Circulating Matrix Metalloproteinases and Their Inhibitors in Patients with Kawasaki Disease

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Background—Accelerated matrix breakdown caused by the increased activity of matrix metalloproteinases (MMPs) and/or the quantitative imbalance between MMP and tissue inhibitor of MMP (TIMP) have been implicated in several pathological conditions. MMP and TIMP may also be involved in the destruction of the coronary arterial wall and the resultant coronary arterial lesions in Kawasaki disease.

Methods and Results—Plasma levels of MMPs, neutrophil elastase, and TIMPs were measured by enzyme-linked immunoassay in 57 patients with Kawasaki disease and no coronary arterial lesions (group 1) and in 8 patients with Kawasaki disease and coronary arterial lesions (group 2). Blood samples were obtained before and after intravenous gamma globulin therapy and in the convalescent stage. Levels of MMPs, neutrophil elastase, and TIMPs were significantly higher in Kawasaki disease patients before gamma globulin therapy than in 18 age-matched afebrile control subjects and 17 age-matched febrile disease control subjects ($P < 0.01$). More importantly, the pre-gamma globulin MMP9 level and MMP9/TIMP2 ratio and post-gamma globulin MMP3 level and MMP3/TIMP1 ratio were significantly higher in group 2 than in group 1 patients ($P < 0.05$). Although MMP levels in febrile disease controls were significantly higher than those of afebrile controls, the MMP/TIMP ratios of febrile disease controls and afebrile controls were comparable.

Conclusions—These data suggest that patients with Kawasaki disease and high levels of MMP and/or MMP/TIMP are susceptible to coronary arterial lesions. Studies of the effects of MMP inhibitors on coronary outcome may provide evidence that MMP is a viable therapeutic target for the prevention of coronary arterial lesions due to Kawasaki disease. (Circulation. 2001;104:860-863.)

Key Words: aneurysm ■ coronary disease ■ metalloproteinases

The physiological condition of the extracellular matrix is maintained by a rigorously controlled balance between the synthesis and breakdown of its component proteins. Matrix metalloproteinases (MMPs) and their endogenous inhibitors, known as tissue inhibitors of MMP (TIMPs), play central roles in this process. Accelerated matrix breakdown caused by increased activity of MMPs and/or a quantitative imbalance between MMP and TIMP can result in pathological conditions, including rheumatoid arthritis, tumor metastasis, and heart failure. Increased levels of MMPs have also been detected in aortic aneurysms in adult humans, suggesting an important role for MMPs in arterial wall destruction and resultant aneurysm formation.

These findings raise the possibility that MMPs may also be involved in coronary arterial wall destruction and the formation of coronary aneurysms in Kawasaki disease (KD). The present study was conducted to test the hypothesis that circulating levels of MMPs and the MMP/TIMP ratio are related to the formation of coronary artery lesions (CAL) in KD.

Methods

The subjects of this study were 57 KD patients without CAL (group 1) and 8 KD patients with CAL (group 2). The blood samples were obtained by nontraumatic needle aspiration from the antecubital vein, with no hemolysis occurring in any of the samples. Immediately after samples were centrifuged, plasma was cooled to -80°C and stored at that temperature until assays were performed (1 to 2 months later). Plasma levels of MMPs 1, 2, 3, and 9 and TIMPs 1 and 2 were measured by one-step sandwich enzyme-linked immunoassay using monoclonal antibodies. The plasma level of neutrophil elastase was also measured because it has been shown to activate MMPs and degrade TIMPs. Interassay and intra-assay variation was <7% for all variables. All KD patients fulfilled the revised KD criteria published by the KD Research Committee of Japan in 1984. Coronary arteries with diameters ≥4 mm were regarded as exhibiting CAL. All patients were treated with the intravenous administration of gamma globulin (IVGG; 400 mg · kg$^{-1}$ · d$^{-1}$ for 5 consecutive days) combined with 50 mg · kg$^{-1}$ · d$^{-1}$ of oral aspirin. Blood samples were obtained before and after IVGG treatment and in the convalescent stage. Eighteen age-matched afebrile children were included in the study as afebrile control subjects. These included patients who had undergone reparative surgery for total anomalous pulmonary

