drugs used between the patients with and without coronary events during the follow-up period (data not shown). Kaplan-Meier analysis demonstrated a significantly higher probability of developing the clinical coronary events in the patients with the higher levels of $sPLA_2$ than those with the lowest levels, as shown in Figure 3. In univariate Cox proportional hazard model analysis, higher levels of $sPLA_2$ (Table 3), higher levels of CRP (>0.48 mg/dL, 90th percentile of the CRP distribution in controls) (Odds, 1.8; 95% CI, 1.1 to 3.3, $P=0.04$ as compared with lower CRP levels [≤0.48 mg/dL]), stenosis of the left main coronary artery (Odds, 2.3, 95% CI, 1.1 to 4.9, $P=0.02$), and 3-vessel disease (Odds, 2.0, 95% CI, 1.0 to 3.9, $P=0.04$, compared with 1-vessel disease) were a significant

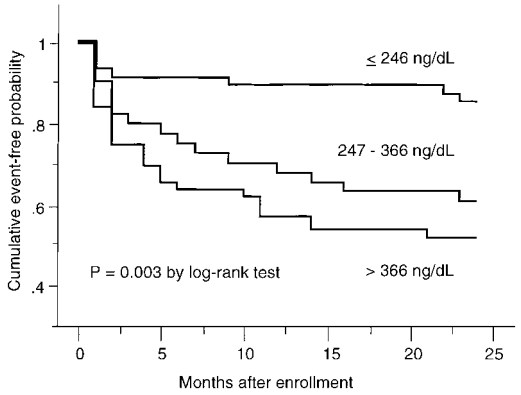

Figure 3. Kaplan-Meier curves comparing the probability of developing coronary events according to the $sPLA_2$ levels during follow-up period maximally for 24 months after enrollment in 142 patients with CAD. End points were revascularization (PTCA and CABG) due to recurrent and refractory angina pectoris, nonfatal myocardial infarction, and cardiac death. Time to first coronary event was prospectively evaluated. $sPLA_2$ levels were divided into tertiles based on 90th and 75th percentiles (366 and 246 ng/dL, respectively) of the distribution of $sPLA_2$ levels in control subjects. n=37, 58, and 47 in the highest (>366 ng/dL), the second (between 247 and 366 ng/dL), and the lowest (≤246 ng/dL) tertile, respectively.

predictor of the clinical coronary events in patients with CAD. Multivariate Cox proportional hazard analysis showed that only higher levels of $sPLA_2$ were a significant predictor of the coronary events independent of the traditional risk factors, left main coronary stenosis, 3-vessel disease, and low LVEF (Table 3). When CRP was added to the covariates in multivariate Cox analysis, $sPLA_2$ but not CRP remained a significant predictor of the future coronary events independent of the other risk factors (Odds, 3.3; 95% CI, 1.3 to 9.2; $P=0.01$ highest versus lowest tertile of $sPLA_2$ levels; odds, 1.3; 95% CI; 0.67 to 2.6; $P=0.43$, higher versus lower CRP levels). The inclusion of previous myocardial infarction and unstable angina at baseline examination into the covariates in the multivariate Cox analysis did not significantly affect the predictive value of higher $sPLA_2$ levels (highest versus lowest tertile of $sPLA_2$ levels, after addition of previous myocardial infarction into the covariates: Odds, 3.5; 95% CI, 1.4 to 8.3; $P=0.006$, after addition of unstable angina into the covariates: odds, 3.3; 95% CI, 1.4 to 8.1; $P=0.008$). Previous myocardial infarction and revascularization therapy before inclusion in this study, presence of unstable angina at baseline examination, low LVEF, and other traditional coronary risk factors did not have significant predictive value for coronary events in the study patients with CAD in either univariate or multivariate Cox proportional hazard analysis.

