Original article

Persistent high fever for more than 10 days during acute phase is a risk factor for endothelial dysfunction in children with a history of Kawasaki disease

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A R T I C L E   I N F O

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A B S T R A C T

Background: Endothelial dysfunction has previously been reported in children with a history of Kawasaki disease, but the determinants of endothelial function in Kawasaki disease patients are still unknown. In this study, we investigated endothelial function in Kawasaki disease patients and attempted to identify risk factors for persistent endothelial dysfunction.

Methods: Using high-resolution ultrasound, we measured the percent flow-mediated dilatation, an arterial response to reactive hyperemia, to evaluate endothelial function in 67 patients with a history of Kawasaki disease and 28 age- and sex-matched control subjects. We divided the Kawasaki disease patients into a group with impaired endothelial function (the percent flow-mediated dilatation below –2 standard deviations of the control group) and a group with normal endothelial function (the percent flow-mediated dilatation more than –2 standard deviations of control). Logistic multiple regression analysis was performed to identify independent predictors of impaired endothelial function.

Results: In Kawasaki disease patients, the percent flow-mediated dilatation was significantly lower than in the control subjects (9.8 ± 3.6%, compared with 13.1 ± 3.4%, p < 0.01). In 13 Kawasaki disease patients (3 patients with coronary artery lesions and 10 patients without coronary artery lesions), the percent flow-mediated dilatation was below –2 standard deviations of control. Logistic multiple regression analysis showed that a febrile period of longer than 10 days during the acute phase was the significant risk factor for endothelial dysfunction (odds ratio: 8.562; 95% confidence interval: 1.366–53.68). Presence of coronary artery lesions was not a determinant of endothelial dysfunction.

Conclusions: Systemic endothelial dysfunction exists in children with a history of Kawasaki disease, and a febrile period of longer than 10 days during the acute phase is an independent predictor of endothelial dysfunction irrespective of coronary artery involvement.

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Introduction

Kawasaki disease is an acute, febrile, pediatric illness that occasionally causes coronary artery lesions [1,2]. This disease is characterized by systemic vasculitis and may be a risk factor for the early progression of atherosclerosis in adolescents, although this has yet to be confirmed by epidemiological studies [3]. Endothelial dysfunction is one of the earliest changes in various types of vascular remodeling, including atherosclerosis [4]. Recently, endothelial function has been investigated noninvasively on the basis of systemic arterial reactivity. Several investigators have reported on the endothelial function of Kawasaki disease patients. Previous studies have demonstrated that systemic endothelial dysfunction exists in Kawasaki disease irrespective of coronary artery involvement [5], and is not influenced by early treatment with high-dose gamma globulin during the acute stage [6]. Because conflicting results have been reported about systemic endothelial function in Kawasaki disease patients [7,8], the actual determinants of endothelial function in this disease are still unknown. In the present study, therefore, we investigated the endothelial...
function of Kawasaki disease patients and attempted to identify risk factors for persistent endothelial dysfunction.

**Methods**

**Subjects**

We studied 67 children with a history of Kawasaki disease (36 boys and 31 girls aged 9.7 ± 2.5 years at examination; range: 6–15 years) and 28 sex- and age-matched controls (16 boys and 12 girls aged 8.6 ± 2.2 years; range: 4–13 years). Subjects with evidence of risk factors for cardiovascular disease such as smoking, hyperlipidemia, hypertension, and diabetes mellitus were excluded. Kawasaki disease was diagnosed from the standard criteria defined by the Japanese Ministry of Health and Welfare. The mean duration from the onset of Kawasaki disease to examination was 7.5 ± 2.4 years. Among the 67 Kawasaki disease patients, in the first month after onset of Kawasaki disease, the maximal coronary artery involvement as noted on echocardiography was described as coronary artery lesions (more than 2 standard deviations above normal and more than 3 mm in diameter) in 10 patients. All subjects refrained from consuming antioxidant-containing foods and beverages, such as Japanese and English tea, fruit juice, and vitamin supplements for at least 24 h before examination. This study was approved by the local ethics committee, and informed consent was obtained from the parents of all subjects.

**Study design**

We took a history from each subject, and performed a physical examination, including the height, weight, and supine blood pressure. The medical records of the Kawasaki disease patients were reviewed to ascertain the details of the acute illness. Total cholesterol and triglyceride levels were measured in all subjects, and high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, random blood glucose, immunoreactive insulin, and *Chlamydia pneumoniae* antibody titers were measured in the Kawasaki disease patients. Blood samples were obtained after an overnight fast. Brachial artery endothelial function was investigated as described below.