Key Words: aneurysm ■ coronary disease ■ metalloproteinases

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Results
Table 1 summarizes the patient characteristics of each group. The only significant difference in data between groups 1 and 2 was that group 2 patients had higher C-reactive protein values than group 1 patients after IVGG therapy. Table 2 shows the baseline levels of MMPs, neutrophil elastase, and TIMPs for each group. The values obtained for the afebrile control group were quite similar to those previously reported for healthy adults. Therefore, although the afebrile control group subjects were not truly healthy subjects, they can be considered comparable to healthy children for the purposes of the present study. Levels of MMPs, neutrophil elastase, and TIMPs were significantly higher in KD patients in the early phase of illness (before treatment) than in afebrile controls (P<0.01). These levels were also significantly higher in febrile disease controls than in afebrile controls, but they were much lower in febrile disease controls than in KD patients (P<0.05). More importantly, levels of MMP9 and neutrophil elastase were significantly higher in patients in group 2 than in those in group 1. Also noteworthy is the fact that there was a significant correlation between MMP9 and C-reactive protein, an inflammatory marker, in febrile disease controls (r=0.51, P<0.05), whereas no such correlation was observed in KD patients; this suggests a causative role for MMP9 in CAL formation.

The Figure shows the serial changes in MMPs, TIMPs, and neutrophil elastase in KD patients. Levels of MMP9 and neutrophil elastase decreased after IVGG in both groups. In contrast, the MMP3 levels in group 2 remained elevated after IVGG and were significantly higher than those in group 1. It is worth noting that there was no difference in MMP levels between groups 1 and 2, despite a difference in MMP levels, suggesting that an imbalance between MMPs and TIMPs contributes to CAL formation. The pre-IVGG MMP9/TIMP2 ratio and the post-IVGG MMP3/TIMP1 ratio were significantly higher in group 2 than in group 1 (Table 3). Notably, although levels of MMPs and TIMPs of febrile patients were significantly higher than those of afebrile controls, MMP/TIMP ratios of febrile patients were equal to those of afebrile controls.

Discussion
To the best of our knowledge, the present results provide the first clear evidence that circulating levels of proteolytic enzymes which act on connective tissue matrix are markedly elevated in KD patients. More importantly, the MMP9 level and the MMP9/TIMP2 ratio at the early stage of illness and the MMP3 level and MMP3/TIMP1 ratio after IVGG therapy were significantly higher in KD patients with CAL than in KD patients without CAL. These data indicate that circulating levels of MMP and the balance between MMP and TIMP may be related to the severity of vascular damage during the acute phase of KD and that KD patients with high MMP levels and/or a high MMP/TIMP ratio are susceptible to CAL.

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### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline (Before IVGG)</th>
<th>After IVGG</th>
<th>Convalescent Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Afebrile</td>
</tr>
<tr>
<td>Age, y</td>
<td>2.5±0.3</td>
<td>2.4±0.4</td>
<td>2.6±0.5</td>
</tr>
<tr>
<td>Days of illness</td>
<td>5.0±0.2</td>
<td>4.8±0.3</td>
<td>...</td>
</tr>
<tr>
<td>WBC, *10^3/μL</td>
<td>17.2±2.1* †</td>
<td>14.6±1.7* †</td>
<td>5.9±0.4</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>13.7±1.4* †</td>
<td>9.6±1.5* †</td>
<td>0.1±0.0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. WBC indicates white blood cell count; CRP, C-reactive protein; and days of illness, the points during the time course of illness that blood samples were obtained. The onset of illness is the day on which the patient developed fever. *P<0.05 vs afebrile controls; †P<0.05 vs febrile disease controls by ANOVA. ‡P<0.05 vs group 1 by unpaired t test.

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### Table 2. Baseline Levels of MMPs, Neutrophil Elastase, and TIMPs

