Relation of Secretory Phospholipase A2 and High-Sensitivity C-Reactive Protein to Chlamydia Pneumoniae Infection in Acute Coronary Syndromes

Norio Miya, MD; Sumito Oguchi, MD; Ikuyoshi Watanabe, MD; Katsuo Kanmatsuse, MD

Background  Recently it has become clear that inflammatory changes play a part in the development of atherosclerosis, including coronary artery disease, and Chlamydia pneumoniae (C. pneumoniae) is thought to be a proinflammatory factor. The plasma concentration of high-sensitive C-reactive protein (hs-CRP) is a potential predictor of outcome in atherosclerotic diseases. Recent interest has focused on secretory group IIA phospholipase A2 (sPLA2) in regard to the progression of atherosclerotic disease.

Methods and Results  The concentrations of sPLA2, hs-CRP, and the titers of C. pneumoniae IgG and IgA antibodies were measured in blood samples. The study groups were an acute coronary syndrome (ACS) group, old myocardial infarction/angina pectoris (OMI/AP) group, and a control group. The concentrations of sPLA2 and hs-CRP in the ACS group and the OMI/AP group were higher than in the control group. The titers of C. pneumoniae IgG and IgA were higher in the ACS group than in the control group. The sPLA2 concentration was higher in those who were positive to C. pneumoniae IgG/IgA than in those who were negative.

Conclusion  Increased concentrations of sPLA2 reflect participation in the progression of coronary artery disease. The sPLA2 concentration was higher in patients positive for C. pneumoniae than in those negative for C. pneumoniae, so C. pneumoniae infection poses a greater risk for ACS in those individuals than in those who are free of such infection. (Circ J 2004; 68: 628–633)

Key Words:  Acute coronary syndromes; Atherosclerosis; Chlamydia pneumoniae; Secretory group IIA phospholipase A2

Recent studies have shown that in addition to the classic risk factors, such as hypertension, hyperlipidemia, and diabetes mellitus, acute and chronic inflammatory changes play a part in the development of atherosclerosis and the pathogenesis of acute coronary syndromes (ACS). Epidemiological research, including the Physicians’ Health Study and Multiple Risk Factor Intervention Trial, have focused on the pathogenetic role of high-sensitivity C-reactive protein (hs-CRP)1–3.

Pathological studies have demonstrated that many macrophages and T lymphocytes are present in unstable atherosclerotic lesions in patients with unstable angina and acute myocardial infarction (MI)4. Inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-γ (TNF-γ), act on macrophages and smooth muscle cells and participate in thrombus formation and the disruption of atheromatous plaques. Therefore, inflammation has an important role in the occurrence of cardiovascular events5–9.

Microorganisms such as Chlamydia pneumoniae (C. pneumoniae), cytomegalovirus10,11 and herpes simplex virus are thought to act as proinflammatory factors contributing to chronic inflammation. Plasma concentrations of oxidized low-density lipoprotein (LDL), C. pneumoniae antibodies, IL-6, TNF-γ, and hs-CRP have received considerable attention as potential predictors of outcome in atherosclerotic diseases, including coronary artery disease (CAD)8–12.

CRP is found in many atherosclerotic lesions in CAD. A positive correlation between CRP immune activity and endothelial thickening suggests that CRP directly participates in the progression of coronary atherosclerosis13–15.

Phospholipase A2 is involved in the degradation of arachidonic acid metabolites and dietary phospholipids and the metabolism of membrane phospholipids.16 Recent interest has focused on secretory group IIA phospholipase A2 (sPLA2) because of its potential role in the accumulation of oxidized LDL in vascular walls as well as in the progression of atherosclerosis and the development of CAD17–20.

The value of sPLA2 as a potential marker for the risk of cardiovascular events has received considerable attention.

In the present study, we measured the concentrations of sPLA2, hs-CRP, and C. pneumoniae IgG and IgA antibodies in peripheral blood samples obtained from patients with ischemic heart disease to gain further insight into the roles of inflammation and C. pneumoniae infection in CAD.

Methods

Study Patients

The study group comprised 268 patients with ischemic heart disease: 160 (134 men, 26 women) had ACS (ACS group) and 108 (81 men, 27 women) had old MI and stable angina pectoris (OMI/AP group). As controls, we studied 178 persons (132 men, 46 women) who underwent a health examination at the healthcare center of the hospital and did...
Patients with ACS had to be admitted within 1 to 6 h of the onset of symptoms; blood samples obtained on admission were used. The OMI/AP group had to have stable angina pectoris and could include patients with an old MI who had not had anginal attacks for at least 6 months before study entry; blood samples obtained at the outpatient clinic were used.

The peripheral blood samples obtained from the subjects in each group were analyzed to determine the concentrations of hs-CRP, sPLA2 and titers of C. pneumoniae IgG and IgA antibodies. Differences in these variables among the groups and the correlations among different variables were analyzed.

The cutoff value for the C. pneumoniae antibody titers was 1.1 for all 3 groups; a value of less than 1.1 was defined as negative and that of 1.1 or more was defined as positive. The concentrations of hs-CRP and sPLA2 were examined in each of these groups.