**Measurement of brachial artery endothelial function**

We determined endothelium-dependent flow-mediated dilation and hyperemic flow of the brachial artery in a quiet and temperature-controlled room, as described previously [9]. The same examiner performed all examinations throughout the study. The diameter of the brachial artery was measured on two-dimensional ultrasound images that were acquired using a 7.0 MHz linear array transducer and a Hewlett-Packard (Palo Alto, CA, USA) Sonos 5500. To determine the baseline diameter, the brachial artery was scanned longitudinally at 2–10 cm above the elbow after the subject had rested for at least 10 min. After inflating a pneumatic tourniquet distal to the target arterial segment and holding the pressure at 250 mm of mercury for 4–5 min, scanning was repeated at 30 s before and 90 s after cuff deflation. Flow velocity was measured using a pulsed Doppler signal at 70° to the vessel wall, with the range gate (1.5 mm) set at the center of the artery. Flow measurements were recorded during the first baseline scan and again during the first 15 s of reactive hyperemia, and the increase in flow was expressed as a percentage of baseline flow. The electrocardiograph was monitored continuously throughout the study. All recordings were stored on supervideo home system videotape for later analysis.

Measurements of the arterial diameter and blood flow were made from the videotapes by a single observer who was blinded to the clinical details and stage of the experiment. The arterial diameter was measured from the anterior to posterior “m-line” at end-diastole, coinciding with the R wave on the electrocardiograph. Four cardiac cycles were analyzed for each scan. Diameter measurements were also obtained at 50–60 s after cuff deflation to assess the effect of reactive hyperemia and the change in diameter was calculated as a percentage relative to the baseline diameter. The baseline diameter was subtracted from the diameter after cuff deflation, the result of which was then divided by the baseline diameter and multiplied by 100, yielding the percent flow-mediated dilation.

**Statistical analysis**

Descriptive data are presented as the mean value (±standard deviation) unless otherwise specified. Differences of the percent flow-mediated dilatation between the Kawasaki disease patients and control subjects were analyzed by the unpaired Student’s t-test.

We divided the Kawasaki disease patients into two groups, which were impaired endothelial function (the percent flow-mediated dilatation below –2 standard deviations of control) and normal endothelial function (the percent flow-mediated dilatation more than –2 standard deviations of control). Differences between these groups were assessed by the unpaired Student’s t-test for continuous variables and by the χ²-test for categorical variables.

Furthermore, logistic multiple regression analysis was performed to identify independent predictors of impaired endothelial function. The variables analyzed were age at the examination, coronary artery lesions, low-density lipoprotein cholesterol, immunoreactive insulin, and febrile (temperature more than 37.5°C) period during the acute phase of Kawasaki disease. Statistical significance was accepted at a p value less than 0.05.

**Results**

The Kawasaki disease patients and the control subjects were well matched for age, gender, and body mass index. Total cholesterol and triglyceride levels did not differ between the Kawasaki disease patients and the control subjects (Table 1).

There were no qualitative differences between the two groups with regard to the appearance of the vessel walls or intima. There were also no significant between-group differences of baseline brachial artery diameter or the increase in flow during reactive hyperemia. However, the percent flow-mediated dilatation of the brachial artery was significantly smaller in the Kawasaki disease patients than in the controls (9.8 ± 3.6%, compared with 13.1 ± 3.4%, p < 0.01) (Table 1, Fig. 1). The percent flow-mediated dilatation of the Kawasaki disease patients without coronary artery

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics and vascular studies in Kawasaki disease patients and control subjects.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>Kawasaki patients (n=67) Control subjects (n=28) p value</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>9.7 ± 2.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>36/33</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>17.2 ± 2.5</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>177.6 ± 29.3</td>
</tr>
<tr>
<td>Random blood glucose, mg/dl</td>
<td>56.8 ± 37.7</td>
</tr>
<tr>
<td>Vessel size, mm</td>
<td>91.9 ± 7.3</td>
</tr>
<tr>
<td>Reactive hyperemia, %</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Flow-mediated dilatation, %</td>
<td>257 (77)</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation).

n, number of patients or subjects; NS, not significant.
lesions (10.4 ± 3.3%) as well as that of the Kawasaki disease patients with coronary artery lesions (8.1 ± 4.4%) was significantly lower than in the control subjects (both p-values < 0.01) (Fig. 1).

Comparison with impaired and normal endothelial function in Kawasaki disease patients

We divided the Kawasaki disease patients into two groups, those who had impaired endothelial function (the percent flow mediated dilatation below −2 standard deviations of control) and normal endothelial function (the percent flow mediated dilatation more than −2 standard deviations of control). In 13 out of 67 Kawasaki disease patients (19%), the percent flow-mediated dilatation was below −2 standard deviations of control. These 13 patients with impaired endothelial function included 3 out of 10 patients with coronary artery lesions (30%) and 10 out of 57 without coronary artery lesions (18%).

