Soluble Forms of P-, E- and L-Selectin in Children with Kawasaki Disease

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Summary: Kawasaki disease (KD) is an acute, self-limiting systemic vasculitis syndrome of unknown origin, that mainly affects small and medium-sized arteries, particularly the coronary artery, which affects primarily infants and young children. Cell adhesion molecules play important roles in the inflammatory process. The aims of this study were to investigate the pathophysiological role of cell adhesion molecules in KD, and to look for the evidence of direct relationship between the plasma levels of soluble cell adhesion molecules and the incidence of coronary artery lesion (CAL). The 52 patients with KD, Group A patients who were clinical responders of initial intravenous immunoglobulin (IVIG) treatment (n=30), Group B patients who did not respond to the initial IVIG treatment (n=22), were studied. The circulating E-selectin (105.6±12.6 ng/ml) in the acute phase of KD, while the peak plasma P-selectin level (238.4±26.8 ng/ml) occurred in the subacute phase of illness (p<0.05, respectively). Plasma L-selectin levels (1557.3±44.3 ng/ml) during the convalescent phase tend to higher than in the acute and in the convalescent phases (p=NS). The analysis of paired samples in Group A patients before (E-selectin: 131.2±9.8 ng/ml, P-selectin: 216.6±13.4 ng/ml) and 48 hour after (E-selectin: 98.9±9.2 ng/ml, P-selectin: 153.9±34.1 ng/ml) IVIG administration revealed significantly lower values of E- and P-selectins, however, no significant differences in those in Group B patients. There were also no significant differences in the values of L-selectins between the 2 groups. Before IVIG treatment, the plasma levels of E- (225.1±46.1 ng/ml) and P-selectin (259.4±76.2 ng/ml) of patients with CAL (n=11) were significantly higher than those of patients (n=41) without CAL (p<0.05, respectively). Plasma L-selectin levels (1596.9±385.0 ng/ml) in patients with CAL tended to be lower than those in patients without CAL (p=NS). E- and P-selectin may have potential as predictors of CAL in patients with KD.

Key words: Kawasaki disease, coronary artery lesion, cell adhesion molecules, high-dose intravenous immunoglobulin therapy

INTRODUCTION

Kawasaki disease (KD) is an acute, self-limiting systemic vasculitis syndrome of unknown origin, that mainly affects small and medium-sized arteries, particularly the coronary artery, that affects primarily infants and young children [1-4]. In untreated patients with KD, there is a 25% risk of coronary artery lesion (CAL) [5-7]. The CAL after KD is an important issue because it may cause myocardial infarction [8,9]. High-dose intravenous immunoglobulin (IVIG) reduces the incidence of CAL by 5-10% [10-15].

Cell adhesion molecules play important roles in the inflammatory process. Such adhesion molecules include P-selectin, which exists on platelet and endothelial cell, E-selectin which exists on endothelial cell, and L-selectin, which exists on leukocyte [16]. These selectins play an active role in mediating cellular interaction in the initial process of inflammation [17]. Selectins such as P-, E-, and L-selectin mediate “Rolling” of leukocytes on the endotheli-
Platelets, leukocytes and endothelial cells have been shown to release soluble adhesion molecules in the blood, and this may be taken as conclusive evidence of those cellular activation [18,19]. Histologically, vascular lesions in the acute phase of KD are associated with evidence of activation and damage to endothelial cells. Thus, inflammatory parameters such as soluble cell adhesion molecules may play an important role in KD vasculitis [20-22]. The aims of this study were to investigate the pathophysiological role of cell adhesion molecules in KD, and to look for the evidence of direct relationship between the plasma levels of soluble cell adhesion molecules and the incidence of CAL.

PATIENTS AND METHODS

Patients

Fifty-two KD patients, 38 males and 14 females, median age 24.0 months, with an age range of 2 months to 80 months, were studied. The diagnosis of KD was made according to criteria established by the Japanese Kawasaki Disease Research Committee. These patients were treated with intravenous immunoglobulin (IVIG; 2 g/kg/day) and with aspirin (30 mg/kg/day). After obtaining informed consent, serial blood samples were taken from all patients with KD in the acute febrile phase (3 to 7 days) before the administration of IVIG and aspirin, as well as at 48 hrs after administration of initial IVIG, each 48 hrs during the febrile phase, and in the afebrile convalescent phase (14 to 30 days), when the C-reactive protein of each patient was less than 0.3 mg/dl. We defined a clinical responder to IVIG treatment as one in which the patient showed a resolution of fever (<37.5 °C) and a fall in C-reactive protein by 50% within 48 hrs after initial IVIG treatment. The patients with KD were retrospectively divided into 2 groups; Group A consisted of 30 patients who were clinical responders of initial IVIG treatment, and Group B consisted of 22 patients who did not respond to the initial IVIG treatment.

