CXCR3 Chemokine Receptor–Plasma IP10 Interaction in Patients With Coronary Artery Disease

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In rat models of transplant vasculopathy, the strongest staining of CXCR3 is observed in the innermost layer of the neointima and because neointimal hyperplasia is seen after coronary angioplasty, the CXC chemokines may be targets for preventing stenosis. The expression of leukocyte surface chemokine receptors (CCR2/CCR5/CXCR2/CXCR3), as determined by flow cytometry, and plasma concentrations of monocyte chemoattractant protein (MCP)-1 and interferon-inducible protein (IP)10, as determined by enzyme immunoassays, were investigated in 55 patients with coronary artery disease (CAD) who underwent percutaneous transluminal coronary angioplasty (PTCA) and 20 patients without significant coronary stenosis based on the results of coronary catheterization during the same period (C group). The patients with CAD were divided into 3 groups: 20 with de novo stenosis (D group), 15 with restenosis (R group) and 20 without restenosis (N group) after PTCA. CXCR3 expression on lymphocytes, but not monocytes, in the R group was significantly lower than that in the C group. Although the plasma concentrations of IP10 in the D and N groups did not differ from that in the C group, the concentration in the R group was significantly higher. Increased plasma concentrations of IP10 were accompanied by a compensatory decrease in the CXCR3 expression on lymphocytes, but not monocytes, suggesting that a high plasma concentration of IP10 strongly induces monocytes signaling. The CXCR3–plasma IP10 chemokine receptor–chemokine interaction on monocytes may affect the development of coronary restenosis, but not de novo stenosis, in patients with CAD.

Key Words: Coronary artery disease; CXCR3; Interferon-inducible protein 10; Restenosis

Chemokines facilitate leukocyte migration and positioning, as well as other processes such as cell activation and differentiation. They and their receptors constitute a large set of proteins and 2 subfamilies, CXC and CC chemokines and their receptors, have been identified. There is increasing evidence from both clinical and animal studies that leukocytes play a central role in restenosis after balloon angioplasty and stent implantation in patients with coronary artery disease (CAD). Plasma concentrations of monocyte chemoattractant protein-1 (MCP-1), which is a ligand for CCR2, are elevated in patients with restenosis after coronary angioplasty and Horvath et al reported that blockade of CCR2 effectively reduced neointimal hyperplasia after stenting in an animal model.

Although there is no clear evidence to show that CXC chemokines are associated with coronary stenosis in patients with CAD, recent studies have shown that they are involved in graft rejection and ischemia–perfusion injury. The interferon-inducible protein 10 (IP10) is a chemo-attractant for human monocytes and lymphocytes and promotes T cell adhesion to endothelial cells. The presence of CXCR3+ T cells and the CXCR3 ligand IP10 in 169 sequential human endomyocardial biopsies strongly associates the chemokine with acute allograft rejection. Interestingly, Horiuchi et al found only weak expression of CXCR3 on cells in the outer layer of the neointima and adventitia and found the strongest staining in the innermost layer of the neointima in rat models of transplant vasculopathy. Because neointimal hyperplasia is seen after coronary angioplasty, CXC chemokines may be a new target for preventing coronary stenosis.

In the present study, we hypothesized that coronary stenosis is associated with a significant expression of leukocyte IP10–CXCR3, although there is no clear evidence that blockade of the IP10–CXCR3 interaction has beneficial effects on coronary stenosis in patients with CAD. Accordingly, in the present study, we investigated the levels of expression of CC and CXC chemokines and their receptors, including the IP10–CXCR3 interaction, in patients with de novo stenosis or restenosis.

Methods

Study Population

The subject group comprised 55 patients with CAD who underwent percutaneous transluminal coronary angioplasty (PTCA) including stent implantation and 20 patients without significant coronary stenosis (defined as >50% luminal narrowing) based on the results of coronary angiography during the same period as a control group (C group, n=20). The patients with CAD were divided into 3 groups: 20 with de novo stenosis (D group), 15 with restenosis (R group) and 20 without restenosis (N group) after PTCA.