The study protocol was approved by the ethics committee of the hospital and all subjects gave written informed consent before enrollment in the study.

**Statistical Analysis**

The statistical significance of differences in clinical characteristics among the 3 groups was determined with the chi-square test. The significance of differences among the groups in the concentrations of sPLA2, hs-CRP and C. pneumoniae IgG and IgA antibodies was determined with the Kruskal-Wallis test. In the event of significant differences among the groups, the Mann-Whitney U test with the Bonferroni correction for multiple comparisons was performed. The significance of correlations among examination variables was tested by Spearman rank correlation coefficient. All study patients were divided into a negative group and a positive group for C. pneumoniae IgG titer and IgA titer, respectively. The statistical significance of differences between these groups in the concentrations of hs-CRP and sPLA2 was determined with the Mann-Whitney U test.

**Results**

**Clinical Characteristics of Study Groups**

The mean age (±SD) of the subjects was 60.5±10.7 years in the ACS group, 64.0±9.5 years in the OMI/AP group, and 58.0±5.5 years in the control group. The classic risk factors (hypertension, hyperlipidemia, smoking, and diabetes mellitus) in each group are shown in Table 1.

The median hs-CRP concentration was 18.90 mg/dl in the ACS group, 0.823 mg/dl in the OMI/AP group, and 0.556 mg/dl in the control group. These values differed significantly (p<0.05). After multiple comparisons, the hs-CRP concentration in the ACS group was significantly higher than the concentrations in the OMI/AP and control groups (p<0.01) (Fig 1A).

The median concentration of sPLA2 was 1.300 ng/ml in the ACS group, 2.000 ng/ml in the OMI/AP group, and 0.900 ng/ml in the control group. The differences among the groups were significant (p<0.05). After multiple comparisons, the sPLA2 concentration in the ACS and OMI/AP groups was significantly higher than that in the control group (p<0.01) (Fig 1B).

The median C. pneumoniae IgG antibody titer differed significantly among the groups: ACS =1.140, OMI/AP = 1.010, and control group =0.830 (p<0.05). After adjustment...
for multiple comparisons, the C. pneumoniae IgG antibody titer was significantly higher in the ACS group than in the control group \( (p<0.01) \) (Fig 2A).

The median C. pneumoniae IgA antibody titer also differed significantly among the groups (ACS: 1.220; OMI/AP: 1.160; control: 0.945; \( p<0.05 \)). After adjustment for multiple comparisons, the C. pneumoniae IgA antibody titer was significantly higher in the ACS group than in the control group \( (p<0.01) \) (Fig 2B).

In all study patients, the hs-CRP concentration significantly correlated with the sPLA2 concentration \((r_s=0.31, p<0.001 \text{ by Spearman rank correlation, } n=446)\) (Fig 3). Significant correlations between the hs-CRP and the sPLA2 concentrations were also obtained in the individual groups (each group, data not shown).

Next, we examined differences in the hs-CRP and sPLA2 concentrations between patients negative and positive for C. pneumoniae IgG antibody titer or IgA antibody titer (cutoff point=1.1) in all study patients \( (n=446) \). For both the C. pneumoniae IgG antibody titer and the C. pneumoniae IgA antibody titer, the sPLA2 concentration was significantly higher in the positive group than in the negative group \( (p<0.05) \) (Fig 4). In contrast, hs-CRP concentration did not differ significantly between patients positive and those negative for either the C. pneumoniae IgG antibody titer or the C. pneumoniae IgA antibody titer. The sPLA2 concentration correlated significantly with the C. pneumoniae IgG and IgA antibody titers (C. pneumoniae IgG titer and sPLA2 concentration, \( r_s=0.10, p=0.04 \); C. pneumoniae IgA titer and sPLA2 level, \( r_s=0.11, p=0.03 \) by Spearman rank correlation; Fig 5). However, there was no significant correlation of the hs-CRP concentration with either the IgG antibody titer or the IgA antibody titer \( (p>0.05 \text{ by Spearman rank correlation}) \). Moreover, background factors (age, hypertension, diabetes mellitus, and smoking) differed significantly among the ACS, OMI/AP, and control groups. We therefore examined the effects of these factors on the study variables. The patients were divided into 2 groups according to age \((<65 \text{ years vs } \geq 65 \text{ years})\) and whether or not they had the risk factors of
hypertension, diabetes mellitus, or smoking. Whether the hs-CRP and sPLA2 concentrations, and the C. pneumoniae IgG and IgA antibody titers differed significantly between these groups was tested by the Mann-Whitney U test. The hs-CRP concentration was found to be significantly higher in patients 65 years or older, hypertensive patients, diabetic patients, and smokers (p<0.05), whereas the sPLA2 concentration was significantly higher only in patients 65 years or older (p<0.05). There was no significant difference between the groups in the C. pneumoniae IgG or IgA antibody titer. The effects of these background factors on the relation between the hs-CRP and the sPLA2 concentrations were examined by multiple analysis of variance. Only age affected this relation (p<0.05); there was no discernible effect of hypertension, diabetes mellitus, or smoking.