Controls

Ten children (5 males, 5 females; median age, 1.8 years; range, 0.9-3.5 years) with fever >38.5 °C and hospitalized for treatment of severe infection were designated as febrile controls. Their diagnoses were septic meningitis (n=3), pneumonia (n=2), colitis (n=2), tonsillitis (n=1), non-specific viral infection (n=1), and group A streptococcal infection (n=1). No medication other than antibiotics and acetaminophen was administered at the time the sample was taken. Eleven age-matched children (6 males and 5 females; median age, 3.0 years; range, 1.2-11 years) with congenital heart disease were studied as afebrile controls. They were diagnosed with atrial septal defect (n=4), ventricular septal defect (n=3), mitral stenosis (n=1), partial anomalous pulmonary return (n=1), and coarctation of the aorta (n=1). None of these patients showed any signs of congestive heart failure, and none were receiving any medication at the time of sampling.

Assay method

The plasma levels of soluble P-, E-, and L-selectin were measured by a sandwich ELISA using a commercially available kit from Takara Biomedical for P-selectin and from R & D Systems, Ltd. for E- and L-selectin. Plasma was stored between −30 and −70 °C prior to analysis. This study was approved by the Kurume University Ethical Committee.

Statistical analysis

Student’s t-test was used to compare the effects of treatment between patients with and without CAL. Correlations between plasma levels of the soluble adhesion molecules and other laboratory values were assessed using the Pearson’s correlation coefficient. P values less than 0.05 were considered to indicate statistically significance. Linear regression analyses were used to determine correlation of plasma E-, P- and L-selectin levels with other laboratory values, including white blood cell, hemoglobin, Platelet, C-reactive protein, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase.

RESULTS

Kinetics of circulating plasma soluble adhesion molecules in Kawasaki disease (Fig. 1)

Values of E-, P- and L-selectin were expressed as the mean ± SD ng/ml. E-selectin levels in the acute phase (5±2 days) were significantly higher than those in the subacute (10±3 days) and in the convalescent phase (22±8 days). Plasma E-selectin levels in both the acute and subacute phase were higher than the plasma those in control patients. Plasma levels of P-selectin in the subacute phase patients with KD were significantly higher than those in patients in acute and the convalescent phases. The
Fig. 1. Kinetics of circulating plasma soluble adhesion molecules in Kawasaki disease. * indicates statistically significance (p<0.05). Gray shadows indicate the normal value obtained from control patients.

Fig. 2. The relationship between plasma E-selectin levels in patients with Kawasaki disease and IVIG treatment. Closed rectangular indicates Group A patients (n=30) who were clinical responders of initial intravenous immunoglobulin (IVIG) treatment. Closed circle indicates Group B patients (n=22) who did not respond to the initial IVIG treatment. Open circle indicates 11 patients who were clinical responders of 2nd IVIG treatment. Closed triangle indicates 11 patients did not respond to the 2nd IVIG treatment. Gray shadow indicates the normal value of plasma E-selectin levels obtained from control patients.

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plasma P-selectin levels in the acute and subacute phase in KD patients were also higher than those in control patients. In other words, circulating E-selectin values peaked in the acute phase of KD, while the peak plasma P-selectin level occurred in the subacute phase of illness. Plasma L-selectin levels during the convalescent phase tend to be higher than in the acute and in the convalescent phases.