Exclusion criteria were acute myocardial infarction and...
unstable angina within the past 4 weeks, surgery or trauma within the past month, known malignant diseases, and febrile conditions. The study protocol was approved by the Institutional Review Board at Fukuoka University, and written or verbal informed consent was obtained from all patients before they entered the study. Blood was drawn in the morning after an overnight fast when patients were admitted before coronary angiography.

**Coronary Angiography**

Coronary angiograms were recorded and divided into 15 segments according to the classification of the American Heart Association Grading Committee. The presence of stenosis was determined using a computer-assisted coronary angiography analysis system after direct intracoronary injection of isosorbide dinitrate, as described previously. Arterial stenosis that produced more than 50% luminal narrowing was considered significant.

**Leukocyte Expression of CCR and CXCR**

Expression of CCR and CXCR on neutrophils, monocytes and lymphocytes was measured using flow cytometric analysis (Beckman Coulter). Blood samples were anticoagulated with EDTA. Staining was performed using whole blood with fluorescein-isothiocyanate (FITC)-conjugated anti-CCR2, anti-CCR5, anti-CXCR2, and anti-CXCR3 monoclonal antibodies (MAbs). Whole blood (100 µl) was incubated with saturating concentrations of FITC-conjugated MAbs for 30 min at room temperature. Erythrocytes were lysed and leukocytes were fixed with commercially available solutions (Immunoprep™, Beckman Coulter) by TQ prep Workstation (Beckman Coulter). The cells were then washed with phosphate buffered saline and the flow cytometric analysis was performed immediately. MAb binding was assessed by flow cytometry with a FACScan (Beckman Coulter) equipped with a 488-nm argon laser. To analyze the monocytes, lymphocytes and neutrophils, a gate was set in the forward angle versus right angle scatter. The fluorescence intensity of 10,000 events was recorded as the mean channel number over a logarithmic scale of 1–1,026 channels. Results are expressed as the mean channel of fluorescence intensity. Instrumental optical alignment and fluidics were verified using Flow-Check Fluorospheres (Beckman Coulter) whereas day-to-day variability in instrument settings was monitored and adjusted using Flow-Set Fluorospheres.

**Enzyme Immunoassay**

The concentrations of IP10 and MCP-1 in plasma were determined in duplicate by specific enzyme immunoassays (R&D Systems) according to the manufacturer’s instructions. In our laboratory, the intra- and interassay coefficients of variation were each 5%.

**Statistical Analysis**

Data are shown as the mean ± standard deviation. Categorical variables between groups were compared by chi-square analysis. Differences in individual variables were

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**Table 1 Baseline Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>C group (n=20)</th>
<th>D group (n=20)</th>
<th>N group (n=20)</th>
<th>R group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>59±8.5</td>
<td>64±9.4</td>
<td>68±3.8*</td>
<td>67±12.4*</td>
</tr>
<tr>
<td>Male, %</td>
<td>50</td>
<td>85</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.4±3.6</td>
<td>23.8±4.1</td>
<td>24.7±2.1</td>
<td>23.2±3.3</td>
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<tr>
<td>Hypertension, %</td>
<td>50</td>
<td>55</td>
<td>65</td>
<td>67</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>20</td>
<td>40</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>55</td>
<td>65</td>
<td>90</td>
<td>67</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>WBC, 10³/µl</td>
<td>5,630±1,464</td>
<td>4,880±1,162</td>
<td>6,045±1,458*</td>
<td>5,433±1,574</td>
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<tr>
<td>CRP, mg/dl</td>
<td>0.2±0.3</td>
<td>0.2±0.3</td>
<td>0.2±0.2</td>
<td>0.1±0.2</td>
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<table>
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<tr>
<th>Medication</th>
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<tbody>
<tr>
<td>ACEI, %</td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>ARB, %</td>
<td>30</td>
<td>25</td>
<td>55</td>
<td>40</td>
</tr>
<tr>
<td>Calcium antagonists, %</td>
<td>50</td>
<td>55</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>β-blockers, %</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Long-acting nitrates, %</td>
<td>30%</td>
<td>85</td>
<td>70</td>
<td>67</td>
</tr>
<tr>
<td>Statins, %</td>
<td>15</td>
<td>40</td>
<td>50</td>
<td>33</td>
</tr>
</tbody>
</table>

*Values are mean ± SD. WBC, white blood cell; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II type 1 receptor antagonist.