Risk factors for endothelial dysfunction

The groups with impaired and normal endothelial function were well matched for gender and body mass index. There was a significant difference of the age at examination, but no differences in the lipid profile (total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol), random blood glucose, immunoreactive insulin, C. pneumoniae antibody titer, blood pressure, and passive smoking between the two groups (Table 2). When independent predictors of impaired endothelial function were assessed by multiple regression analysis, the odds ratio for endothelial dysfunction was significantly higher in patients with a febrile period of longer than 10 days during the acute phase of Kawasaki disease (odds ratio: 8.562; 95% confidence intervals: 1.366–53.680) (Table 3). In contrast, coronary artery lesions were not a determinant of endothelial dysfunction (Table 3).

Discussion

Endothelial function in Kawasaki disease patients

It is already known that coronary artery involvement occurs in a minority of infants and children with acute Kawasaki disease [10], and considerable attention has been paid to the long-term outcome of this subgroup [11]. However, intimal hyper trophy of the coronary arteries has been detected by histopathological examination [12,13] or intravascular ultrasound even in children without aneurysms on echocardiography or angiography [14–16]. Furthermore, previous studies have demonstrated that the apparently normal coronary arteries of children with Kawasaki disease showed significant constriction in response to acetylcholine provocation, indicating the impairment of endothelium-dependent relaxation [7,17].

In acute Kawasaki disease, histopathological examination shows widespread vascular inflammation with endothelial edema, necrosis, and leukocyte infiltration involving the coronary arteries and other medium-sized muscular arteries [18,19]. Thus, Kawasaki disease is characterized by the existence of systemic vasculitis. Endothelial dysfunction, as indicated by systemic arterial reactivity, has been reported in children with a history of Kawasaki disease. Dhillon et al. [5] showed that systemic endothelial dysfunction was present many years after the resolution of acute Kawasaki disease, even in patients without detectable early coronary artery involvement. Deng et al. [6] found that systemic endothelial dysfunction exists after acute Kawasaki disease and is not prevented by early treatment with high-dose gamma globulin in the acute stage. However, several investigators have reported conflicting results regarding systemic endothelial function in Kawasaki disease patients. Mitani et al. [7] found no difference between Kawasaki disease patients and normal subjects with regard to the femoral artery response to reactive hyperemia, although

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics of Kawasaki disease patients with normal and impaired endothelial function.</th>
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<tbody>
<tr>
<td>Endothelial function</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td>Impaired (n = 13)</td>
</tr>
<tr>
<td>Age, year</td>
<td>11.5 (2.5)</td>
</tr>
<tr>
<td>Gender, Male/Female</td>
<td>7/6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>18.0 (2.9)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>174.9 (31.4)</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>52.0 (20.4)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>98.0 (22.8)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>66.3 (12.6)</td>
</tr>
<tr>
<td>Random blood glucose, mg/dl</td>
<td>91.9 (7.6)</td>
</tr>
<tr>
<td>Immunoreactive insulin, µIU/l</td>
<td>9.0 (4.8)</td>
</tr>
<tr>
<td>Chlamydia pneumoniae antibody titer (lgG)</td>
<td>1.1 (1.3)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>120 (13)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>72 (7)</td>
</tr>
<tr>
<td>Passive smoking, plus/minus</td>
<td>9/4</td>
</tr>
<tr>
<td>Vessel size, mm</td>
<td>3.0 (0.5)</td>
</tr>
<tr>
<td>Reactive hyperemia, %</td>
<td>242 (84)</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation). n, number of patients; NS, not significant.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Logistic multiple regression analysis of determinants of impaired endothelial function in Kawasaki disease patients.</th>
</tr>
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<tbody>
<tr>
<td>Odds ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Febrile period, day &gt; 10 versus less</td>
<td>8.562</td>
</tr>
<tr>
<td>Coronary artery lesions ±</td>
<td>2.099</td>
</tr>
<tr>
<td>Age &gt; 13 versus younger</td>
<td>7.25</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl &gt; 120 versus less</td>
<td>1.159</td>
</tr>
<tr>
<td>Immunoreactive insulin, µIU/l &gt; 15 versus less</td>
<td>0.715</td>
</tr>
</tbody>
</table>