Discussion

The study demonstrated that the increase in plasma levels of $sPLA_2$ was an independent risk factor for the presence of CAD and that the increase in the levels predicted the development of clinical coronary events in patients with CAD. There is increasing evidence that atherosclerosis is an inflammatory disease that develops in response to the coronary risk factors.[7–9,12] In this context, the present study further showed that the circulating levels of $sPLA_2$ were significantly correlated with the levels of CRP, a marker of systemic inflammation, and with the number of the traditional coronary risk factors associated with individuals. These imply that the increase in $sPLA_2$ levels in patients with CAD may reflect the inflammatory activity in the development of atherosclerosis. $sPLA_2$ may be one of the inflammatory markers, but $sPLA_2$ is a better inflammatory predictor of coronary events than CRP, a well established inflammatory prognostic marker because $sPLA_2$ but not CRP remained a significant and independent predictor in the multivariate Cox hazard analysis when addition of both factors into the covariates.

$sPLA_2$ is shown to be induced in vascular cells by cytokines such as interleukin-1 and tumor necrosis factor-α,[2,3,19–21] which abundantly exist in atherosclerotic arterial walls and have crucial roles in the inflammatory and immunological features in atherosclerotic development.[7,9,11] These cytokines could stimulate synthesis of $sPLA_2$ in the atherosclerotic arterial walls and release into circulation, resulting in the elevation of the $sPLA_2$ levels in patients with CAD.

The lipid products generated through PLA_2 and their related products, such as lysophosphatidylcholine and modified LDL, are proatherogenic and proinflammatory[2,3,10,11] and they activate vascular cells to produce plasminogen activators (PA), PA inhibitor-1, adhesion molecules, various proatherogenic cytokines and growth factors, and oxygen free radicals,[10,22–24] leading to atherothrombotic development and plaque instability in the atherosclerotic arterial walls.[8,9,12,25] This atherothrombogenic role of $sPLA_2$ may result in the association of the increased levels of $sPLA_2$ with the high frequency of future coronary evens in patients with CAD, as observed in our study. The causative role of $sPLA_2$ levels in human plaque unstabilization is now under investigation in our laboratory.

TABLE 3. Cox Proportional Hazard Model Analysis of Risks of Developing Coronary Events According to the Levels of $sPLA_2$ in Patients with CAD

	No. of Patients with Coronary Events	Univariate			Multivariate		
$sPLA_2$ levels	(%)	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*
>366 ng/dL (n=37)	18 (49)	3.88	1.7–9.0	0.002	3.46	1.4–8.3	0.006
247–366 ng/dL (n=58)	23 (39)	3.04	1.2–7.4	0.01	2.89	1.1–7.5	0.03
≤246 ng/dL (n=47)	7 (15)	1			1		

$sPLA_2$ levels were divided into tertiles based on 90th and 75th percentiles (366 and 246 ng/dL, respectively) of the distribution of $sPLA_2$ levels in control subjects. $sPLA_2$ levels, stenosis of left main coronary artery, number of diseased coronary arteries, age (≥70 y), male sex, histories of smoking, diabetes mellitus, and hypertension, hypercholesterolemia, high levels of LDL-cholesterol, low levels of HDL-cholesterol, and low LV ejection fraction were included as categorical covariates on multivariate analysis.

Recent reports have demonstrated that the increase in levels of CRP was associated with CAD.[26,27] CRP is hepatically derived and has an uncertain physiological role in atherosclerotic development and in CAD. $sPLA_2$ could be also one of acute phase reactants.[2,3,14,15] However, unlike CRP, the lipid mediators produced through $sPLA_2$ and their related lipids in the arterial walls can stimulate T cells and macrophages to synthesize and release the proatherogenic and proinflammatory cytokines,[2,3,7–11,28] which may in turn induce $sPLA_2$ production in the atherosclerotic arterial walls.[2,3,19–21] These positive feedback mechanisms could amplify this sequence of events in the atherosclerotic arterial walls, thereby $sPLA_2$ present in the arterial walls may play an important role in the pathogenesis of CAD, not simply as a marker of inflammation.