*p<0.05 vs C group; ′p<0.05 vs D group; †p<0.05 vs D, N, and R group.

**Table 2 Angiographic Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>C group (n=20)</th>
<th>D group (n=20)</th>
<th>N group (n=20)</th>
<th>R group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stent, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20 (100)</td>
<td>13 (87)</td>
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<tr>
<td>Lesion of stenosed vessel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD/LCX/RCA, n</td>
<td>0/0/0</td>
<td>16/13/14</td>
<td>18/6/10</td>
<td>10/9/7</td>
</tr>
<tr>
<td>No. of stenosed vessels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, n (%)</td>
<td>0 (0)</td>
<td>6 (30)</td>
<td>8 (40)</td>
<td>7 (47)</td>
</tr>
<tr>
<td>2, n (%)</td>
<td>0 (0)</td>
<td>4 (20)</td>
<td>10 (50)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>3, n (%)</td>
<td>0 (0)</td>
<td>10 (50)</td>
<td>2 (10)</td>
<td>4 (27)</td>
</tr>
</tbody>
</table>

LAD, left anterior descending coronary artery; LCX, left circumflex artery; RCA, right coronary artery.
analyzed by repeated measures one-way analysis of variance followed by Fisher’s PLSD. Relationships between variables were tested by Pearson and Spearman correlations. A value of p<0.05 was regarded as significant. Data were analyzed using commercially available statistical software (Statview-J 5.0; Abacus Concepts Inc).

Results

Patient Demographics

The baseline clinical characteristics of the 4 groups are shown in Table 1. Although there were no differences among the groups with respect to body mass index (BMI) or the prevalence of hypertension, diabetes mellitus, hyperlipidemia, smoking or C-reactive protein, the C group had a higher percentage of younger patients. Although patients had been treated with various combinations of medication, there was no difference in drug administration among the groups, except for long-acting nitrates in the C group, which did not have significant coronary stenosis.

There were no differences in the lesion of the stenosed vessel or the number of stenosed vessels among the D, N and R groups (Table 2). The ejection fraction by echocardiography was similar among the 4 groups (data not shown).

CCR2/CCR5/CXCR2/CXCR3 Expression

CXCR3 expression on lymphocytes, but not monocytes and neutrophils, was significantly lower in the R group than in the C group (Figs 1, 2). Because there were differences in age and sex between these 2 groups, we analyzed the correlation between lymphocyte CXCR3 expression and sex or age. There was no difference in CXCR3 between females and males (C group: p=0.626; R group: p=0.845) and there was no correlation between CXCR3 expression on lymphocytes and age in either group (C group: r=0.369, p=0.145; R group: r=0.099, p=0.738). There was no significant difference in the expression of CCR2 or CXCR2. CCR5 was undetectable.

Plasma IP10 and MCP-1

Although the plasma concentrations of IP10 in the D and N groups were not different from that in the C group, the concentration in R group was significantly higher (Fig 3). The plasma concentration of MCP-1 was higher in the R group than in the C and D groups. Despite differences in age and sex, there was no difference in IP10 between females and males (C group: p=0.626; R group: p=0.845) and there was no correlation between CXCR3 expression on lymphocytes and age in either group (C group: r=0.369, p=0.145; R group: r=0.099, p=0.738). There was no significant difference in the expression of CCR2 or CXCR2. CCR5 was undetectable.

