IVIG Reduced Vascular Oxidative Stress in Patients With Kawasaki Disease

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**Background:** Oxidative stress (OS) contributes to the acute phase of Kawasaki disease (KD) in a manner that is as yet unknown. In the present study OS in the acute phase of KD was investigated by measuring urinary 8-iso-prostaglandin F2α (8-iso-PG) and evaluating its correlation to the efficacy of intravenous immunoglobulin (IVIG) administration.

**Methods and Results:** The 62 patients with acute phase of KD were enrolled, as well as 20 healthy children (HC) and 20 with acute febrile illness (FI). Urinary samples were obtained before and after administration of IVIG. The HC and FI groups also had inflammatory markers evaluated at the same time. The 8-iso-PG was significantly elevated in the 62 KD patients (719±335 pg/mg Cr) without IVIG administration compared with those with FI (583±213 pg/mg Cr) as well as HC (443±288 pg/mg Cr) (P<0.01). 40 patients were given 3 different regimens of IVIG: 16 received 2 g/kg for 1 day; 17 received 1 g/kg for 1 day; 7 received 400 mg·kg⁻¹·day⁻¹ for 5 days. All regimens of IVIG reduced the 8-iso-PG level at 7 days after initiation.

**Conclusions:** OS provokes vasculitis in KD, the activation of which was reduced by IVIG. The urinary level of 8-iso-PG is a useful marker of the effectiveness of IVIG in the acute phase of KD. (Circ J 2009; 73: 1315–1318)

**Key Words:** Intravenous immunoglobulin; 8-iso-prostaglandin F2α; Kawasaki disease; Oxidative stress

Oxidative stress (OS) plays an important role in vascular diseases. Endothelial dysfunction activates the pathway that leads to elevated OS! In particular, oxidative damage occurs when the delicate balance between pro- and antioxidant molecules, which act against free radical injury, is upset. This balance may be destroyed by certain risk factors; for example, atherosclerosis, hyper tension, hyperlipidemia, diabetes and cigarette smoking.

The 8-isoprostaglandin F2α (8-iso-PG) is a nonenzymatic oxidation product of arachidonic acid and is widely recognized as a reliable marker of lipid peroxidation both in vitro and in vivo. Enhanced endothelial dysfunction, as reflected by increased 8-iso-PG excretion, has been reported previously.

Kawasaki disease (KD) is a systemic vasculitis and an acute febrile illness (FI) in children. Recent studies have shown activation of the immune system is involved and multiple factors are likely to cause the pathologic changes seen in KD! How OS contributes to the acute phase of KD is as yet unknown.

In the present study, we investigated OS in the acute phase of KD by measuring urinary 8-iso-PG and evaluated its correlation to the efficacy of intravenous immunoglobulin (IVIG) treatment.
Results

Clinical Characteristics of Patients and Controls
The baseline characteristics of the patients and control subjects are detailed in Table. No significant differences between patients with KD, FI and HC were found. Two patients in the KD group had coronary artery abnormalities (1 had a coronary artery lesion (CAL), 1 had coronary artery dilatation).

8-iso-PG Levels in the KD and Control Groups
The 8-iso-PG was significantly elevated in the 62 KD patients (719±335 pg/mg Cr) before IVIG administration compared with the 20 patients with FI (583±213 pg/mg Cr) and the 20 HC (443±288 pg/mg Cr) (P<0.01 1-way ANOVA) (Figure 1). The normal value of 8-iso-PG in children is <650 pg/mg Cr.

8-iso-PG and Inflammatory Parameters
We evaluated inflammatory parameters such as CRP, albumin, WBC count, neutrophil count and ESR, but there were no significant correlations between 8-iso-PG and any of these.

8-iso-PG Levels in the Acute Phase of KD After IVIG Therapy
We evaluated 8-iso-PG levels in 40 patients with acute KD before and after IVIG treatment. The 8-iso-PG levels at 7 days after a single administration of IVIG were significantly decreased compared with before treatment (707±356 pg/mg Cr vs 426±214 pg/mg Cr; P<0.01 paired t-test) (Figure 2). The 8-iso-PG levels before IVIG administration tended to be decreased compared with after 14 days (707±356 pg/mg Cr vs 556±324 pg/mg Cr; P=0.05 paired t-test).

We also evaluated the 8-iso-PG levels in 40 patients given 3 different regimens of IVIG: 16 patients received 2 g/kg for 1 day, 17 patients received 1 g/kg for 1 day and 7 patients received 400 mg · kg⁻¹ · day⁻¹ for 5 days. All treatment regimens of IVIG reduced the 8-iso-PG level at 7 days after administration. The 8-iso-PG levels before IVIG administration were not significant different between the 3 regimens. The 8-iso-PG tended to decrease in the patients

Table. Clinical Characteristics of Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>KD group</th>
<th>FI group</th>
<th>HC group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>62</td>
<td>20</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>Age (median)</td>
<td>2 months–11 years (4 years)</td>
<td>1–10 years (5 years)</td>
<td>1–11 years (4 years)</td>
<td>NS</td>
</tr>
<tr>
<td>M/F</td>
<td>37/25</td>
<td>12/8</td>
<td>10/10</td>
<td>NS</td>
</tr>
</tbody>
</table>

KD, Kawasaki disease; FI, febrile illness; HC, healthy children.