This study showed that diabetes mellitus, hypertension, and high $sPLA_2$ levels were significant and independent variables differing between patients with CAD and the control subjects. However, in the present prospective study, only high $sPLA_2$ levels (but not other traditional coronary risk factors including diabetes and hypertension) had predictive values for coronary events. The lack of the predictive significance of diabetes and hypertension in the present prospective study may be partly explained by the modification of these traditional risk factors during the follow-up by medications and improvement of lifestyle. Our study also showed that extensive coronary diseases (left main coronary disease and 3-vessel disease) had weak but significant probability for clinical coronary events in the univariate analysis. However, presence of the extensive coronary diseases alone may not be related directly to the significant probability of coronary events, which will not necessarily be provoked at the sites of severe coronary stenosis on baseline angiograms.[25] Other associated risk factors may additionally contribute to the significant probability because the extensive coronary diseases did not remain significant in the multivariate analysis.

This study is limited by the small number of the studied patients. Also, a case-control study has cross-sectional nature and it may have inherent selection bias of cases and controls. A trial with specific inhibitors of $sPLA_2$ activity in a large number of study patients with homogeneous risk is required to assess the precise role of $sPLA_2$ in the pathogenesis of CAD.

In conclusion, high levels of $sPLA_2$ in the circulation have an independent risk factor for the presence of CAD and predict future coronary events in patients with CAD.

Acknowledgments

This study was supported in part by grants-in-aid for C09670730 from the Ministry of Education, Science, and Culture and the ONO Medical Research Foundation, Osaka, Japan. We thank Professor Michio Ogawa, MD, PhD, Second Department of Surgery, Kumamoto University School of Medicine, for helpful discussion.