Discussion

To our knowledge, the present study is the first to provide evidence that an increased plasma concentration of IP10 is accompanied by decreased CXCR3 expression on lymphocytes, but not monocytes, in patients with restenosis.

Vessel injury may initiate an inflammatory response that accelerates the recruitment of leukocytes, which play a crucial role in healing and remodeling. IP10 has been identified as a major activator and attractant of lymphocytes: it induces chemotaxis, Ca^{2+} mobilization and adhesion. It is
produced by monocytes, endothelial cells and fibroblasts, and is a ligand for CXCR3. In a rat model of transplant vasculopathy, CXCR3 is expressed only weakly in the outer layer of the neointima and adventitia and the strongest staining is found in the innermost layer of the neointima. Although intracoronyal stenting has reduced the rate of stenosis, in-stent restenosis is still a major clinical problem that is almost exclusively caused by neointimal hyperplasia. It is possible that enhanced levels of IP10 may have important direct and indirect pathophysiological consequences in patients who undergo PTCA. IP10 has chemotactic activity for monocytes and lymphocytes. In animal models of balloon angioplasty and stenting, monocytes are recruited early and abundantly to the denuded vessels where they stimulate smooth muscle cell migration and proliferation leading to neointimal hyperplasia. In addition, a strong correlation has been observed between the extent of inflammatory reaction, characterized by monocyte infiltration, and late neointimal thickening. Therefore, monocytes/macrophages may be prevalent in human restenotic lesions.

We found that increased plasma concentrations of IP10 were accompanied by a compensatory decrease in CXCR3 expression on lymphocytes, but not monocytes, suggesting that a higher plasma concentration of IP10 induces strong signaling on monocytes. Although it is not known definitively whether an increase in the IP10 concentration downregulates the expression of CXCR3 on lymphocytes, but not monocytes, our data suggest this may be possible. Because there is a linear correlation between monocyte adherence and neointimal hyperplasia, the IP10–CXCR3 interaction on monocytes may induce signaling for neointima hyperplasia in coronary restenosis, but not de novo stenosis, after PTCA. Therefore, blockade of CXCR3 should be effective for reducing neointimal hyperplasia after PTCA. Our study was a small observational study and we simply examined the association between IP10–CXCR3 and coronary stenosis. An in vivo trial suggested that antagonising the CXCR3 function may be a target for effective treatment of restenosis, as in T-cell-mediated autoimmune disease.

Previous studies have reported that MCP-1 production in a balloon-injured vessel may play a pivotal role in restenosis after PTCA. Moreover, the expression of MCP-1 in atherectomy specimens from restenotic lesions is significantly higher than that in those from de novo lesions. In the present study, we also observed that plasma concentrations of MCP-1 in the R group were significantly higher than those in the C group. CCR2 is a receptor for various other chemokines (eg, MCP-2, -3, -4), and the downregulation of CCR2 receptors on monocytes could serve to maintain the cells in the inflammation area until other chemokine signals are transmitted. Because CCR2 expression may vary according to the stage of arteriosclerosis and/or restenosis, an increase in MCP-1 may not have downregulated CCR2 expression in this study.

Study Limitations

Although no correlation was observed between CXCR3 or IP10 and age, there was a significant difference in age between the C and R groups. A prospective examination of the association between IP10–CXCR3 and coronary stenosis with more patients seems warranted to investigate this possible association.

Conclusion

Our present observations suggest that blockade of the IP10–CXCR3 interaction on monocytes may have beneficial effects in coronary restenosis in patients with CAD.

Acknowledgments

This work was supported by a grant-in-aid from the Ministry of Education, Science and Culture of Japan (No. 12670712), and research grants from the Central Research Institute of Fukuoka University (No. 026001), the Uehara Memorial Foundation (2002), the Clinical Research Foundation (2001), Fukuoka University School of Medicine, the Eboshi Association (2001) and the Japan Research Foundation for Clinical Pharmacology (2002).

References


Circulation Journal Vol.67, October 2003