**Relationship circulating plasma soluble adhesion molecules to IVIG treatment**

In Group A patients who responded well to initial IVIG treatment, the plasma levels of E- and P-selectins in the acute phase were higher than in afebrile controls, but were not found to be significantly different than those in comparison in febrile controls (Figs 2 and 3). The analysis of paired samples before and 48 hrs after IVIG administration revealed significantly lower values of E- and P-selectins. In Group B patients who did not respond to the initial IVIG treatment, the plasma levels of E- and P-selectins in the acute phase were higher than in either afebrile or febrile controls. Before IVIG treatment, the plasma levels of E-selectins in Group B were significantly higher than those in Group A (Figs 2 and 3). In the analysis of paired samples before and 48 hrs after IVIG administration, there were no significant differences in the values of E- and P-selectins in Group B patients. In the six Group-B patients who did not respond to 2nd IVIG treatment, the E- and P-selectin levels were still higher at 48 hrs after 2nd IVIG treatment than in patients who responded well to 2nd IVIG treatment. In Group A and Group B patients, no significant differences in the plasma levels of L-selectins in the acute phase were found between afebrile controls and febrile controls (Fig. 4). In the analysis of paired samples before and 48 hrs after IVIG administration, there were also no significant differences in the values of L-selectins between the 2 groups. However, in the six patients who did not
**Fig. 4.** The relationship between plasma L-selectin levels in patients with Kawasaki disease and IVIG treatment. Closed rectangular indicates Group A patients (n=30) who were clinical responders of initial intravenous immunoglobulin (IVIG) treatment. Closed circle indicates Group B patients (n=22) who did not respond to the initial IVIG treatment. Open circle indicates 11 patients who were clinical responders of 2nd IVIG treatment. Closed triangle indicates 11 patients did not respond to the 2nd IVIG treatment. Gray shadow indicates the normal value of plasma L-selectin levels obtained from control patients.

**Fig. 5.** Plasma E-, P-, L-selectin levels in patients with coronary artery lesion (closed rectangular) and those in patients with no coronary artery lesion (closed circle) before IVIG treatment.
TABLE 1.

Correlation between soluble adhesion molecules and laboratory values

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Hb: hemoglobin; WBC: white blood cell; PLT: platelet; GOT: glutamic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase; CRP: C-reactive protein.

Fig. 6. Plasma E-, P-, L-selectin levels in patients with coronary artery lesion (closed rectangular) and those in patients with no coronary artery lesion (closed circle) at 48 hrs after IVIG treatment.

Relationship between cell adhesion molecules to the coronary artery lesion

No patients in Group A had CAL. However, 11 patients in Group B had CAL, including 2 patients who had a giant aneurysm, 3 patients who had a small-to-moderate sized aneurysm, and 2 patients who showed transient dilatation during the acute phase. Before IVIG treatment, the plasma levels of E- and P-selectin of patients with CAL were significantly higher than those of patients without CAL and both afebrile and febrile controls (Fig. 5). Plasma
L-selectin levels in patients with CAL tended to be lower than those in patients without CAL, but the difference was not statistically significant. At 48 hrs after the 1st IVIG treatment, the plasma E- and P-selectin levels of patients with CAL were significantly higher than those of patients without CAL and both afebrile and febrile controls (Fig. 5). The plasma levels of L-selectin of patients with CAL tended to be higher than those of patients without CAL, but the difference was not statistically significant (Fig. 5). At 48 hrs after the 1st IVIG treatment, the plasma E- and P-selectin levels of patients with CAL were significantly higher than those of patients without CAL and those of both afebrile and febrile controls (Fig. 6). The plasma L-selectin levels of patients with CAL tended to be higher than those of patients without CAL, but the difference was not statistically significant (Fig. 6).

Correlation between soluble adhesion molecules and laboratory values (Table 1)

Among patients with Kawasaki disease, within Kawasaki disease patients, there was a weak positive correlation between plasma E-selectin levels and WBC (r=0.37, P=0.0009). There was a significant positive correlation between plasma E-selectin levels and C-reactive protein (r=0.51, P<0.0001). Values of P-selectin showed no significant correlation with platelet count. No significant correlation was found between cell adhesion molecules levels and other laboratory values.

DISCUSSION

The time points of the peak levels of the soluble forms of adhesion molecules varied among our KD patients. The finding of elevated plasma levels of adhesion molecules in patients with KD provides additional evidence of a relationship between acute vasculitis and plasma selectins.

Mechanism of changes in plasma selectin levels

The selectin family consists of 3 proteins designated by the prefixes E (endothelial), P (platelet and endothelial), and L (leukocyte). These adhesion molecules are shed following proteolytic cleavage near the transmembrane domain or by expression of alternatively spliced mRNA lacking a transmembrane domain from the cell surface, following activation with cytokines or other stimuli, such as endotoxin. It has been suggested that the soluble molecules can regulate cell adhesion by downregulation as competitive inhibitors, or by upregulation as co-signaling factors [18,19]. More recent studies have provided evidence that soluble isoforms can be detected in the circulation, and that increased levels may be a key to understanding the prognosis and inflammatory processes of certain diseases [23-26]. When the endothelial cells are activated by various stimuli including cytokines, intercellular adhesion molecules (ICAMs) can be expressed on the cell surface. ICAMs between endothelial cells and leukocytes can be classified into three classes: the immunoglobulin superfamily, integrin family and selectin family [27,28]. The selectin family includes E-selectin, L-selectin, and P-selectin. The physiological roles of soluble selectins in KD are still unknown. E-selectin may represent a marker for endothelial damage or activation in KD vasculitis. In the acute phase, E-selectin may play a role as a mediator of the cytokine network in which immune effector cells are markedly activated [20-22]. In our study, there was no correlation between P-selectin levels and platelet counts, the soluble form may represent a marker for endothelial damage and may not be mainly shed from platelet. Although the roles of L-selectins are still uncertain, it is known that L-selectins are immediately shed after activation. It is unclear whether the decreased L-selectin levels may contribute to the pathogenesis of vasculitis in KD.