Figure 1. The 8-iso-prostaglandin F2α in patients with Kawasaki disease (KD) or febrile illness (FI) and in healthy controls. Gray zone shows the normal range in children. HC, healthy children.

Figure 2. The 8-iso-prostaglandin F2α levels before and after intravenous immunoglobulin therapy (7 and 14 days).
with the single 2 g/kg administration compared with those who received 1 g/kg of IVIG infusion for 1 day (1 g/kg vs 2 g/kg, -214 pg/mg Cr –14% vs –329 pg/mg Cr –35%; P= 0.2 ANOVA), although this was not statistically significant (Figure 3). We had only 2 cases of CAL and could not evaluate the 8-iso-PG level in those patients.

**Discussion**

Cardiovascular disease is related to increased systemic OS; established risk factors of systemic vascular disease, such as diabetes, have been associated with elevated levels of markers of OS. Measurement of the urinary excretion of 8-iso-PG has been characterized as a method of investigating lipid peroxidation in vivo and has been shown to reflect enhanced OS, regardless of the underlying pathophysiological trigger. Systemic vascular disease in which endothelial dysfunction is postulated on the basis of recent data has been reported to be associated with increased 8-iso-PG levels.

Palombo et al demonstrated that hypercholesterolemic animals had increased circulating levels of 8-iso-PG and increased deposition on the intimal surface of vessels. The association between increased circulating levels and increased intimal deposition of 8-iso-PG supports the pathogenetic role of 8-iso-PG in vascular damage. This finding leads to a hypothesis of intimal disease as a dynamic process involving the arterial wall in the early stages of atherosclerosis, where the morphologic abnormality may be related to possibly reversible biochemical changes more than to permanent structural abnormalities.

Kato et al reported that baseline percent flow-mediated vasodilatation (FMD) was lower and the baseline 8-iso-PG level higher in smokers than in nonsmokers, that %FMD decreased and the 8-iso-PG level increased in nonsmokers to the levels in smokers after exposure to 30 min of passive smoking, and that %FMD negatively correlated with the 8-iso-PG level. It has already been established that vascular endothelial function is impaired and plasma lipid peroxidation products are elevated in nonsmokers exposed to passive smoke. This data showed of 8-iso-PG products immediately after exposure to OS. Our results demonstrate that isoprostane products are present in the acute phase of KD. In our group of KD patients, the urinary 8-iso-PG level was higher than that of healthy subjects, which suggests that in the acute phase of KD there is endothelial peroxidation, which may be associated with increased OS in this setting.

Moreover, the normal range of 8-iso-PG in childhood is less than 650 pg/mg Cr and our results showed that the 8-iso-PG level in children was higher than that in healthy adults. We did not differentiate between infants and older children. The 8-iso-PG level in the KD patients was also higher than in those with a febrile disease caused by a mild viral infection. The 8-iso-PG level in patients with a severe bacterial infection may be higher than in those patients.

We observed that IVIG reduced the 8-iso-PG levels in patients with acute phase of KD who had significantly increased 8-iso-PG levels before treatment, which suggests that IVIG may be an anti-oxidant, as are vitamins E and C. Measurement of the 8-iso-PG concentration has proven to be valuable in assessing OS in vivo, because it is a specific product of lipid peroxidation. Although an increase in the 8-iso-PG level is indirect evidence of endothelial dysfunction, 8-iso-PG is a specific marker of oxidative injury in vasculitis. Our study demonstrated that OS activation plays an important role in the pathological process of the acute phase of KD. In addition, 8-iso-PG is also a reliable marker of OS in the acute phase of KD and may be a sensitive marker of the effects of IVIG therapy.

Interestingly, our results showed that there is no correlation between 8-iso-PG and inflammatory markers such as CRP, WBC count and ESR. This observation may indicate that 8-iso-PG is an independent parameter of endothelial injury in the acute phase of KD. Furthermore, we hypothesize that IVIG may have antioxidative effects that prevent the generation of 8-iso-PG.

We had 2 patients with coronary artery abnormalities, but only 2 cases are too few to assess OS in patients with CAL and further study of such patients should be needed. In a recent study, 8-iso-PG promoted platelet aggregation and induced platelet adhesion. Moreover, 8-iso-PG is a powerful constrictor of the vasculature and so increased generation of bioactive 8-iso-PG may be responsible for damage.

Another important point is that measurement of urinary 8-iso-PG is a totally noninvasive method and is superior to measurement of 8-iso-PG in serum, because urinary 8-iso-PG is a stable molecule.

Our data showed that measurement of an OS marker could represent oxidative capacity in vascular disease. Further studies are needed to evaluate 8-iso-PG against antioxidants such as vitamin C in acute KD.
Conclusions

Our study revealed OS provokes vasculitis in KD, the activation of which was reduced by IVIG administration. We conclude that the urinary 8-iso-PG level is a sensitive and useful marker of the effectiveness of IVIG therapy in the acute phase of KD.

Acknowledgments

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References