References

1. Dennis EA. Diversity of group types, regulation, and function of phospholipase A_2. *J Biol Chem*. 1994;269:13057–13060.
2. Pruzanski W, Vadas P. Phospholipase A_2 - a mediator between proximal and distal effectors of inflammation. *Immunol Today*. 1991;12:143–146.
3. Murakami M, Kudo I, Inoue K. Secretory phospholipase A_2. *J Lipid Mediat Cell Signal*. 1995;12:119–130.
4. Menschikowski M, Kasper M, Lattke P, Schiering A, Schiefer S, Stockinger H, Jaross W. Secretory group II phospholipase A_2 in human atherosclerotic plaques. *Atherosclerosis*. 1995;118:173–181.
5. Hurt-Camejo E, Andersen S, Standal R, Rosengren B, Sartipy P, Stadberg E, Johansen B. Localization of nonpancreatic secretory phospholipase A_2 in normal and atherosclerotic arteries: activity of the isolated enzyme on low-density lipoproteins. *Arterioscler Thromb Vasc Biol*. 1997;17:300–309.
6. Elinder LS, Dumitrescu A, Larsson P, Hedin U, Frostegård J, Claesson HE. Expression of phospholipase A_2 isoforms in human normal and atherosclerotic arterial wall. *Arterioscler Thromb Vasc Biol*. 1997;17:2257–2263.
7. Hansson GK, Jonasson L, Seifert PS, Stemme S. Immune mechanisms in atherosclerosis. *Arteriosclerosis*. 1989;9:567–578.
8. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993;362:801–809.
9. Libby P. Molecular bases of the acute coronary syndromes. *Circulation*. 1995;91:2844–2850.
10. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest*. 1991;88:1785–1792.
11. Hajjar DP, Pomerantz KB. Signal transduction in atherosclerosis: integration of cytokines and the eicosanoid network. *FASEB J*. 1992;6:2933–2941.
12. Selwyn AP, Kinlay S, Ganz P. Atherogenesis and ischemic heart disease. *Am J Cardiol*. 1997;80:3H–7H.
13. Lin MKS, Farewell V, Vadas P, Bookman AA, Keystone EC, Pruzanski W. Secretory phospholipase A_2 as an index of disease activity in rheumatoid arthritis: prospective double blind study of 212 patients. *J Rheumatol*. 1996;23:1162–1166.
14. Minami T, Tojo H, Shinomura Y, Tarui S, Okamoto M. Raised serum activity of phospholipase A_2 immunochemically related to group II enzyme in inflammatory bowel disease: its correlation with disease activity of Crohn's disease and ulcerative colitis. *Gut*. 1992;33:914–921.
15. Matsuda Y, Ogawa M, Sakamoto K, Yamashita S, Kanda A, Kohno M, Yoshida N, Nishijima J, Murata A, Mori T. Development of a radioimmunoassay for human group-II phospholipase A_2 and demonstration of postoperative elevation. *Enzyme*. 1991;45:200–208.
16. Kanda A, Ono T, Yoshida N, Tojo H, Okamoto M. The primary structure of a membrane-associated phospholipase A_2 from human spleen. *Biochem Biophys Res Commun*. 1989;163:42–48.
17. Kugiyama K, Doi H, Motoyama T, Soejima H, Misumi K, Kawano H, Nakagawa O, Yoshimura M, Ogawa H, Matsumura T, Sugiyama S, Nakano T, Nakajima K, Yasue H. Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. *Circulation*. 1998;97:2519–2526.
18. The Expert Committee. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 1997;20:1183–1197.
19. Nakano T, Ohara O, Teraoka H, Arita H. Group II phospholipase A_2 mRNA synthesis is stimulated by two distinct mechanisms in rat vascular smooth muscle cells. *FEBS Lett*. 1990;261:171–174.
20. Murakami M, Kudo I, Inoue K. Molecular nature of phospholipase A_2 involved in prostaglandin I_2 synthesis in human umbilical vein endothelial cells. *J Biol Chem*. 1993;268:839–844.
21. Kuwata H, Nakatani Y, Murakami M, Kudo I. Cytosolic phospholipase A_2 is required for cytokine-induced expression of type IIA secretory phospholipase A_2 that mediates optimal cyclooxygenase-2-dependent delayed prostaglandin E_2 generation in rat 3Y1 fibroblasts. *J Biol Chem*. 1998;273:1733–1740.
22. Kugiyama K, Sakamoto T, Misumi I, Sugiyama S, Ohgushi M, Ogawa H, Horiguchi M, Yasue H. Transferable lipids in oxidized low-density lipoprotein stimulate plasminogen activator inhibitor-1 and inhibit tissue-type plasminogen activator release from endothelial cells. *Circ Res*. 1993;73:335–343.
23. Kume N, Gimbrone MA Jr. Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. *J Clin Invest*. 1994;93:907–911.
24. Ohara Y, Peterson TE, Zheng B, Kuo JF, Harrison DG. Lysophosphatidylcholine increases vascular superoxide anion production via protein kinase C activation. *Arterioscler Thromb*. 1994;14:1007–1013.
25. Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation*. 1995;92:657–671.
26. Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB, Maseri A. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. *N Engl J Med*. 1994;331:417–424.
27. Berk BC, Weintraub WS, Alexander RW. Elevation of CRP in "active" CAD. *Am J Cardiol*. 1990;65:168–172.
28. Asaoka Y, Yoshida K, Sasaki Y, Nishizuka Y, Murakami M, Kudo I, Inoue K. Possible role of mammalian secretory group II phospholipase A_2 in T-lymphocyte activation: implication in propagation of inflammatory reaction. *Proc Natl Acad Sci U S A*. 1993;90:716–719.

Circulating Levels of Secretory Type II Phospholipase A_2 Predict Coronary Events in Patients with Coronary Artery Disease

Kiyotaka Kugiyama, Yasutaka Ota, Keiji Takazoe, Yasushi Moriyama, Hiroaki Kawano, Yuji Miyao, Tomohiro Sakamoto, Hirofumi Soejima, Hisao Ogawa, Hideki Doi, Seigo Sugiyama and Hirofumi Yasue

Circulation. 1999;100:1280-1284
doi: 10.1161/01.CIR.100.12.1280
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539