Analysis of paired samples demonstrated that 48 hrs after initial IVIG treatment, there were significant decreases in the levels of plasma E- and P-selectin of patients in Group A. However, in Group B patients, no significant difference in the levels of plasma E- or P-selectin was found between before and after IVIG treatment. This might reflect downregulation of cytokines by IVIG treatment. The elevated levels of E- and P-selectin before and 48 hrs after initial IVIG treatment were significantly correlated with disease severity of KD vasculitis, since both higher levels or persistent elevation were associated with the presence with CAL. It is likely, however, that these molecules play a significant role in localization of leucocytes to the site of vascular injury in KD, since they have been shown to be important in other immunologically mediated diseases. In addition, our study suggests that the kinetics of these circulating molecules may differ from those in the acute through the convalescent phases of KD, and that elevated levels of these molecules may reflect the inflammatory process.
Clinical implications of adhesion molecules

Previous studies have demonstrated the efficacy of IVIG treatment in the acute stage of KD. Nonetheless, approximately 10% of patients experience a persistent or recurrent fever despite the initial IVIG treatment [7,13]. A subgroup of patients with KD is resistant to IVIG treatment, and these patients are at greatest risk of developing CAL. To our knowledge, there are no useful predictors of the risk of CAL development before IVIG administration. In the present study, E- and P-selectin levels in patients with CAL before IVIG treatment (Group B) were significantly higher than those in patients without CAL (Group A). These results suggested that E- and P-selectin may reflect disease activity and may represent a marker for endothelial damage or activation in KD vasculitis. Our interest in E-selectin has extended to a consideration of its fate subsequent to placement in the plasma membrane. It was shown earlier that the endothelial cells induced to express E-selectin by stimulation with cytokines, such as IL-1 and TNF-α, peaked in their expression at 4-6 hrs, and that by 24 hrs the molecules were no longer detectable on the cell surface [27,28]. Interestingly, endothelial cells have been shown to release E-selectin following in vitro activation. Therefore, the demonstration of soluble E-selectin in the blood may be taken as conclusive evidence of endothelial activation. In the acute phase of KD, E-selectin may play a role as a mediator of the cytokine network in which immune effector cells are markedly activated [20-22]. E-selectin is particularly interesting because it is found only on the activated endothelium, in contrast to other adhesion molecules. Even more interesting is that E-selectin can be detected in the blood in a soluble form, shed from the cell membrane, and this form could be used as an index of the severity of vasculitis in inflammatory disease [20]. In KD patients, early identification of the predictors of CAL is important because early treatment with IVIG is necessary to prevent CAL. Numerous investigators have attempted to correlate clinical findings and laboratory findings in patients with CAL. Previously, however, the best predictors of CAL have depended on measurements in the subacute phase and thus have not been helpful in determining the risk of CAL in the acute phase. In our present study, it is interesting that all KD patients with CAL showed markedly increased E- and P-selectin levels before IVIG treatment. E- and P-selectin may thus become a useful predictor of CAL in patients with even acute-phase KD.

Several reports have indicated the potential use of cell adhesion molecules as new genetic therapies for certain diseases. For example, P-selectin antibody has been shown to be useful in therapy for lung hemorrhage, and L-selectin antibody has proven useful in the treatment of baboons [29,30]. Not only may soluble cell adhesion molecules of the selectin family become useful as predictors of CAL, but their antibodies may also one day be useful in genetic therapy for KD patients.

CONCLUSIONS

Alterations in plasma selectin levels are important clinical molecular markers for the understanding of the pathophysiology of patients who may need closer follow-up. E- and P-selectin molecules may have potential as predictors of CAL in patients with KD. The present results suggest the need for further investigation into alterations in plasma selectin levels as a source of baseline information in the design of future, selectin-family based genetic therapy for KD.

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REFERENCES