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Afebrile Controls</th>
<th>Febrile Disease Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP1</td>
<td>9.9±0.9*</td>
<td>11.9±1.4*</td>
<td>5.1±0.3</td>
<td>7.6±1.0</td>
</tr>
<tr>
<td>MMP2</td>
<td>934±29†</td>
<td>1106±157†</td>
<td>460±21</td>
<td>781±39*</td>
</tr>
<tr>
<td>MMP3</td>
<td>40.9±6.3*</td>
<td>49.0±21.3*</td>
<td>11.0±1.3</td>
<td>33.1±6.4*</td>
</tr>
<tr>
<td>MMP9</td>
<td>289.3±50.8†</td>
<td>830.0±164.7†∥</td>
<td>27.0±1.8</td>
<td>67.8±11.8*</td>
</tr>
<tr>
<td>TIMP1</td>
<td>359.5±23.8†</td>
<td>299.6±63.4*</td>
<td>95.0±5.0</td>
<td>253±19.1*</td>
</tr>
<tr>
<td>TIMP2</td>
<td>59.8±2.9*</td>
<td>56.3±3.3*</td>
<td>41.0±1.5</td>
<td>51.2±3.3*</td>
</tr>
<tr>
<td>Neutrophil elastase</td>
<td>377.6±44.0*∥</td>
<td>687.3±119.3*∥∥</td>
<td>27.0±4.3</td>
<td>209.6±33.6*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P<0.05 vs afebrile controls; †P<0.05 vs febrile disease controls by ANOVA. ‡P<0.05 vs group 1 by unpaired t test.
Histopathological studies of CAL in KD have demonstrated a destruction of the coronary arterial wall during the acute phase of KD. Infiltration by inflammatory cells begins within the intima, with degradation of endothelial cells and disruption of elastic lamina, and then this infiltration advances into the media and adventitia, resulting in diffuse vasculitis and aneurysm formation. MMP9 has elastinolytic activity and collagenolytic activity. A recent study of an experimental model of arterial aneurysm demonstrated a destruction of elastic lamina as a result of increased MMP9 activity. Thus, elevated MMP9 levels in the early phase of KD could contribute to the initial destruction of the intima of the arterial wall, thereby triggering CAL formation.

In addition to the markedly elevated MMP9 levels in group 2, neutrophil elastase levels were also significantly higher in group 2 than in group 1. Neutrophil elastase is known to activate MMPs, and it may also suppress TIMP activity. Thus, neutrophil elastase could accelerate coronary arterial wall destruction by tipping the balance in favor of MMP activation in the early stage of illness. Investigation of elastase inhibitors, including α1-antitripsin, could help clarify the pathological significance of the elevated elastase levels observed in KD.

After the rapid decline in MMP9 after IVGG therapy, KD patients with CAL showed significantly higher MMP3 levels and MMP3/TIMP1 ratios than those without CAL. MMP3, like MMP9, has elastinolytic and collagenolytic activity. It also degrades laminin and fibronectin, which are important components of the basement membrane. Thus, MMP3 may further accelerate arterial wall damage and contribute to the development of CAL in KD.

Although our data showed significantly higher MMP levels and MMP/TIMP ratios in KD patients with CAL than in patients without CAL, it is also true that there was some overlap in values between the 2 groups. In addition to MMPs, serine proteases have been shown to play significant roles in the development of aortic aneurysm in adults. The importance of inflammatory cytokines in the pathogenesis of several inflammatory diseases has also been demonstrated. Thus, it is possible that many factors other than MMP and TIMP could be involved in CAL formation in KD. In addition, there may be genetic factors that modify the risk of aneurysm formation in KD. Future studies of the roles of MMP and TIMP in CAL formation in KD should take into account interactions among the various factors mentioned above.

### TABLE 3. MMP/TIMP Ratios

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Afebrile Controls</th>
<th>Febrile Disease Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP9/TIMP2 (pre-IVGG for KD)</td>
<td>5.5±0.9†</td>
<td>11.5±1.4‡‡</td>
<td>0.7±0.1</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>MMP3/TIMP1 (post-IVGG for KD)</td>
<td>0.08±0.01</td>
<td>0.44±0.16‡‡</td>
<td>0.12±0.02</td>
<td>0.13±0.03</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05 vs afebrile controls; †P<0.05 vs febrile disease controls by ANOVA. ‡P<0.05 vs group 1 by unpaired t test.
Although the present data may be evidence of a causative role for MMP in CAL formation, it is also possible that the elevated MMP levels we observed in KD patients were a result of the inflammatory process in the coronary arteries. Further studies of the effects of MMP inhibition on coronary outcome are necessary to define the roles of MMP and TIMP in CAL formation in KD, and such data may support the use of MMP inhibitors for the prevention of coronary artery complications in patients with KD.

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References
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The four articles listed below have been retracted due to ethical violations. The corresponding author’s institution, Saitama Medical University, reported to the editors of Circulation, that Dr. Hideaki Senzaki did not receive approval for these studies from the institutional internal ethics committee. Furthermore, in each of the articles referenced below, it was determined that Dr. Senzaki misinformed the editors and readers of Circulation by stating that the studies had received the necessary approval from his institutional review board.