**Discussion**

The ACS encompass unstable angina pectoris, acute MI, and sudden death from ischemic heart disease. Recent studies have shown that ACS result from rupture of the fibrous cap of an atheromatous plaque, exposing the lumen to the lipid-rich atheromatous plaque core and leading to sudden thrombus formation. Inflammatory markers of coronary risk, such as hs-CRP, cytokines (IL-1, IL-6, etc), TNF-α, vascular-cell adhesion molecule 1, and intercellular adhesion molecule 1, have been used to evaluate the vulnerability of atheromatous plaques and to predict the risk of ACS. Such inflammatory markers are expected to contribute to the identification of patients at increased risk for ACS.

Recent studies have reported that among the various types of lipoprotein-associated phospholipases, sPLA2 is present in high concentrations in inflammatory exudate, plays a particularly important role in ischemia and atherosclerosis. Secretory PLA2, is stored in the secretory granules of immune cells such as leukocytes and mast cells and participates in arachidonic acid metabolism during the progression of inflammation. High concentrations of sPLA2 are found in inflammatory exudate. Presently, expression of sPLA2 is thought to be promoted by IL-1, as well as by inflammatory cytokines such as interferon and IL-6, lipopolysaccharides, and substances that elevate intracellular concentrations of cyclic adenosine monophos-
phate, such as forskolin. In contrast, the expression of sPLA2 is inhibited by transforming growth factor-β, platelet-derived growth factor, insulin-like growth factors, and anti-inflammatory steroids. It is currently thought that sPLA2 hydrolyzes LDL cholesterol in the vessel wall, thereby participating in the progression of atherosclerosis and the pathogenesis of CAD.

This enzyme has attracted attention because of its potential as a marker of coronary events. Macrophages are known to take in negatively charged substances. Oxidized LDL is recognized by scavenger receptors on macrophages and internalized to form foam cells. These cells contribute to the formation of fatty streaks, one of the earliest lesions of atherosclerosis. Experimental studies have shown that atherosclerotic lesions stained with sPLA2 antibodies exhibit strongly positive staining of foam cells and extracellular matrix. Many T lymphocytes are present at the sites of atherosclerotic lesions. Together with the T-interferon produced by T lymphocytes, sPLA2 stimulates the production and release of inflammatory cytokines and proteases leading to plaque rupture and thrombus formation and causing ACS.

The mechanism by which C. pneumoniae participates in atherosclerosis and CAD remains incompletely understood. C. pneumoniae infecting vessel walls is phagocytosed by macrophages and these macrophages release larger quantities of inflammatory cytokines (TNF-α, IL-1, IL-6) and matrix metalloproteinases than macrophages that have not phagocytosed C. pneumoniae, thereby increasing the risk of plaque disruption. Increased release of inflammatory cytokines is thought to stimulate the release of sPLA2 from vascular smooth muscle cells. Our results showed the blood concentration of sPLA2 was significantly higher in the ACS and OMI/AP groups than in the control group. Moreover, the sPLA2 concentration in patients with ischemic heart disease was much lower than that associated with severe acute inflammation, such as pancreatitis. The blood concentration of sPLA2 is thus considered to be a good inflammatory marker, similar to hs-CRP, for diagnosing the very mild inflammation occurring in patients with coronary disease. Although the hs-CRP concentration correlated with the sPLA2 concentration, the lack of a significant difference in the hs-CRP concentration between patients with a negative C. pneumoniae IgG or IgA titer and those with a positive C. pneumoniae IgG or IgA titer suggested that the hs-CRP concentration, in contrast to the sPLA2 concentration, is influenced by risk factors such as hypertension, diabetes mellitus, and smoking. Previous epidemiological studies have provided evidence that the hs-CRP concentration may be a predictor of the risk of ACS. Because the sPLA2 concentration is unaffected by risk factors such as hypertension, diabetes mellitus, and smoking, this variable may provide slightly different information than hs-CRP for predicting the risk of ACS.

**Study Limitations**

The plasma concentrations of hs-CRP and sPLA2 were measured after the onset of symptoms in the ACS group, and we cannot determine whether elevation of these markers was a cause or an effect of the coronary events. However, because the patients with ACS were admitted within 6 h of symptom onset, time-related effects on the concentration of inflammatory markers were considered minimal. In addition, background factors (age, hypertension, diabetes mellitus, and smoking) differed significantly among the ACS, OMI/AP, and control groups.

**Conclusion**

The sPLA2 concentration was significantly higher in patients positive for C. pneumoniae than in those negative for C. pneumoniae, which suggests that the vascular proinflammatory organism is a factor in the elevation of the sPLA2 concentration. Infection of vessel walls with C. pneumoniae can cause the release of cytokines, promoting the release and expression of sPLA2 in the vessels wall. Increased concentrations of sPLA2 participate in the progression of atherosclerosis and the rupture of plaque. Therefore, patients with C. pneumoniae infection are at a greater risk for ACS than those free of such infection.

**References**

4. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994; 89: 36–44.