CI, confidence intervals; LDL, low-density lipoprotein.
they reported endothelial dysfunction in the epicardial coronary arteries. In addition, Silva et al. [8] found no significant differences in endothelial function, as indicated by brachial artery reactivity, between Kawasaki disease patients and a normal control group. These previous studies only assessed a small number of subjects, but Kawasaki disease covers a broad spectrum of severity from a self-limiting vasculitis to the development of severe coronary artery lesions. Such wide variation in disease severity combined with small study populations may have led to conflicting results with respect to the endothelial function of Kawasaki disease patients. In the present study, we enrolled 67 subjects, a larger number than in previous studies. Since the percent flow-mediated dilatation is a fairly sensitive parameter that is affected by many factors, such as dietary antioxidants [20] and mental stress [21], we prohibited the intake of antioxidant-containing foods or beverages prior to the examination and performed the study in a quiet room to allow the subjects to relax.

As a result, we demonstrated that endothelial function was impaired regardless of the presence/absence of significant coronary artery involvement. This finding corroborated the previous results of Dhillon et al. and Deng et al. Furthermore, we found that the percent flow-mediated dilatation was below –2 standard deviations of the control group value in 19% of our Kawasaki disease patients and in 30% of the patients with coronary artery lesions. Even among the patients without coronary artery lesions, the percent flow-mediated dilatation was reduced in 18%. These findings may reflect the wide variation in the severity of Kawasaki disease.

Determinants of endothelial dysfunction in Kawasaki disease

Although previous studies have shown that systemic endothelial dysfunction was independent of coronary artery lesions or acute treatment with high-dose gamma globulin Kawasaki disease, the risk factors for chronic endothelial dysfunction are still unknown. In the present study, we divided our Kawasaki disease patients into a group with systemic endothelial dysfunction (the percent flow-mediated dilatation below –2 standard deviations of control) and a group with normal endothelial function (the percent flow-mediated dilatation more than –2 standard deviations of control). Meyer et al. [22] performed similar analyses in their study with endothelial function in obese children. Since the data of percent flow-mediated dilatation in the control group were normally distributed in the present study, these analyses are rational.

There were no significant between-group differences of gender, body mass index, and factors that might impair endothelial function, but the subjects with endothelial dysfunction were slightly older than those with normal endothelial function. Multiple regression analysis showed only one determinant of endothelial dysfunction in patients with Kawasaki disease patients, which was a febrile period of longer than 10 days during the acute phase. In contrast, coronary artery lesions were not a determinant of endothelial dysfunction in this study population.

It is well known that the cytokine network is activated during acute Kawasaki disease and various growth factors and cytokines are elevated at this time [23]. Adhesion molecules also show increased expression [24], while hypercoagulation and reduced fibrinolysis have been reported [25]. These data suggest that endothelial cell damage occurs in acute Kawasaki disease. A protracted febrile period means a longer duration of endothelial damage in the acute phase, which may cause endothelial impairment to persist for a long period after the onset of Kawasaki disease even without causing coronary artery lesions.

Limitations

In arteries lined by healthy endothelium, increased flow causes vasodilation via release of nitric oxide, an endothelium-derived relaxing factor, while this mechanism fails in the presence of endothelial dysfunction. In contrast, nitroglycerin causes vasodilatation by a direct action on smooth muscle and its effect is independent of the endothelium. In the present study, the youngest subject was only 4 years old. Sublingual administration of nitroglycerin may raise concerns in such young children, so we did not test nitroglycerin-induced dilation. However, most of the previous studies of the percent flow-mediated dilatation in Kawasaki disease patients have shown no differences of nitroglycerin-induced dilation between patients and controls. Leeson et al. [26] omitted nitroglycerin-induced dilation in their percent flow-mediated dilatation study of young children after a pilot study on a smaller number of subjects. Considering such results, assessment of nitroglycerin-induced dilation does not seem to be necessary, although the participation of smooth muscle in vasodilatation cannot be completely excluded in the present study.

We investigated 67 patients with Kawasaki disease in the present study, which was a considerably larger number than in previous studies. However, the wide variation in the severity of Kawasaki disease means that further investigation of a larger number of subjects may be necessary.

Conclusions

We demonstrated that systemic endothelial dysfunction is present in children with a history of Kawasaki disease, and that a febrile period of longer than 10 days during the acute phase was an independent predictor of impaired endothelial function regardless of the presence/absence of significant coronary artery involvement. The present results suggest that severe acute impairment of endothelial function may persist for a long period after the onset of Kawasaki disease. The relevance of Kawasaki disease to cardiovascular disease in adults is still unknown and the method of follow-up for Kawasaki disease patients without coronary artery lesions is controversial. The information provided by the present study may help to resolve these issues.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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References


